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human reproduction



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Abstracts of the 33rd Annual Meeting of the European Society of Human Reproduction and Embryology

Geneva

Switzerland

2 to 5 July 2017

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Abstracts

33rd Annual Meeting of the
European Society of
Human Reproduction and Embryology,
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INVITED SESSION

SESSION 01: KEYNOTE SESSION

Monday 3 July 2017 Plenary I 08:30–09:30

O-001 Human Reproduction keynote lecture - Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study

X. Santamaría[1], I. Cervelló[2], S. Cabanillas[3], C. Arbona[4], F. Raga[5], J. Ferro[6], J. Palmero[7], J. Remohi[8], A. Pellicer[8], C. Simon[9]

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Study question: Could cell therapy using autologous peripheral blood CD133+ bone marrow-derived stem cells (BMDSCs) offer a safe and efficient therapeutic approach for patients with refractory Asherman's syndrome (AS) and/or endometrial atrophy (EA) and a wish to conceive?

Summary answer: In the first 3 months, autologous cell therapy, using CD133+ BMDSCs in conjunction with hormonal replacement therapy, increased the volume and duration of menses as well as the thickness and angiogenesis processes of the endometrium while decreasing intrauterine adhesion scores.

What is known already: Asherman's syndrome (AS) is characterized by the presence of intrauterine adhesions and endometrial atrophy (EA) prevents the endometrium from growing thicker than 5 mm, resulting in menstruation disorders and infertility. Many therapies have been attempted for these conditions, but none have proved effective.

Study design, size, duration: This was a prospective, experimental, non-controlled study. There were 18 patients aged 30–45 years with refractory AS or EA recruited, and 16 of these completed the study. Medical history, physical examination, endometrial thickness, intrauterine adhesion score and neoangiogenesis were assessed before and 3 and 6 months after cell therapy.

Participants/materials, setting, methods: After the initial hysteroscopic diagnosis, BMDSC mobilization was performed by granulocyte-CSF injection, then CD133+ cells were isolated through peripheral blood aphaeresis to obtain a mean of 124.39 million cells (range 42–236),which were immediately delivered into the spiral arterioles by catheterization. Subsequently, endometrial treatment after stem cell therapy was assessed in terms of restoration of menses, endometrial thickness (by vaginal ultrasound), adhesion score (by hysteroscopy), neoangiogenesis and ongoing pregnancy rate. The study was conducted at Hospital Clinico Universitario of Valencia and IVI Valencia (Spain).

Main results and the role of chance: All 11 AS patients exhibited an improved uterine cavity 2 months after stem cell therapy. Endometrial thickness increased from an average of 4.3 mm (range 2.7–5) to 6.7 mm (range 3.1–12) ($P = 0.004$). Similarly, four of the five EA patients experienced an improved endometrial cavity, and endometrial thickness increased from 4.2 mm (range 2.7–5) to 5.7 mm (range 5–12)($P = 0.03$). The beneficial effects of the cell therapy increased the mature vessel density and the duration and intensity of menses in the first 3 months, with a return to the initial levels 6 months after the treatment. Three patients became pregnant spontaneously, resulting in two babies born, and a miscarriage. Furthermore, seven pregnancies were obtained after fourteen embryo transfers, resulting in three biochemical pregnancies, one miscarriage, one ectopic pregnancy, and two babies born.

Limitations, reasons for caution: Limitations of this pilot study include the small sample size and the lack of control group. It is consider as a phase I study for this advance cell therapy

Wider implications of the findings: This novel autologous cell therapy is a promising therapeutic option for patients with these incurable pathologies and a wish to conceive

Study funding/competing interest(s): This study was funded by the Spanish Ministry of Science and Innovation (SAF 2012–31017, Principal Investigator C.S.), Spanish Ministry of Health (EC11-299, Principal Investigator C.S.) and Regional Valencian Ministry of Education (PROMETEOII/2013/018, Principal Investigator C.S.). Four authors (X.S., I.C., A.P. and C.S.) are co-inventors of the patent resulting from this work (Application number: 62/013,121). S.C., C.A., F.R., J.F., J.P. and J.R. have in relation to this work.

Trial registration number: This study was approved by the Ethical Committee at the Hospital Clinico Universitario, Valencia, Spain and was registered with ClinicalTrials.gov (NCT02144987).

O-002 Non-invasive prenatal testing (NIPT) - state of the art

Y.M.D. Lo[1]

[1]The Chinese University of Hong Kong, Li Ka Shing Institute of Health Sciences Prince of Wales Hospital, Shatin- New Territories, Hong Kong

Abstract text

We explored the limit of noninvasive prenatal testing (NIPT) by sequencing the plasma DNA of a pregnant woman to 270X haploid genome coverage. This sequencing depth represents the deepest that any case has been sequenced to date. By using such a depth of sequencing and a custom-built bioinformatics pipeline, we were able to detect fetal de novo mutations on a genomewide level at a sensitivity of 85% and a positive predictive value of 74%. These results indicate that we have solved a hitherto unsolved challenge in NIPT. Furthermore, we have shown that at such a depth of sequencing, we were also able to elucidate the maternal inheritance of the fetus without using haplotype-based approaches. This development has allowed us to increase the resolution of determining the maternal inheritance of the fetus by 90-fold. Furthermore, we have observed that there are recurrent DNA sites that plasma DNA molecules tend to preferentially end on. We called such positions "preferred DNA ends". Interestingly, circulating DNA fragments derived from the fetus and

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those derived from the pregnant woman have sets of different preferred ends. This development allows one to predict the likelihood that a plasma DNA fragment is of fetal or maternal origin without using DNA polymorphisms. This approach also allows one to estimate the fetal DNA fraction without using DNA polymorphisms or size analysis. We believe that such 'second generation noninvasive fetal genomics' would have many exciting diagnostic applications and may facilitate an increased understanding of the biology of circulating DNA.

SELECTED ORAL COMMUNICATIONS
SESSION 02: NOVEL IDEAS IN EMBRYOLOGY

Monday 3 July 2017 Plenary 1 10:00–11:30

O-003 Influence of opening the incubator on morphokinetics in mouse embryos

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Study question: Does the twice daily opening of the incubator door for 10 seconds affect mouse embryo development, cleavage, blastocyst formation and morphokinetic analysis of cell division compared to undisturbed culture?

Summary answer: This study underlines the importance of culture conditions as small and brief changes through simple door openings lead to significant changes in morphokinetics.

What is known already: It has been well established that using time lapse systems lead to increases in pregnancy rates. It remains yet an open question whether this is due to improved selection or to stable and undisturbed culture conditions. In standard laboratory practices incubators are opened and embryos removed from the incubator to assess cleavage and morphology once a day. During embryo manipulation and monitoring, both temperature and pH of the medium change slightly. Stress is thereby not only exposed sequentially to the removed embryos, it also affects the embryos cultured in the same incubator.

Study design, size, duration: To mimic laboratory practice and to evaluate minimal changes in culture conditions we designed a randomized parallel group study employing mouse embryos. 176 zygotes were arranged randomly in control and intervention group. The control group was cultured in an incubator that was completely closed for 5 days, while the intervention group was interfered by two door openings of 10 seconds per day. Environmental factors and morphokinetic analysis of cell division were recorded via a Primovision-System.

Participants/materials, setting, methods: Hybrid Bl6/CBAca mouse embryo assay (MEA) was used to identify morphokinetic cleavage parameters derived from time-lapse imaging. Female mice were sacrificed and zygotes were isolated 20 hours post-insemination. Zygotes were cultured in Primovision micro well group culture dishes with GTL Medium (Vitrolife). Zygotes were observed in the Primovision time lapse system that integrates a digital inverted microscope into a standard Memmert IVF incubator. Pictures of embryos were taken at 10 minute intervals for 5 days.

Main results and the role of chance: Zygotes did not significantly differ in baseline characteristics as age of parents, number of cells derived per mouse, procedures or media.

There was no significant difference in blastocyst rate (86,7% and 88,2%) and blastocyst size (104 and 103 mm) between control and intervention Group. The embryos in the intervention group showed significantly quicker development. They reached 3-cells stage at 1898 versus 2025 minutes, $p = 0.002$; 5-cells stage at 2637 versus 2833 minutes, $p = 0.002$; morula stage at 3247 versus 3557 minutes, $p = 0.001$, blastocyst stage at 4464 versus 4692 minutes, $p < 0.001$ and hatching at 4960 versus 5250 minutes, $p < 0.001$ comparing to control group.

Limitations, reasons for caution: The present study used surrogate parameters as morphokinectics and blastocyst rates. No data on pregnancy or birth rates can be provided as blastocysts were not transferred. The results may differ in different laboratory settings as type of incubator and laboratory procedures may also have significant impact on embryo development.

Wider implications of the findings: The findings underline the importance of further evaluation of culture conditions in IVF. As even small and short environmental changes can significantly impact the characteristics of development, the evaluation of time lapse parameters as morphokinetics in this context may further improve our understanding of these factors.

Trial registration number: N/A.

O-004 Image analysis of human embryos grown in vitro as a new non-invasive tool to determine embryo health

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Study question: Which embryo criteria have the most potential for automatic monitoring and embryo quality assessment using images captured during embryo growth in vitro?

Summary answer: Detection of syngami, timing of mitosis, blastocyst formation and blastomere number are quantifiable attributes. Embryo activity and fragmentation are also detectable but not quantifiable.

What is known already: Non-invasive imaging has recently made it possible to monitor embryos continuously without any known consequences to their health. It has been shown that the timing of key occurrences within the embryo can vary greatly between embryos that have similar morphological appearance at the conclusion of the recording period and that embryo morphology can change in a matter of hours, emphasizing the fact that dynamic monitoring is preferred over intermittent monitoring of embryos. Dynamic monitoring of embryos requires computerized analysis but few studies have systematically attempted to map automatically extracted embryo imaging criteria to manual detection of the same.

Study design, size, duration: Time lapse image series of human embryos fertilized *in vitro* were acquired as anonymized sequences donated to research with ethical approval. Images were analysed for a number of embryo trait known to correlate with embryo health. Results from computerised analysis were compared to manual detection of the same trait. The study consisted of seven studies performed on the same imaging material over a course of three years.

Participants/materials, setting, methods: Embryos were cultured in 25 µl culture media (Origio, Redhill, UK) under mineral oil for up to 6 days, incubated at 37°C in an atmosphere of 5%CO2, 5%O2 and 90%N2. The images were captured using the Embryoscope® time-lapse system (Vitrolife, Gothenburg, Sweden), with 9 focal depth planes, 15–25 µm apart, recorded at 20 minute intervals using a HMC optical set up and a 635 nm LED as light source. Images were analyzed using Matlab® 7.12.0.635 (R2011a).

Main results and the role of chance: Detection accuracy of of syngami was 83%, timing of mitosis 80.8% (1–6 cells), blastocyst formation 71.8% and blastomere number 80.8% (1–6 cells). Using the timing information, it was possible to measure the time elapsed between divisions to 10.27 h (2–3 cells), and 1.11 h (3–4 cells), respectively. Detection accuracy for mitosis reduced by cell number. 100% of divisions from 1 to 2 cells were detected, 73% from 2 to 3 (or 4) cells, 30% from 3 to 4 cells, and 59% from 4 to 5 (or 6) cells. Using the timing of blastocyst and morula formation, we calculated the duration of the morula stage to 1/7 of the duration of the cleavage stage, with some patient variability. Embryo activity and fragmentation were detectable in images but the accuracy not quantifiable, due to the lack of a standardized way to measure these traits manually. The automatic counting of blastomere number was semiautomatic and required manual selection of typical images prior to analysis. The

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counting was tested up to the 8 cell stage, in which case the accuracy was 74.9%. The accuracy was less than for other imaging techniques, e.g. using fluorescent markers.

Limitations, reasons for caution: Images were obtained from a range of microscopy settings using different protocols for embryo cultivation and different lighting conditions in the microscopy. However, the study cohort was small (38 embryos from 7 patients from 3 laboratory settings at the largest).

Wider implications of the findings: Continuous imaging of human embryos growing in vitro is being routinely generated as part of IVF cultivation. The material poses a method to study dynamic properties of embryos without having to result to experimentation on human tissue and has the possibility to provide new methods to improve IVF success rates.

Trial registration number: Ethical approval by Coventry Research Ethics Committee (04/Q2802/26) and the Human Fertilisation and Embryology Authority (R0155).

O-005 Study of embryo surface variation and contraction pattern as potential predictors of embryo chromosomal content

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Study question: Do embryo contraction behaviour and surface variation have a predictive value on embryo chromosomal content?

Summary answer: Blastocyst contraction pattern shows to be related to embryo chromosomal content.

What is known already: Time-Lapse monitoring (TLM) together with next-generation sequencing (NGS) are considered to be two main techniques for selecting the embryos with the potential to result in a healthy pregnancy. Together with morphology based criteria, many selection parameters for embryonic competence based on TLM have emerged to refine this selection process. First reported by Lewis and Gregory in 1929, blastocyst contractions during blastulation were first related to the ability for an embryo to hatch. Other studies reported a reduced implantation and live birth rates in embryos presenting contractions. The physiological role and the cause for this phenomenon remains to be fully understood.

Study design, size, duration: 141 good quality blastocysts generated after ICSI or IMSI between January 2016 to January 2017, derived from 46 patients (mean age 38.6 (SD 2.3) years), were included in the study. Two different outcomes were considered after the NGS testing (Normal and Abnormal) in this retrospective study of TLM embryos after day 5/6 blastocyst biopsy.

Participants/materials, setting, methods: Different morphokinetic parameters were assessed together with some contraction pattern variables in the different groups. Embryo contraction was defined as the event during blastulation where the perivitelline space is observed by retraction of trophectoderm cells. Surface variation was determined with EmbryoViewer software measuring tools. Univariable and Multivariable Analysis of the data was performed using STATA software, considering that one or more embryos belonged to the same patient (random effect analysis).

Main results and the role of chance: A total of 104/141 (73.8%) blastocysts were proven to be chromosomally abnormal (not mosaic) while 37/141 were chromosomally normal embryos (29.2%). A total of 77 blastocysts presented contractions (54.6%), of these 26 presented more than one contraction during development (18.4%). Data analysis shows no statistical significance on the total number of contractions between both groups. Univariable logistic regression analyses showed significance in time of first contraction (Tc1), surface loss in first contraction (loss1) and Surface Gain after Blastulation (Gain) at the 5% level between both groups. Followed by a multivariable logistic regression analysis only tc1 and loss1 showed significance at the 5% level. Our data suggests that a unit increase of time of Tc1 and loss1 reduces the odds of a Normal genetic result by about 16% and 31%, respectively after adjusting for the other variables in the model.

Limitations, reasons for caution: Larger studies with an increased number of embryos are needed to confirm our findings.

Wider implications of the findings: According to our results we could suggest that blastocyst contraction pattern could possible be used as a potential predictor of embryo chromosomal content. Surface measurements during blastulation could be an extra check-point on embryo quality.

Trial registration number: N/A.

O-006 Oliana Strings: Perivitelline threads that originate from corona radiata cells and cause fragmentation in human embryos

O. Oliana Membrado[1], R. Derrick[2], C. Hickman[3], T. Wilkinson[1], D. Gwinnett[1], R. Fawthrop[1], A. Carby[4], S. Lavery[5]

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[5]*The Fertility Partnership, Clinical, London, United Kingdom*

Study question: Where do Oliana Strings (OS) originate from? What is their function? Do OS affect ploidy or implantation potential?

Summary answer: OS are observed in most embryos, originate from corona-radiata, are mostly visible at 2-cell stage, significantly correlate with fragmentation but not with ploidy or implantation.

What is known already: Embryoscope Plus optics has improved the visibility of certain embryonic structures. OS are amongst the structures which have become more clearly visible with this type of incubator. OS are defined as thin filaments that extend across the perivitelline space connecting the zona pellucida with the oogenic. OS were first described by our group at Fertility 2017 conference in Edinburgh. This abstract presents further work conducted by our group to clarify the origin and role of OS in early embryo development.

Study design, size, duration: Retrospective assessment of time-lapse imagery from 525 human embryos cultured for clinical IVF treatment. The assessor was blinded to ploidy and implantation potential.

Participants/materials, setting, methods: Time-lapse videos were reviewed for the presence of OS, the cell stage when OS were first observed, association with fragmentation, ploidy or implantation potential. ICSI videos were also reviewed to determine the origin of OS.

Main results and the role of chance: OS were observed in the majority (77%,404/525) of embryos. There was no difference in incidence of OS in embryos that were euploid (78%,61/78) versus aneuploid (83%,91/109); or those that implanted (73%,64/88) versus those that did not (75%,195/261). In the embryos where OS were observed, 98%(396/404) were first observed at the two-cell stage. OS were observed to directly pull fragments from the embryo in most embryos (95%, 384/404). Fragmentation occurred significantly less frequently in embryos without OS (67%,81/121;$p < 0.001$). ICSI videos demonstrated string-like structures extending from corona cells, through the zona, interacting with the oolemma through perivitelline debris.

Limitations, reasons for caution: It is expected that the true incidence of OS be higher than observed given that embryos could not be rotated within the time-lapse incubator and OS may have occurred outside the view of the seven focal planes, or may have been smaller than detectable by the resolution of the images.

Wider implications of the findings: This data suggests that OS may be a cause of fragmentation, although the high incidence of fragmentation in string-free embryos suggests OS are not essential for fragmentation to occur. OS may be useful in differentiating between fragments and cells.

Trial registration number: not applicable.

O-007 Does the extent of blastocyst hatching at time of trophectoderm biopsy affect PGD clinical outcome?

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Guy's and St. Thomas' hospital, Assisted Conception Unit, London, United Kingdom

Study question: Does the extent of blastocyst hatching at time of trophectoderm biopsy (TB) affect PGD clinical outcome?

Summary answer: Implantation of fully hatched blastocysts is significantly reduced compared to hatching blastocysts following TB and vitrification for PGD.

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What is known already: High rates of survival and implantation have been reported after blastocyst biopsy and vitrification for PGD (Galn *et al.* 2003; Van Landuyt *et al.* 2010). The effect of TB for PGD at different degrees of blastocyst hatching followed by vitrification on blastocyst implantation potential has not been adequately explored. Data investigating the fate of vitrified blastocysts at different stages of hatching are limited (Zech *et al.* 2005).

Study design, size, duration: Retrospective analysis was undertaken for all PGD cycles that took place between January 2014 and June 2016. Following zona ablation on day 3, trophectoderm cells were biopsied from hatching and fully hatched blastocysts on day 5/6; biopsied blastocysts were vitrified. Genetically suitable embryos were warmed and transferred individually. Implantation rates were compared for blastocysts biopsied at different stages of hatching (Group A: < 50% hatched; Group B: ≥50% hatched; Group C: fully hatched).

Participants/materials, setting, methods: Following ICSI, pronucleate stage embryos were cultured in single-step medium (SAGE; Origio, USA) and incubated in Embryoscope incubators (Unisense-FertiliTech, Denmark) in 5%O_2:6%CO_2:89%N_2. After laser-assisted zona ablation on day 3, 5–10 trophectoderm cells were biopsied on day 5/6 followed by vitrification (Cryotop/Kitazato; Dibimed, Japan); genetically suitable embryos were warmed and transferred individually. Retrospective scoring of herniation/hatching prior to biopsy was carried out by a single, independent operator using time-lapse images.

Main results and the role of chance: The mean age of participants, number of PGD cycles, eggs collected and fertilised normally, and type of genetic test (aCGH or PGH) were not significantly different between the three groups ($p > 0.05$). From 407 egg collection/PGD cycles, 614 embryos were warmed for 340 patients, resulting in 542 single embryo transfers (overall survival rate 88% [542/614]). Survival and implantation rates (SR, IR) per embryo were not statistically different between Groups A and B (90% [393/438] *vs* 88% [127/145]; $p = 0.54$, and 41% [179/438] *vs* 32% [47/145]; $p = 0.08$ respectively), but were significantly lower in Group C compared to both Group A (71% [22/31]; $p = 0.005$ *vs* 19% [6/31]; $p = 0.02$ respectively). The clinical pregnancy rates (CPR) per transfer for Groups A, B and C were 42% [164/393], 35% [45/127] and 18% [4/22] respectively. There was no significant difference between Groups A and B; $p = 0.21$, but CPR was significantly reduced in Group C compared to Group A; $p = 0.04$. The mean interval between the time of ICSI and the time of TB, as evaluated from time-lapse images, was 123.7hrs (Group A; n = 438), 128.7hrs (Group B; n = 145) and 136.5hrs (Group C; n = 31).

Limitations, reasons for caution: This is retrospective study; the number of embryos in Group C is relatively small; the impact of additional morphological and morphokinetic differences between embryos have not been evaluated.

Wider implications of the findings: In order to maximise implantation and clinical pregnancy rates, TB for PGD/PGS should be carried out before the blastocyst hatches fully. This finding has important implications for the management and scheduling of egg collection, ICSI and TB procedures for PGD/PGS cases.

Trial registration number: N.A.

O-008 Complete zona pellucida removal facilitates embryo attachment and outgrowth by upregulating the integrin α5 and β1 expression in human blastocysts: in vitro outgrowth model

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Study question: Does complete removal of the zona pellucida (ZP) as a method of assisted hatching improve the adhesion and outgrowth of vitrified-warmed human blastocysts?

Summary answer: Complete ZP removal increases the chance of blastocyst adhesion and promotes subsequent outgrowth by upregulating the integrin α5 and β1 expression after the vitrification-warming procedure.

What is known already: Laser-assisted hatching (LAH) has become the primary assisted hatching method used in many IVF laboratories. Generally, in LAH method, ZP is partially removed using laser system. However, in some cases, low-viability blastocysts cannot hatch even after partial ZP removal. Furthermore, escaping through the assisted hatching holes still requires a considerable energy-consuming effort from blastocysts and cause a marked distortion of their shape. Binding of cells to fibronectin is mediated through fibronectin receptor, Integrin heterodimer α5β1. In addition, human blastocysts express integrin α5 and β1 mRNA during the process of implantation.

Study design, size, duration: A total of 217 discarded cryopreserved human blastocysts donated for research by consenting couples (Woman's age: 35.4 ± 0.3 years, Blastocyst morphology: 3CC or greater) were warmed and subjected to assisted hatching to remove the ZP partially (n = 79) or completely (n = 79), or did not undergo assisted hatching (ZP intact controls, n = 59). Blastocyst adhesion rate, outgrowth speed and mRNA expression level of integrin α5β1 after plating on fibronectin coated dish were compared among the groups.

Participants/materials, setting, methods: Human blastocysts were warmed using the Cryotop^R method. Laser drilling was performed to remove 30–40% of ZP of blastocysts (Partial ZP removal). Subsequently, the blastocyst was gently denuded of ZP by pipetting (Complete ZP removal). These blastocysts were plated on fibronectin-coated dish and cultured for 96 hours in time-lapse incubator to monitor hatching, adhesion and outgrowth. qRT-PCR assay was performed to measure mRNA expression of integrin α5 and β1 in blastocysts during in vitro outgrowth.

Main results and the role of chance: Blastocyst hatching rate was improved by partial ZP removal group (64%, 32/50) compared with ZP intact group (10%, 3/30), still, 34% (18/50) blastocysts in the partial ZP removal group failed to hatch despite assisted hatching treatment. The blastocyst adhesion was initiated earlier in complete ZP removal group compared with the other groups. The blastocyst adhesion rate at 96 hours after the culture was significantly higher in complete ZP removal group (80.0%, 40/50) than in partial ZP removal (60%, 30/50) and ZP intact group (10%, 3/30). The outgrowth speed of the ZP intact, partial removal and complete removal groups were 2.4×10^3 μm^2/h, 4.7×10^3 μm^2/h and 5.8×10^3 μm^2/h, respectively and complete removal group tended to show faster outgrowth. The expression levels of integrin α5 and β1 at 24 hours after plating in complete ZP removal group were significantly higher than ZP intact and partial ZP removal groups (complete ZP removal: 1.9 ± 0.3 and 3.1 ± 0.4, ZP intact:1.0 ± 0.2 and 1.0 ± 0.2 and partial ZP removal: 1.1 ± 0.5 and 1.5 ± 0.4).

Limitations, reasons for caution: The use of the in vitro blastocyst outgrowth assay was a weakness in this study since hatching process may vary between in-vitro and in-vivo conditions. Therefore, further clinical studies are required to explore the clinical efficacy of the complete ZP removal.

Wider implications of the findings: The complete ZP removal as an assisted hatching method prevents hatching failure and is advantageous for blastocyst adhesion and its outgrowth as assessed in an in-vitro model. Therefore, when a blastocyst is chosen for vitrified-warmed blastocyst transfer, complete ZP removal may help to increase the chance of blastocyst attachment.

Trial registration number: IRB approval number: 14–19

SELECTED ORAL COMMUNICATIONS
SESSION 03: REFINING PROTOCOLS
Monday 3 July 2017 Plenary 2 10:00–11:30

O-009 Ovarian stimulation in IUI cycles in couples with unexplained subfertility: follicle stimulating hormone (FSH) or clomiphene citrate (CC)?

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[10]UMCU, Reproductive Medicine, Utrecht, The Netherlands
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Study question: Is 75 IU FSH or 100 mg CC the best ovarian stimulation (OS) regimen in couples with unexplained subfertility undergoing intra uterine insemination (IUI)?

Summary answer: In IUI-OS with adherence to strict cancellation criteria, FSH is not superior to CC to achieve ongoing pregnancies, while multiple pregnancy rates are comparable.

What is known already: IUI-OS is the first line treatment in couples with unexplained or mild male subfertility. OS aims to increase the number of dominant follicles per cycle, thus increasing pregnancy chances, but at the expense of multiple pregnancies. To reduce the risk of multiple pregnancy, IUI-OS with strict cancellation criteria, i.e. when ≥3 dominant follicles develop, has been suggested. With such a strategy, it is unclear whether the ovarian stimulation should be done with FSH or with CC.

Study design, size, duration: We performed a multicentre, non-blinded, superiority randomised controlled trial in the Dutch Consortium for Healthcare Evaluation in Obstetrics and Gynaecology. Based on a power calculation, we needed to include 732 women to be able to prove a difference in ongoing pregnancies of 10%. We randomly allocated 738 couples with unexplained or mild male subfertility and an unfavourable prognosis according to the model of Hunault for natural conception, to 4 cycles of IUI-FSH or IUI-CC.

Participants/materials, setting, methods: The primary outcome was ongoing pregnancy within six months after randomisation. Secondary outcomes were multiple pregnancy, live birth, time to ongoing pregnancy, cancellation rates and number of cycles with monofollicular growth. We analysed the data on an intention to treat basis. We calculated relative risks and 95% CI.

Main results and the role of chance: Between August 2013 and March 2016, we randomised 369 women to FSH and 369 women to CC. 115 women (31%) had an ongoing pregnancy following IUI-FSH and 100 women (27%) had an ongoing pregnancy following IUI-CC (RR 1.15, 95% CI .92 to 1.44, $p = .23$). 6 women (5%) had a multiple pregnancy following IUI-FSH and 9 women (9%) had a multiple pregnancy following IUI-CC (RR 0.58, 95% CI 0.21 to 1.57, $p = .28$).

Limitations, reasons for caution: At this moment, we have access to 92% of the data regarding ongoing pregnancy and 90% of the data regarding multiple pregnancies. Full data will be available before June 2017.

Wider implications of the findings: In view of the data, we suggest to use the least expensive and least invasive stimulation agent, which is CC. A formal cost-effectiveness analysis for the ultimate interpretation of the data is planned.

Trial registration number: Nederlands Trial Register *NTR4057*

O-010 A randomized controlled trial of intrauterine insemination with clomiphene citrate stimulation compared with expectant management for women with unexplained infertility (The TUI study)

C.M. Farquhar[1], E. Liu[2], S. Armstrong[1], N. Arroll[1], S. Lensen[2], J. Brown[3]

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Study question: Does 3 cycles of IUI with clomiphene (IUI-C) increase cumulative livebirth rates (CLBR) versus 3 cycles of expectant management (EM) in couples with unexplained infertility.

Summary answer: LBR was increased following 3 cycles of IUI-C compared with three cycles of EM in couples with unexplained infertility with low probability of natural pregnancy in next 12 months.

What is known already: There are only two published randomised controlled trials (RCT) of IUI with and without stimulation compared with no treatment. In one trial the IUI did not include ovarian stimulation. In the other trial the IUI included gonadotropin stimulation and patients had a favourable probability of natural pregnancy in the next 12 months between 30 and 40%. Neither trial suggested benefit. In 2013 NICE Fertility guidelines recommended that intrauterine insemination should not be routinely offered, with or without ovarian stimulation for couples with unexplained infertility. In spite of this recommendation many UK clinics still offer IUI. 100/95

Study design, size, duration: A RCT of three cycles of IUI-C compared with three cycles of EM in couples with unexplained infertility. Computer generated randomized numbers in sequentially numbered opaque sealed envelopes were opened by a third party study coordinator after consent signed. We needed to include 90 women in each arm to demonstrate an increase from the predicted 8% to 22% in LBR (80% power, 5% level).

Participants/materials, setting, methods: From March 2013 - May 2016, 201 couples with unexplained infertility were recruited from two fertility clinics. Inclusion criteria were ovulatory women with patent tubes, partners with normal semen analysis (>15 mil/l, motility > 32%) and probability of natural pregnancy in the next 12 months <30%. 101 couples were allocated to three cycles of IUI-C and 100 couples were allocated to three cycles of expectant management. The couples with expectant management completed a diary recording sexual activity.

Main results and the role of chance: IUI: 31 live births includes 3 LB who were pregnant at study entry and 5 LB that occurred prior to or between IUI cycles

EM: 9 LB including 1 who was pregnant from IUI at study entry and 1 from IVF.

Two ectopic pregnancies and two multiple pregnancies were reported in the IUI group and one stillbirth at 20 weeks gestation was reported in the EM group.

Using an intention to treat analysis for live births (LB):

IUI-C was associated with an increase in CLBR compared to EM group (31 of 101 [31%] vs. 9 of 100 [9%], P = 0.0003; risk ratio (RR) for cumulative live birth, 3.41; 95% confidence interval (CI), 1.71 to 6.79)). There were two sets of twins, both in the IUI-CC group (one from a cancelled cycle for overresponse).

Using a per protocol analysis for LB: excluding treatment protocol violations, the cumulative live birth rate was 22/85 (25.6%) with IUI-C and 6/88 (6.8%) with EM and the RR was 3.80, 95% CI, 1.62 to 8.90) (P = 0.005).

Number needed to treat: Five women (95% CI, 3–12) would need to undergo 3 cycles of IUI-C for one additional live birth.

Limitations, reasons for caution: Protocol breaches included 5 women who conceived before and between IUI-C cycles, 5 women in the EM group who had IUI or IVF (two conceived), and 8 women in each group who had prediction scores > 30% and in both groups 2 conceived. In addition, three women conceived on CC without undergoing insemination.

Wider implications of the findings: Intrauterine insemination is associated with a three-fold increase in live birth rate when compared to EM. IUI-C may be offered to couples with unexplained infertility as a safe and effective treatment.

Trial registration number: U1111-1134–9722 24/09/2012.

O-011 Intrauterine insemination: does timing matter? A multicenter randomised controlled trial

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[6]Laurentius Hospital, Gynaecology, Roermond, The Netherlands
[7]Zuyderland Hospital, Gynaecology, Sittard, The Netherlands
[8]Maastricht University, Epidemiology, Maastricht, The Netherlands

Study question: Does the ongoing pregnancy rate differ between intrauterine insemination (IUI) at the moment of ovulation triggering ('immediate IUI") and IUI performed 32–36 hours after that ('traditional IUI")?

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Summary answer: Immediate IUI, at the moment of human chorionic gonadotropin (hCG), results in an ongoing pregnancy rate that is not significantly different from traditional IUI.

What is known already: IUI is a widely used treatment for couples with unexplained and mild male factor subfertility. Although IUI is a common treatment, its timing is poorly investigated and is usually performed 32–36 hours after hCG. The largest probability of pregnancy in natural cycles, however, occurs with intercourse one day before ovulation, with chances rapidly decreasing thereafter. Järvelä et al (2010), showed significantly higher pregnancy rates when IUI was performed immediately after hCG (19.6%) in comparison to 24–36 hours after hCG (10.9%). Aydin et al (2013) however did not show any difference in clinical pregnancy rates between the two groups

Study design, size, duration: Between February 2013 en July 2016 we conducted an open-label randomized controlled trial in seven Dutch fertility clinics. We included 165 couples for immediate IUI and 207 couples for traditional IUI.

Participants/materials, setting, methods: Ovarian Stimulation (OS) was performed by subcutaneous FSH (according to local protocol and in a personalized dosage), starting between cycle day three and five. The 'Immediate IUI" group had IUI the day after the largest follicle had reached 16–18 mm, immediately followed by administration of hCG for triggering ovulation. The"Traditional IUI" group received care as usual, namely hCG for triggering ovulation when the largest follicle reached 16–18 mm, followed by IUI 32–36 hours later.

Main results and the role of chance: After randomisation six couples did not start with IUI-OS treatment (five because of spontaneous pregnancy before the start of IUI-OS and one started with another fertility treatment). In total 163 couples (immediate IUI) and 205 couples (traditional IUI) started IUI-OS treatment. We did not receive information about three couples (one in the immediate IUI group and two in the traditional IUI group).

The groups did not show any difference in BMI, age, type of subfertility (primary versus secondary subfertility (respectively 69.3% versus 30.6 % in the immediate IUI group and 64.4% versus 35,6% in the traditional IUI group), indications for IUI, semen analysis outcome or number of follicles (average 1,34 in the immediate IUI group and 1,31 in the traditional IUI group).

After one cycle, there was no difference in ongoing pregnancy rate: 6.79% (11/162) in the immediate IUI group and 7.88% (16/203) in the traditional IUI group yielding a relative risk of 0.86 (95% confidence interval 0.41–1.80).

Limitations, reasons for caution: This study compared two timing strategies for IUI: IUI immediately followed by hCG-triggering versus traditional timing, 32–36 hours after hCG triggering. We cannot say anything about the chances of ongoing pregnancy with other timing strategies.

Wider implications of the findings: We did not find any difference in ongoing pregnancy rate between the two different timing strategies. Our findings imply that scheduling IUI relatively to the hCG trigger in everyday practice can be more flexible.

Trial registration number: Portal ToetsingOnline Kenmerk: NL39738.068.12, approval October 31st 2012.

O-012 Anovulatory women not conceiving after six ovulatory cycles with clomiphene citrate – should we switch to gonadotrophins and/or add IUI? A 2 by2 factorial RCT

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[10]The Robinson Institute- University of Adelaide, School of Paediatrics and Reproductive Health, Adelaide, Australia

Study question: What is the effectiveness of extended treatment with clomiphene (CC) compared with FSH and what is the additional value of IUI in women who had six ovulatory cycles after CC?

Summary answer: Extended ovulation induction with CC resulted in less ongoing pregnancies compared to FSH, while addition of IUI had no impact on ongoing pregnancy chances

What is known already: CC is a first line treatment in women with World Health Organization (WHO) type II anovulation and polycystic ovary syndrome (PCOS). If women ovulate but do not conceive after several cycles CC, medication is usually switched to FSH with or without IUI. At present, it is unclear whether such a switch to ovulation induction with gonadotropins with or without IUI is effective

Study design, size, duration: We performed a 2 by 2 factorial multicenter RCT in the Netherlands from November 2008 until November 2015. The study aimed to compare CC and FSH and IUI with no IUI in a superiority design. The data safety monitoring board reviewed the data after inclusion of 320 women and advised to include at least 600 women

Participants/materials, setting, methods: We included women with WHO type II anovulation who had been ovulatory for six cycles CC without conception. Women were randomly allocated in a factorial design to receive ovulation induction with CC or FSH with IUI or intercourse for six cycles. Women were treated for six cycles or until an ongoing pregnancy occurred. Primary outcome was live birth rate at 12 weeks gestation within eight months after randomisation. Primary analysis was by intention to treat

Main results and the role of chance: We randomised 666 women to CC (N = 173), CC plus IUI (N = 162), FSH (N = 165), or FSH plus IUI (N = 166). Included women were on average 29 years old with a BMI of 25. At the moment of writing this abstract, the ongoing pregnancy data for 95% of the couples was known. For the comparison of CC with FSH the ongoing pregnancy rates were 42% for CC and 53% for FSH (relative risk (RR) of 0.79 (95%CI: 0.68–0.92)). For the comparison IUI to no IUI the ongoing pregnancy rates were 49% for IUI and 45% for no IUI (RR 1.08 (95%CI: 0.93–1.26)). There were 7 multiple pregnancies following CC and 7 multiple pregnancies following FSH. There were 9 multiple pregnancies following IUI and 5 multiple pregnancies following no IUI. Average time leading to an ongoing pregnancy was 4.6 months following CC and 4.2 months following FSH. Average time leading to an ongoing pregnancy was 4.3 months with IUI and 4.5 months without IUI

Limitations, reasons for caution: These results are based on 95% of the data, while data on live birth are currently being collected.

Reason for caution is that a cost-effectiveness has not been done as of yet. This will decide the ultimate interpretation of the data

Wider implications of the findings: These preliminary results suggest that in type II anovulatory women with CC failure, a switch to FSH slightly increases ongoing pregnancy chances over a 42% with CC alone. Addition of IUI is not useful in these women.

Trial registration number: Netherlands Trial Register NTR1449.

O-013 Corifollitropin alfa followed by hp-HMG versus recombinant FSH in young poor ovarian responders: a multicenter randomized controlled clinical trial

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Study question: Does administration of corifollitropin alfa followed by hp-HMG result in higher ongoing pregnancy rates compared with daily recombinant FSH in young Bologna poor responders?

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Summary answer: Corifollitropin alfa followed by hp-HMG does not increase ongoing pregnancy rates compared with rFSH in young poor responders. However, more supernumerary cryopreserved embryos are obtained.

What is known already: Poor ovarian response (POR) remains one of the main therapeutic challenges in women undergoing ovarian stimulation, given that very low live birth rates of 6% have been reported in this special group of infertile patients. Nevertheless, concerns have been raised that a degree of heterogeneity remains, as the prognostic effect of individual factors is still unclear, particularly for the young poor responder group. The rationale for conducting the current randomized trial lies to a previous pilot study demonstrating promising results with the administration of hp-HMG following corifollitropin alpha in women less than 40 years of age, fulfilling the "Bologna" criteria.

Study design, size, duration: This is a multicenter, phase III superiority randomized trial using a parallel two –arm design. The study included 152 patients <40 years old, fulfilling the "Bologna" criteria for POR from 1 tertiary referral center in Europe and 1 tertiary referral center in Asia, who underwent ovarian stimulation for ICSI from March 2013 to May 2016. Randomization sequence was performed using a computer generated randomization list, stratified by center, using 1:1 allocation.

Participants/materials, setting, methods: Eligible patients were randomized to either administration of 150 μg corifollitropin alfa followed by 300 IU hp-HMG (Group A) or to 300 IU of daily recombinant FSH (Group B) in a fixed GnRH antagonist protocol. The primary outcome was ongoing pregnancy rates (defined as presence of intrauterine gestational sac with an embryo with cardiac activity at 9-10 weeks of gestation). Secondary outcomes included clinical /biochemical pregnancy rates and number of oocytes retrieved cryopreservation rates.

Main results and the role of chance: Overall, 152 poor ovarian responders defined by the "Bologna" criteria were included in the study. Using an intention-to treat analysis the ongoing pregnancy rates did not differ significantly between Group A 11/77 (14.3%) and Group B 11/75 (14.7%), OR = 1.03 (0.4–2.5). Biochemical/clinical pregnancy rates and the number of oocytes retrieved were also comparable between the two groups. Nevertheless, more patients in the corifollitropin alfa group had cryopreserved embryos compared to recombinant FSH [14 (31.1%) versus 6 (15.8%), OR = 2.4 (1.04–5.5)]. Furthermore, Asian poor responders had significantly lower cancellation rates compared to European poor responders [2/64 (3.1%) versus 17/83 (20.4%), OR = 0.12 (0.03–0.5)]. This discrepancy could be explained by the fact that Asian women were of better prognosis than European patients, with significantly lower FSH [9.8(5.3) versus 11.5(5.4), p = 0.017] and significantly higher AMH [1.1(0.9) versus 0.4(0.3), p value <0.001].

Limitations, reasons for caution: Although we failed to identify differences between the two randomized arms, we cannot exclude that smaller differences might exist, which our study was underpowered to detect.

Wider implications of the findings: POR represents a challenge and although specific protocols may increase the number of cryopreserved embryos, no difference is observed in ongoing pregnancy rates. Our study, being one of the largest RCTs in Bologna poorresponders, highlights that baseline characteristics may play a crucial role in the clinical prognosis of this population.

Trial registration number: The EUDRACT number of the trial was 2013-000583-29 and the study was registered to clinicaltrials.gov (NCT01816321).

O-014 Letrozole step-up protocol: the effect of a novel superovualtion induction protocol to enhance pregnancy rate in a couple with unexplained infertility undergoing intrauterine insemination

A. Galal[1]

[1]*Alexandria, Egypt,*

Study question: investigate success of novel step-up protocol of letrozole as an attempt to achieve multifollicular development and to assess its effect on outcome of IUI cycles in couples with unexplained infertility.

Summary answer: letrozole step up protocol is potentially valid novel induction protocol, has privilege of similar pregnancy rate to standard HMG ovulation induction with lower cost

What is known already: Objective Empiric ovarian stimulation with clomiphene citrate, aromatase inhibitors (Letrozole) or exogenous gonadotropin is commonly combined with IUI in the treatment of couples with unexplained infertility. The successful use of the aromatase inhibitor,letrozole, for ovarian stimulation was documented when administered as a single dailydose or multiple daily fixed dose. To our knowledge,achieving consistent multifollicular development with letrozole, without the addition of FSH, has not been reported except in a retrospective trial.

Study design, size, duration: Design a prospective randomized controlled study.

Materials and Methods 100 couples with unexplained infertility undergoing IUI were randomized by computer number system into two groups.The sample size was calculated using Epi-Info 2002 software. Using a power of 80% to detect a significant difference at conception rate after IUI between the two groups = 19% with 5% precision and alpha error = 0.05, the sample size was 50 couples per group.

Participants/materials, setting, methods: ovulation stimulation by letrozole step up protocol from day 2, 3 of menstrual cycle starting by dose 2.5 mg increased daily by 2.5 mg for next 3 days.Group B, HMG ampoules 75 iu, tailred according to response,HCG when leading follicle 17 mm, IUI done 36 hours later.luteal support by vaginal progesterone, serum B HCG was measured after 14 days, clinical pregnancy by fetal heart pulsation 6–8 weeks

Main results and the role of chance: The step-up letrozole protocol was associated with multifollicular ovarian development with a mean of 1.5 ± 0.7 that was less than this detected with HMG 3.1 ± 1.0 however this did not affect so much clinical pregnancy rate that was statistically insignificant (16% in letrozole group versus 18% in HMG group) that may reflect the good quality of oocyte in addition to the good receptivity suggested by non significant difference in endometrial thickness. The cost of letrozole cycles was significantly lower than HMG group.

Limitations, reasons for caution: small scale

Wider implications of the findings: can be applied on a large number of patients

Trial registration number: ACTRN12614000024640

SELECTED ORAL COMMUNICATIONS

SESSION 04: THE ROLE OF GENES TESTIS STRUCTURE IN MALE INFERTILITY

Monday 3 July 2017 Room A 10:00–11:30

O-015 Blood-testis barrier organization in a prepubertal and peripubertal boys' cohort: correlation with Sertoli cell maturation, clinical puberty and testicular anatomopathology

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Study question: How do blood-testis barrier (BTB) proteins correlate with Sertoli cell maturation, testicular histopathological and clinical characteristics during transition from prepubertal to pubertal stages?

Summary answer: We observed that BTB proteins expression and organization were correlated to Sertoli cell maturation and to increasing age, in association with histopathological and clinical puberty

What is known already: The BTB consists of tight and gap junctions, which preserve paracrine interactions between Sertoli and germ cells as well as the

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migration of differentiating germ cells towards the seminiferous lumen. Claudin 11 and connexin 43 are main proteins of tight and gap junctions, respectively. In connexin 43 knockout mice, Sertoli cells do not fully differentiate, implying thus that the BTB is essential for their maturation. In humans knowledge on the BTB is limited to adult tissue, where abnormal BTB formation was associated with impaired spermatogenesis. However, before puberty and during the pubertal transition, BTB establishment has not yet been described.

Study design, size, duration: The study was designed to assess the dynamic evolution of the BTB in a cohort of pre- and peripubertal boys. 49 patients, aged 0–15 years, who underwent a testicular biopsy to preserve their fertility before gonadotoxic treatment and had no previous history involving risks for infertility were selected. Correlations between the presence of BTB proteins, patient's age and Sertoli cell maturation were analyzed.

Participants/materials, setting, methods: Connexin 43 and claudin 11 immunostaining was performed to evaluate the BTB. A scoring system was used to assess their absence or disorganized-organized presence. Sertoli cell maturation was evidenced by Anti Mullerian hormone (AMH) immunohistochemistry. Tanner stages and the histological presence of haploid cells were recorded. AMH evolution, association between age and BTB, and correlation between AMH and BTB proteins were analyzed with linear regression, Fischer's test and Spearman correlation, respectively.

Main results and the role of chance: Connexin 43 and claudin 11 expressions increased significantly with age ($p = 0.04$ and $p \leq 0.01$, respectively). Connexin 43 was expressed in a disorganized state from the first year of age and its organized expression was only observed after 12 years of age, simultaneously with the onset of claudin 11 expression, the presence of haploid germ cells and the progression towards more advanced Tanner stages. AMH staining decreased significantly with age ($p \leq 0.01$), showing a progressive maturation of Sertoli cells. Moreover, we observed an inverse correlation between the expression of AMH and both connexin 43 ($p = 0.05$) and Claudin 11 ($p < 0.01$), indicating that Sertoli cell maturation is linked to the organization of the BTB. We showed for the first time, in a cohort of pre- and peripubertal boys, that the progression through puberty, demonstrated by Tanner stages and by testicular histological analysis, was simultaneous to the establishment of an organized BTB and Sertoli cell maturation. Further studies on the association between BTB components and onset of spermarche during the pubertal transition period are required to increase knowledge on differentiation of prepubertal testicular tissue and achieve in vitro maturation of immature testicular tissue.

Limitations, reasons for caution: Assessment of more BTB proteins may help to fully understand its establishment. The size of the population of peripubertal boys should be increased to study the correlation between germ cells at all stages of differentiation and the BTB and understand how alterations of the formation of BTB can affect spermatogenesis.

Wider implications of the findings: Since the knowledge on the human BTB in a pre-peripubertal cohort was lacking, our data provide a control population which can serve to assess in vitro maturation protocols for prepubertal testicular tissue. Furthermore, it may also be useful for in vivo applications as male contraception.

Trial registration number: not applicable.

O-016 Gradient system for testicular organoids generation – a novel system to model germ to somatic cell association in vitro

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Study question: Can germ and somatic testicular cells reorganize *in vitro* in a close to *in vivo* association if co-cultured in a three dimensional gradient system (3DGS)?

Summary answer: Germ and Sertoli rat cells reorganized in seminiferous-like structures when co-cultured in the 3DGS allowing the study of germ-to-somatic cell interactions *in vitro*.

What is known already: Germ cell proliferation and differentiation are delicate and complex processes governed by a broad network of factors and somatic cells. These signaling pathways and cell-to-cell interactions have been exhaustively studied, but a lot still remain unknown. Different approaches such as organ culture or *de novo* formation of seminiferous-like structures from primary testicular cells have been applied to investigate the mechanisms that govern germ cell fate decision into proliferation or differentiation. However, a more efficient and controlled model which recapitulate the germ-to-somatic cell associations is still need to study the germ cell niche *in vitro*.

Study design, size, duration: Primary testicular cells from 20 *dpp* rat were culture for 10 and 21 days using the 3DGS in basic culture condition. The effect of the treatment for 10 days with retinoic acid (RA) IL-1α, TNFα and RA inhibitors in germ cell maintenance and BTB organization was compared to the control culture conditions for the same period of time.

Participants/materials, setting, methods: For the gradient system setting, 3 concentric drops of Corning® Matrigel® diluted 1:1 with culture medium were sequentially applied on the bottom membrane surface of a hanging cell insert. The middle drop had a final cell concentration of 44 million cells/mL. DMEM-α supplemented with 1% pen/strep and 10% KnockOut serum replacement was used as basic culture medium. Evaluation of the results was done by bright-field microscopy and by confocal microscopy after whole-mount staining.

Main results and the role of chance: Sertoli and germ cells reassembled in spherical-tubular structures (STSs) showing similarities to seminiferous tubules organization. The characterization of STSs revealed that they are mainly formed by epithelized Sertoli cells. Moreover, the formation of a blood-testis barrier (BTB) *in vitro* was demonstrated by the detection of Zo-1 and occluding proteins between Sertoli cells and by the impermeability of the spherical-tubular structures to Evans Blue, a small molecule that cannot cross healthy BTB *in vivo*. Additionally, germ cells could be maintained for 21 days on the STSs. Furthermore, undifferentiated germ cells were observed to proliferate and formed cellular chains in a similar way as observed *in vivo*.

In order to validate the 3DGS to investigate signaling pathways and cell-to-cell interactions in the germ cell niche, we verify the role of retinoic acid (RA), IL-1α, TNFα and RA inhibitors in germ cell maintenance and BTB organization in vitro. RA treatment had a positive effect in germ cell maintenance compared with control conditions. Furthermore, IL-1α and TNFα were observed to impair the formation of testicular organoids and germ cell maintenance. Thus, we demonstrated our 3DGS to be a new model to explore germ cell niche *in vitro*.

Limitations, reasons for caution: The testicular organoids do not completely mimic testicular physiology yet. More specifically, progression in spermatogenesis was not observed in the basic culture conditions utilized mainly due to the lack of knowledge regarding the factors involved in germ cell differentiation.

Wider implications of the findings: The 3DGS constitutes a new method to generate testicular organoids representing a unique model of germ-to-somatic cell association *in vitro* with possible application to search for factors involved in the germ cell niche regulation. Moreover, the model might be applied to generate organoids and study organogenesis in other scientific fields.

Trial registration number: Not applicable.

O-017 In vitro re-assembly of human primary testicular cells into seminiferous cord-like structures

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Study question: Can enzymatically dispersed testicular cells from adult men re-organize into seminiferous cords *in vitro*?

Summary answer: Adult human testicular somatic cells re-assembled into testicular cord-like structures consisting of Sertoli and peritubular cells showing dynamic interactions.

What is known already: Attempts to induce spermatogenesis *in vitro* have a long lasting history with no success in human so far. Current evidence from animal studies suggests that an intact testicular somatic microenvironment is required to support germ cells. The capacity of testicular cell suspensions from adult prostate cancer patients to self-organize in spheroid testis-like units, albeit

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not displaying typical testicular architecture, has been recently shown. Our study aims at describing in more detail the cellular and molecular mechanisms involved in human testicular tubulogenesis.

Study design, size, duration: Testes from 15 adult gender dysphoria patients (mean age 35 ± 9.3, SD) of varying spermatogenic status (spermatogonia arrest to complete spermatogenesis), were used for this study after sex reassignment surgery. *In vitro* primary testicular somatic cell cultures were generated to investigate the self-organizing ability of testicular cells to form cord-like structures over a three-week period. Morphological appearance, marker expression by immunohistochemistry, phenotype and dynamics of cell reorganization were analyzed.

Participants/materials, setting, methods: Cell suspensions obtained by two-step enzymatic digestion which were plated onto glass inserts in 24-well plates for two days. To enrich for adherent somatic cells, the supernatant was discarded and the culture of the attached cell population was continued. Reassembly into cord-like structures was analyzed daily by microscopic observations. Endpoints were qualitative changes in morphology. Cell types were characterized by phase-contrast and immunohistochemistry. Dynamics of cord formation were recorded by time lapse microscopy.

Main results and the role of chance: Primary human testicular cells underwent a stepwise cascade of re-assembly into distinctive morphological patterns, resulting in cord-like structures after two weeks. Already at day two testicular somatic cells had aggregated into irregular- and spherically-shaped structures. Between days 3 and 6 adjacent aggregates connected with each other by 'bridges' of elongated spindle-shaped cells. Then, aggregates fused to form multi-layered spherical or elongated cord-like structures between days 7 and 14. Time-lapse imaging between days 3 and 6 confirmed the dynamics of cord formation. Processes observed were cell migration, compaction and fusion via contraction of spindle-shaped cells. Immunohistochemical analysis revealed that both SOX9-positive Sertoli and α-SMA-positive peritubular myoid cells interacted and contributed to cord-like structure formation. By day 14, peritubular cells had aligned in single layer, acquired flattened appearance, surrounding the cord-like structures.

Limitations, reasons for caution: Due to scarcity of normal human testicular tissue, testes from gender dysphoria patients were used in the study, which tissue might differ from the normal one. Despite sharing morphological features with *in vivo* testicular cords, further refinement of this *in vitro* model is required.

Wider implications of the findings: The proposed *in vitro* culture system can be developed further into a tool for the examination of testicular cell interactions during testis organogenesis under controlled experimental conditions.

Trial registration number: Not applicable.

O-018 Oxidation reduction potential: a valuable tool for male fertility evaluation

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[1]*Hamad Medical Corporation, Urology, Doha, Qatar*
[2]*American Center for Reproductive Medicine- Cleveland Clinic Foundation, Urology, Cleveland, U.S.A.*

Study question: Is there a significant correlation between seminal oxidation reduction potential (ORP) and total motile sperm count (TMSC)?

Summary answer: Seminal ORP measures have a significant positive correlation with TMSC.

What is known already: Oxidative stress results from imbalance between oxidants and reductants. Its detrimental effects on sperm production proposed its use a biomarker for overall semen quality. While previous attempts assessing OS in semen samples measured single features of the redox system, ORP can be considered a better measure of OS as it determines the balance between oxidants and reductants. TMSC is considered to be the single most important parameter of the semen analysis result that can predict the severity of male infertility. Examining the correlation between ORP and TMSC should provide solid information on the usefulness of ORP in male fertility evaluation.

Study design, size, duration: This is a cross sectional study of 1162 patients presenting to the male infertility unit of a tertiary medical center over a period of 12 months. After the collection of demographic and clinical data, patients were asked to provide a semen sample for analysis after >=2 days of sexual abstinence.

Participants/materials, setting, methods: Semen samples were analyzed according to the 5th Edition WHO manual. TMSC was calculated using the formula = volume (ml) x sperm concentration (million/ml) x total motility (%)/100. Seminal ORP levels were assessed using the MiOXSYS system. Pearson's correlation was used to assess the relationship between ORP and TMSC. Using a TMSC threshold of 20 million, receiver operator characteristic (ROC) analysis was utilized to determine the ORP cutoff associated with highest predictive values.

Main results and the role of chance: The patients mean age ± standard error of mean was 35.9 ± 0.2 years. Infertility was primary in 69.6% and secondary in 30.4% of patients. After a mean abstinence time of 3.7 ± 0.04 days, semen analysis results revealed a sperm concentration of 32.7 ± 0.78 million/ml, total motility of 50.1 ± 0.57% and normal morphology of 5.7 ± 0.22%. The mean calculated TMCS was 55.5 ± 1.7 million. A significant negative correlation exists between TMSC and the ORP result (r -0.36, CI -0.12 - -0.42, $p < 0.001$). Using a TMSC threshold of 20 million, ROC curve analysis determined a ORP cutoff value of 2.34 mV/10^6 sperm/mL to be associated with a sensitivity of 83.5%, specificity of 82.5%, negative predictive value of 81.4%, positive predictive value of 82.9% and overall accuracy of 79.9% (area under the curve 0.9).

Limitations, reasons for caution: Results were obtained from semen samples of patients presenting with primary or secondary infertility and hence were not compared with a control group or with men of proven fertility.

Wider implications of the findings: Using a single drop of semen (30ul) applied to a sensor, ORP measurement with the MiOXSYS system is a simple, quick and user friendly method that can reliably measure OS in biologic samples. The significant correlation between ORP and TMSC allows its use as a predictor of fertility potential.

Trial registration number: Not applicable.

O-019 High prevalence of PLCζ mutations among cases of failed oocyte activation, but not anomalous fertilization, after IVF/ICSI

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Study question: Is oocyte activation failure (OAF) associated with PLCz mutations in sperm?

Summary answer: Mutations in PLCz sequence are associated with OAF and could explain cases of fertilization failure.

What is known already: Total fertilization failure (FF) occurs in 1–3% of ICSI cycles, often due to oocyte activation failure (OAF). PLCz, a soluble factor responsible for oocyte activation, possess X and Y catalytic domains, an X-Y linker region, and a C2 domain at the C-terminus. PLCz hydrolyzes PIP2 in the oocyte, leading to intracellular calcium release and oocyte activation. So far, 4 PLCz point mutations have been reported (H233L, H398P, I489F and S500L), all linked to cases of fertilization failure and associated to a decrease of either PLCz levels or activity. Nevertheless, the impact of PLCz mutations on OAF cases specifically is unknown.

Study design, size, duration: All consecutive cases (n = 34) of low (<20%) to complete FF in cycles with donor oocytes between 2014 and 2016 were included in the study; of these, 14 cases presented OAF (undetected pronuclei), while 20 presented anomalous fertilization (AF, 1PN or 3PN). As control group, 13 sperm donors with fertilization rate >75% were analyzed. The percentage of mutated cases within groups was assayed by Bernouilli binomial test ($P < 0.05$).

Participants/materials, setting, methods: Sperm genomic DNA was isolated; the exonic regions of PLCζ were amplified by PCR and sequenced, and compared to the published sequence of the genomic PLCζ locus (Homo sapiens 12 BAC RP11-361I14) by BLAST analysis. To characterize PLCζ mutations in silico, 3D protein structure modelling (SWISS-MODEL) was performed. To analyze PLCζ protein in sperm, protein expression and localization was analyzed through Western blot and immunofluorescence.

Main results and the role of chance: 8 of the 14 (57.14%) OAF patients carried at least one PLCz mutation, versus 1 out of 20 in AF group (5%; $p < 0.05$)

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and none of the 13 sperm donors (0%; $p < 0.05$). In total, 5 mutations were identified. Four were single nucleotide missense mutations; previously unreported R197H and L223P, with H233L, were located in the X catalytic domain; S500L was located in the C2 N-terminal domain. One OAF patient presented 2 mutations (S500L and R197H), while single S500L mutations were found in 4 patients with OAF and 1 with AF. All patients presented mutations in heterozygosis, except 1 from OAF group (S500L, in homozygosis) and no relationship was found between PLCζ mutations and protein expression levels or subcellular localization. 3D protein structure modelling did not predict significant changes in the overall structure or the ligand recognition motif in any of the single missense mutations. The fifth mutation, an unreported frameshift, was found in one patient with complete OAF (fertilization rate: 0/5), leading to a truncated protein at the X-Y linker region, resulting in the loss of the Y- and C2-domains. This patient underwent assisted oocyte activation (fertilization rate: 6/9), and a healthy baby was born.

Limitations, reasons for caution: This study reports a clear association between OAF and PLCz mutations; although further functional studies are needed to test mutant PLCz activity. Donor oocytes were used in all IVF cycles included, significantly decreasing the chances of a concurrent oocyte factor.

Wider implications of the findings: Almost 60% of patients presenting OAF carried at least one mutation in PLCz. We reported 3 new and 2 previously described PLCz mutations, and suggest that PLCz gene sequencing might be used as a tool for counseling and treatment of couples presenting OAF.

Trial registration number: NA.

O-020 Klinefelter syndrome and fertility – impact of X-chromosomal inheritance on spermatogenesis

S. Franik[1], D. Smeets[2], G. Van de Zande[2], I. Gomes[2], K. D'Hauwers[3], D. Braat[1], K. Fleischwer[1], L. Ramos[1]

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Study question: This study investigates if paternal or maternal inheritance of the additional X-chromosome determines the absence or presence of spermatogenesis in males with Klinefelter syndrome.

Summary answer: The maternal or paternal origin of the additional X-chromosome in men with KS cannot predict the presence or absence of spermatogenesis.

What is known already: Klinefelter syndrome (KS) is one of the most common chromosomal disorders leading to azoospermia in men. However, with the use of intracytoplasmic sperm injection (ICSI) in combination with testicular sperm extraction (TESE), in about 50% of men diagnosed with KS spermatogenesis is present in the testicular biopsies and they can potentially still father their own children. The reason for absence or presence of spermatozoa in half of men with KS remains unknown.

Study design, size, duration: For this retrospective study, all men with Klinefelter sydrome who have had a TESE in the Radboud University Medical Center from 2010 to present were eligible for inclusion in this study. From 9 participants and their parents, buccal swabs were taken in order to be able to compare X-chromosomal markers to determine the parental origin of both X-chromosomes in the males with KS.

Participants/materials, setting, methods: The testicular biopsies were obtained by our standard procedure. Biopsies were prepared and evaluated at the fertility laboratory for presence or absence of spermatozoa (wet –prep). The presence of at least one spermatozoon was considered a positive sperm retrieval (TESE+). Histopathological analysis of the spermatogenesis and cytology evaluation by scoring the ratios between spermatozoa, pachytene spermatocytes and Sertoli cells was also performed on the TESE samples.

Main results and the role of chance: The results of our analysis show that in 8 of 35 (23%) patients spermatozoa were retrieved by single or double TESE. Different levels of spermatogenesis appeared to be present in 16 of 35 (46%) participants by combining the results of spermatozoa retrieval, and cytological as well as histological analysis of the retrieved testicular tissue, meaning that spermatogonia are present but not always can mature up to spermatozoa. From the 9 KS-trios that were further genetically analyzed for inheritance origin, no evidence of a correlation between the maternal or paternal origin of the extra X-chromosome and the presence of spermatogenesis was found. The hormonal levels (testosterone, LH and FSH) measured prior to the TESE did not differ between the TESE positive and negative subgroups. A combination of high serum testosterone levels > 7.5 nmol/l and low serum LH <17.5U/l was a weak positive predictive marker for presence of spermatogenesis in our study group.

Limitations, reasons for caution: Based on the small number of participants in this study, a larger study is needed to confirm our findings that a combination of high serum testosterone and low luteinizing hormone is a positive predictive marker for the presence of spermatozoa upon TESE.

Wider implications of the findings: Unfortunately, determining the origin of the extra X-chromosome in men with Klinefelter Syndrome cannot predict the presence or absence of spermatogenesis. Although of weak predicting value, a combination of high serum testosterone and low serum LH levels have been identified as prognostic markers for the presence or absence of spermatogenesis.

Trial registration number: No Clinical Trial.

SELECTED ORAL COMMUNICATIONS
SESSION 05: IMPROVING IMPLANTATION

Monday 3 July 2017 Room B 10:00–11:30

O-021 Endometrial function in women with diabetes: role of the hexosamine biosynthetic pathway (HBP)

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[2]*University of Manchester, Faculty of Medical and Human Sciences, Manchester, United Kingdom*

Study question: Does action of the HBP, and subsequent modification of proteins with O-linked β-N-acetylglucosamine (O-GlcNAcylation), act to link glucose metabolism with early pregnancy success/failure?

Summary answer: Expression of the O-GlcNAc transferase EOGT is negatively correlated with BMI. Knockdown of EOGT impedes expansion of endometrial stem-cells, leading to reduced regenerative capacity.

What is known already: Animal studies suggest that high glucose levels are detrimental to endometrial function; however, the molecular mechanisms that translate abnormal glucose availability into altered endometrial function are unknown. In glucose-sensing tissues, the HBP is a nutrient driven regulator of cellular function. HBP activation results in the synthesis of uridine diphosphate N-acteyl glucosamine (UDP-GlcNAc), which is used to glycosylate proteins post-translationally. O-GlcNAcylation is catalysed either by O-GlcNAc transferase (OGT), or EGF-domain specific-OGT (EOGT). EOGT targets include Notch ligands and receptors which are known to regulate the endometrial perivascular stem cell niche, which is critical for endometrial function and successful implantation.

Study design, size, duration: Primary cultures were derived from endometrial biopsies obtained from consenting women recruited from the Implantation Clinic, a dedicated research clinic at University Hospitals Coventry and Warwickshire NHS Trust. A total of 45 fresh endometrial biopsies were processed for human endometrial stromal cell (HESC) primary cultures. The average age (± SD) of the participants was 33 ± 1.8 years. Data were obtained from at least 3 independent cultures for all experiments.

Participants/materials, setting, methods: HESCs were isolated from endometrial biopsies and expanded in vitro. Treatment with 8-bromo-cAMP and medroxyprogesterone was used to induce a decidual phenotype. HESCs were transfected using a jetPRIME Polyplus kit with either EOGT siGENOME or non-targeting (NT) small interfering RNA. Gene and protein expression were assayed by real-time quantitative (qRT)-PCR and Western blots, respectively. Clonal ability was assessed by seeding cells at low density and maintaining in culture for 10 days before staining with hematoxylin.

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Main results and the role of chance: Decidualization of HESCs, an obligatory process for embryo implantation, is associated with altered expression of key HBP enzymes, including down-regulation of OGT and up-regulation of O-GlcNAcase (OGA). The most striking observation, however, is the marked induction of EOGT in decidualizing HESCs. Small interfering RNA-mediated knockdown of EOGT in primary HESC cultures did not affect the induction of decidual marker genes, including PRL, IGFBP1 and HSD11B1. However, EOGT knockdown enhanced NOTCH signalling by stabilising NOTCH1 and NOTCH3, which in turn blunted activation of endometrial mesenchymal stem cells in colony-forming assays ($P < 0.01$). Furthermore, loss of EOGT enhanced the pro-inflammatory cytokine profile in decidualizing HESCs. Analysis of 80 mid-luteal endometrial biopsies revealed an inverse correlation between EOGT mRNA levels and body mass index (Spearman rank test, $\rho = -0.3260$; $P = 0.049$), which was confirmed at protein level by Western blot analysis of whole endometrial tissues from 48 women (Spearman rank test, $\rho = -0.335$, $P = 0.020$). Taken together, these observations suggest that endometrial EOGT deficiency associated with obesity reduces the regenerative capacity of the endometrium and prolongs the pro-inflammatory endometrial environment at implantation.

Limitations, reasons for caution: In vitro assays are associated with inherent limitations as there are difficulties in extrapolating perturbed pathways in vitro to effects in vivo. Further studies are required to characterise interactions between different cell types in the endometrium upon O-GlcNAc cycling and responses to varying glucose conditions.

Wider implications of the findings: These observations link increased BMI to impaired endometrial function. This will increase our understanding of the impact of diabetes on reproductive function and underpin the development of (i) biomarkers to identify women at high risk of early pregnancy problems and (ii) interventions to optimise endometrial function in these women.

Trial registration number: N/A.

O-022 Improving the pre-conceptual uterine natural killer (uNK) cell test to predict pregnancy outcome in recurrent miscarriage

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[2]*University of Warwick, Biomedical Research Unit in Reproductive Health, Coventry, United Kingdom*

Study question: Can we improve the pre-conceptual endometrial uNK cell test, in its ability to predict subsequent pregnancy outcome in women with recurrent miscarriage?

Summary answer: A pre-conceptual endometrial test that measures synchronicity of stromal and glandular decidualisation can improve prediction of pregnancy outcome in recurrent miscarriage.

What is known already: Decidualisation of the endometrium is crucial in creating a micro-environment suitable for embryo selection, implantation and sustaining pregnancies. Impaired decidualisation is strongly associated with recurrent miscarriage (RM). Our endometrial uNK cell test correlated with in-vitro decidualisation but has limited ability to predict pregnancy outcome. Our recent research, found that impaired decidualisation in RM was characterised by accelerated senescence and a lack of endometrial mesenchymal stem cells (eMSC).

Study design, size, duration: This was a cohort study of women with RM who had a mid-luteal phase endometrial biopsy and a pregnancy event of ongoing/livebirth or first trimester miscarriage within one year of biopsy (2012–16). An exploratory study used immunohistochemistry (IHC) to identify senescence and proliferation markers that differentiated women who had a miscarriage from those who had a successful pregnancy ($n = 20$). A further 89 patients were used to confirm or refute findings from the exploratory phase.

Participants/materials, setting, methods: This study was performed in a specialist tertiary referral, Implantation Clinic, where mid-luteal endometrial biopsies are taken for uNK cell assessment. Biopsies from women with known pregnancy outcome were retrieved from our tissue bank for serial sectioning then immunohistochemistry (IHC) staining with antibodies to detect uNK cells-CD56, proliferation-Ki67 and senescence-HMGB2 and p16. The density and localisation of each marker was measured with colour de-convolution and thresholding using Panoramic Viewer and Image J software.

Main results and the role of chance: As cells senesce HMGB2 density is lost and p16 expression is increased. HMGB2 facilitates DNA flexibility and repair, and P16 is a tumour suppressor protein that decelerates cell progression from G1 to S phase.

uNK and p16 density measurements were normalised to the day of cycle using centile reference ranges developed in our laboratory ($n = 1{,}800$). uNK centiles did not differentiate miscarriage from pregnancy success. However, glandular p16 centile was significantly higher in those with a subsequent live birth than those who miscarried ($p = 0.005$).

The best differentiation of those who had a further miscarriage from those who had a successful pregnancy was the difference between uNK cell and p16 (glandular) centile 'uNK-p16'. The larger positive values appear to be related to subsequent pregnancy loss ($p = 0.0163$).

The validity of 'uNK-p16' was assessed by receiver operative characteristics curve. It improves prediction of subsequent miscarriage compared to uNK alone but is still not robust enough for clinical application.

However, our data does suggest that synchronicity in stromal and glandular decidualisation are important for pregnancy success.

Limitations, reasons for caution: Any prediction of pregnancy outcomes in RM is limited by the fact that losses of both normal and abnormal pregnancies occur. We have yet to determine whether endometrial defects account for both or one of these. Another confounding factor is that the endometrial biopsy itself could alter pregnancy outcomes.

Wider implications of the findings: The findings support the concept that co-ordinated decidualisation in stromal and glandular compartments is needed for successful pregnancy outcome. It may be not be possible to predict pregnancy outcome with a pre-conceptual endometrial test however an endometrial test could select women who would benefit from pre-pregnancy therapeutic interventions.

Trial registration number: not applicable.

O-023 Acetylsalicylic acid for prevention of pregnancy loss: a randomized trial

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Institution for Clinical Research- Sahlgrenska Academy- University of Gothenburg, Obstetrics & Gynecology, Gothenburg, Sweden

Study question: Does low dose acetylsalicylic acid (ASA) prevent miscarriage in subsequent pregnancy in women with at least three consecutive miscarriages without a known etiology?

Summary answer: In women with at least three consecutive miscarriages, treatment with ASA from early gestation compared with placebo, does not increase live birth.

What is known already: One percent of fertile couples suffer from recurrent pregnancy loss. Immunologic factors, chromosomal aberrations, disturbed placental circulation and anomalies of the uterus are suggested risk factors, although the condition is of unknown etiology in the vast majority of couples. Without proper evidence for an effect, treatments like ASA, low molecular weight heparin, immunoglobulin, progesterone and immunization have been used when no etiological factor has been detected. In couples with at least three consecutive miscarriages, ASA is sparsely investigated in randomized controlled trials and has been included only together with other drugs.

Study design, size, duration: The study is randomized, double-blind, placebo-controlled, stratified for age and conducted at a single center between 2008 and 2015. Recurrent pregnancy loss was defined as at least three consecutive miscarriages within the couple. Randomization was through a third party, who manufactured and delivered the study drugs. Group allocation was concealed until all study patients had a pregnancy outcome registered. Primary outcome was live birth.

Participants/materials, setting, methods: Couples were referred to one center. Women <40 years old with a BMI <35 were eligible if the work-up was negative. They were randomized after 42 gestational days when fetal heartbeat was detected by transvaginal ultrasound. Active treatment arm received 75 mg ASA and controls received placebo pills with identical appearance. Patients

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were seen by the same gynecologist at gestational weeks 9, 13, 30 and 36. The study drug was discontinued at the last check-up.

Main results and the role of chance: Of 594 screened patients, 505 were eligible and 200 were randomized to each group. The study group was in mean 32.3 years (SD 4.3) old, had a mean BMI of 23.9 (SD 3.8), and had experienced three (median) miscarriages (range 3–7). Primary recurrent pregnancy loss within the couple was present in 52%, secondary being present i 48%. Thirty (7.5%) women were smokers. Women were randomized at gestational age 45.5 (SD 4.6) days. Conception was spontaneous in 356 (89.0%), through IVF in 31 (7.8%) and as a result of ovarian stimulation in 13 (3.2%) women. All demographic variables were evenly distributed between randomization groups. Live birth rates were 83.0% and 86.0% in patients having received ASA and placebo, respectively ($p = 0.24$). Ninety-eight percent of deliveries were singletons, the remaining being duplex. Premature birth before 37 gestational weeks occurred in 3.0% and 7.6% having received ASA and placebo, respectively ($p = 0.08$). There were no differences between randomization groups in pregnancy loss during first and second trimesters.

Limitations, reasons for caution: New miscarriage during current pregnancy was not studied by histological/pathological or chromosomal examination. Uterine evacuation would have been a prerequisite, but was not a part of the study protocol. Such a procedure is not included in the routine management and would have implied an additional invasive procedure for the patient.

Wider implications of the findings: Treatment with ASA, starting at detection of fetal heart beat, did not prevent recurrent miscarriage in women with at least three consecutive miscarriages in the same relationship. The fertility prognosis is very good, the live birth rate being more then 80 percent with or without ASA.

Trial registration number: Clinicaltrials.gov NCT02823743.

O-024 A placebo-controlled, randomized, double-blind study of pregnancy and live birth rates after single oral administration of a novel oxytocin antagonist, nolasiban, prior to embryo transfer

H. Tournaye[1], A. Humberstone[2], V. Lecomte[2], P. Terrill[3], P. Pierzynski[4], E. Loumaye[5]

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[2]*ObsEva SA, Clinical Operations, Geneva, Switzerland*
[3]*CROS NT, Statistics, Maidenhead, United Kingdom*
[4]*Medical University of Bialystok, Reproduction Endocrinology and Gynecology, Bialystok, Poland*
[5]*ObsEva SA, Medical, Geneva, Switzerland*

Study question: Does the oral administration of the novel oxytocin antagonist, nolasiban, prior to Day 3 (D3) fresh embryo transfer (ET) improve pregnancy and live birth rates?

Summary answer: Overall live birth rate was 40% after administration of different oral doses of nolasiban compared to 29% in the placebo group.

What is known already: Infusion of atosiban at the time of ET has been reported to improve pregnancy rates in several clinical studies. These studies have included D3 and D5 fresh transfers and frozen ET. It has been hypothesized that antagonism of oxytocin and/or vasopressin V1a receptors expressed in uterus at the time of ET could enhance uterine receptivity and improve pregnancy rates possibly by decreasing uterine contractions and by improving endometrial receptivity and perfusion. Good endometrial blood flow on the day of embryo transfer was associated with higher pregnancy success after in vitro fertilization (IVF).

Study design, size, duration: Multinational, prospective, double-blind, dose-finding, randomized, parallel group, placebo-controlled study assessing a single oral dose of nolasiban (100, 300 or 900 mg) or placebo, administered 4 hours before ET following IVF or intracytoplasmic sperm injection (ICSI). 247 subjects (≥60/arm) were recruited from 26 fertility clinics in Europe from Jan–Jul 2015. Following ET, pregnancy status was assessed at 2, 6 and 10 weeks. Pregnancies were followed until birth and neonatal/infant outcomes at 1 and 6 months.

Participants/materials, setting, methods: Eligibility criteria included age ≤ 36 years, ≤ 1 failed ART cycle, use of a gonadotropin releasing hormone antagonist cycle, luteal support with vaginal micronized progesterone (P4), and > 1.5 uterine contractions/min assessed by time-lapse ultrasound before ET. One or two embryos (at least one of good quality) were transferred. The primary analysis was performed on the full data set and an additional post-hoc sub-set analysis was performed excluding subjects in the top P4 quartile.

Main results and the role of chance: The pregnancy rates at week 10 in the placebo, 100, 300 and 900 mg groups were 29%, 44%, 35%, 45% (trend test: $p = 0.15$) and the live birth rates were 29%, 40%, 35%, 43% ($p = 0.17$). When all of the active treatment groups were pooled the live birth rate was 40% compared to 29% for placebo ($p = 0.18$). Demographics were generally comparable between treatment groups (e.g. mean age 31; oocytes retrieved 11; good quality embryo 3.5; single ET: 60%; double ET: 40%). However, more oocytes and higher pre-treatment serum P4 were recorded in the 300 mg group, and P4 level negatively predicted pregnancy ($p < 0.05$, as covariate in logistic regression model). In a post-hoc analysis excluding subjects in the top P4 quartile, live birth rates were 31%, 38%, 49% and 51% (trend test: $p = 0.025$). There was a slight reduction in uterine contractions after treatment compared to placebo, which was highest in the 900 mg group (median change: -13%, $p = 0.051$). However, overall there was no correlation between pregnancy outcome and uterine contraction rate. Single dose administration of nolasiban at doses up to 900 mg appeared to be well tolerated in this study and did not result in increased occurrence of adverse events or neonatal outcomes.

Limitations, reasons for caution: This study investigated D3 fresh transfers in relatively young women without repeat implantation failure. It was a relatively small study with about 60 patients per group and there was some imbalance in baseline characteristics including serum P4 which appeared to have a confounding effect on pregnancy outcomes.

Wider implications of the findings: The results indicate a potential 10–20% absolute increase in pregnancy and live birth rates compared to placebo after administration of a single oral dose of nolasiban before ET. If confirmed in larger prospective trials this finding has important potential for improving live birth rates following IVF/ICSI.

Trial registration number: ClinicalTrials.gov: NCT02310802.

O-025 High-quality human preimplantation embryos actively influence endometrial stromal cell migration

R.P. Berkhout[1], C.B. Lambalk[2], J. Huirne[2], V. Mijatovic[2], S. Repping[1], G. Hamer[1], S. Mastenbroek[1]

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[2]*VU University Medical Center, Obstetrics and Gynecology, Amsterdam, The Netherlands*

Study question: Do human preimplantation embryos regulate endometrial stromal cell (hESC) migration?

Summary answer: High-quality embryos stimulate migration of decidualized hESCs and suppress migration of non-decidualized hESCs, irrespective of embryonic developmental stage.

What is known already: Most embryos fail to implant in IVF/ICSI. Little is known about the interaction between the embryo transferred to the uterus and the endometrium. From the embryo point-of-view it is clear that not every embryo has similar chances of implantation. To estimate the developmental potential of each embryo, morphological assessment is most commonly used to rank and select embryos for transfer. Transferring higher ranked embryos results in higher live birth rates. However, whether higher ranked embryos achieve these improved live birth rates by actively interacting with the endometrium has not been elucidated.

Study design, size, duration: An *In vitro* study using primary hESCs from fertile patients undergoing hysterectomy for benign conditions (uterine scar niche n = 3, dysmenorrhea n = 2; no hormonal treatment) subjected to embryo conditioned medium (ECM). Per assay ECM was pooled from 5 individually cultured embryos with similar developmental stage and morphological quality. Per developmental stage, high-quality embryos were defined by <20% fragmentation and low-quality embryos were defined by >20% fragmentation. ECM was collected at day 4 after fertilization.

Participants/materials, setting, methods: Primary hESCs were decidualized with cAMP and medroxyprogesterone acetate (MPA) for 5 consecutive

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days. Migration assays were performed by culturing hESCs in the presence of ECM from high-quality embryos (n = 34), low-quality embryos (n = 23) or in an equal volume of non-conditioned medium (control, n = 21). To quantify the migration response of hESCs, the surface area of cells entering the migration zone was measured.

Main results and the role of chance: ECM from high-quality embryos, i.e. with low fragmentation, actively stimulated decidualized hESCs migration ($p < 0.0001$). This effect was consistent throughout embryonic development from cleavage stage embryos with 2–7 cells (high-quality vs. control; $p = 0.030$), 8–18 cells (high-quality vs. control; $p < 0.0001$) to morulae (high-quality vs. control; $p = 0.002$). Additionally, linear regression analysis showed that hESCs migration was influenced by embryo quality (fragmentation: β -0.299; $p = 0.025$) and not developmental stage (cell number: β 0.177; $p = 0.176$) or maternal age (β -0.036; $p = 0.78$). Opposite to decidualized hESCs, the migration response of non-decidualized hESCs was inhibited by ECM from high-quality embryos ($p = 0.019$). ECM from low-quality embryos, i.e. with high fragmentation, did not cause an altered migration response in decidualized hESCs ($p = 0.860$) or non-decidualized hESCs ($p = 0.986$). Furthermore, ECM of both high- and low-quality human embryos did not influence proliferation of both decidualized and non-decidualized hESCs ($p = 0.375$).

Limitations, reasons for caution: Limitations of this study include the *in vitro* design and pooling of five individual embryo conditioned media.

Wider implications of the findings: This study reveals a mechanism by which the high-quality human preimplantation embryo interacts actively with its surroundings to increase its chances of successful implantation. Our study contributes to the further understanding of human embryo implantation, with the ultimate aim to develop more effective treatment strategies in IVF/ICSI.

Trial registration number: not applicable.

O-026 Evidence base for investigation and management for unexplained Recurrent Implantation Failure (RIF): A systematic review and meta-analysis

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[5]*Derby Fertility Unit- Royal Derby Hospital- Derby, Fertility Unit, Derby, United Kingdom*

Study question: To compare the prevalence of immunological conditions between women with RIF and controls (non-RIF) and evaluate the effect of empirical treatments for unexplained RIF.

Summary answer: Prevalence of certain immunological conditions were higher in RIF, but evidence to support most of the currently practised investigations and treatments for RIF is limited.

What is known already: RIF poses a complex medical challenge for clinicians and scientists alike; its devastating consequences affect approximately 10% of all women undergoing IVF treatment worldwide. The explorative manner in which medical investigations and treatments are often prescribed for RIF is a consequence of its ill-understood aetiology and it bears a significant economic burden to both the public and private sector along with significant emotional burden for the couple.

Study design, size, duration: Design – Systematic Review & Meta-analysis.

We identified 30 studies, comprising 5146 women, that compared the prevalences of positive immunological test outcomes between women with RIF and non-sufferers. A further 17 studies, comprising 2247 women, were identified that compared IVF outcome between women with RIF who received empirical treatment/s before IVF and those with the condition that had received a placebo or no empirical treatment. Medline and The Cochrane Library were searched; commencement to June 2016.

Participants/materials, setting, methods: Our case group comprised women with RIF; we used RIF criteria as described by the authors of individual studies. The review included two control groups; For the prevalence study, the control group comprised both fertile and subfertile women with out a history of RIF whereas for the treatment study, it comprised women with RIF who were either administered a placebo or no empirical treatment during IVF.

Main results and the role of chance: The prevalence of positive antiphospholipid antibody test outcomes was significantly higher in women with RIF than in controls (OR = 3.35; 95%CI 2.05–6.05, $p < 0.001$); more specifically lupus anticoagulant (OR = 5.03; 95%CI 1.81–13.99, $p = 0.002$). Similar results were seen for inherited thrombophilias; more specifically Factor V Leiden (OR = 2.69; 95%CI 1.28–5.63, $p = 0.009$). While meta-analyses for the prevalences of high NK cells and Th1:Th2 ratio were not possible, there was no difference in the prevalence of anti-thyroid antibodies. Endometrial injury performed in the previous menstrual cycle was shown to benefit clinical pregnancy rate in women with RIF (OR = 3.38; 95%CI 2.26–5.06, $p < 0.001$). Similar results were seen for peripheral blood mononuclear cell administration to the uterus (OR = 3.68; 95%CI 2.01–6.64, $p < 0.001$) and intravenous immunoglobulin therapy on live birth rate (OR = 10.51; 95%CI 1.52–76.66, $p = 0.02$), although the data need to be interpreted with caution due to small sample sizes and low quality evidence. There was no significant benefit of empirical low molecular weight heparin on pregnancy rate or live birth rate for unexplained RIF.

Limitations, reasons for caution: The presence of heterogeneity; our exclusion criteria were not conservative leading to the inclusion of a highly heterogeneous group of women. The meta-analyses specific to objective one were limited by the control groups comprising both fertile and subfertile women with no history of RIF introducing confounding to our results.

Wider implications of the findings: Our results support considering testing for congenital and acquired thrombophila but evidence for testing various other immunological factors is limited in cases of RIF. Empirical treatments for unexplained RIF require robust randomised controlled trials to establish appropriate recommendations.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 06: DEVELOPMENTS IN GENETIC TESTING

Monday 3 July 2017 Room W+X 10:00–11:30

O-027 Spent Blastocyst Media and Blastocoel Fluid are not reliable DNA sources for preimplantation genetic diagnosis of aneuploidies and monogenic disorders

V. Romanelli[1], L. Girardi[1], D. Cimadomo[2], C. Patassini[1], C. Scarica[2], M. Albricci[2], M. Stoppa[2], A. Cecchele[1], M. Poli[3], F.M. Ubaldi[2], L.F. Rienzi[2], A. Capalbo[1]

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Study question: Is DNA from Spent Blastocyst Media (SBM) and Blastocoel Fluid (BF) a reliable template for preimplantation aneuploidy testing (PGD-A) and/or PGD for monogenic disorders (PGD-M)?

Summary answer: PGD-A/PGD-M analysis using BF and SBM-derived DNA is unreliable due to high diagnostic failure rate and inconsistency with results derived from trophectoderm biopsy.

What is known already: BF has been suggested as a reliable source of embryonic DNA (eDNA) for the assessment of blastocyst chromosomal constitution. BF aspiration entails a smaller degree of invasiveness compared to trophectoderm (TE) biopsy. The literature on the use of BF as source of eDNA for PGD/PGA reports extremely conflicting results and recommendations. Recently, attempts to use eDNA collected in SBM as a template for PGD have been made with poor results. To add further data on both these novel applications, we performed a prospective study investigating the reliability of BF and SBM for clinical diagnostics in PGD cycles.

Study design, size, duration: Prospective cohort blinded study performed between December 2015 and December 2016. Three groups of samples were

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analyzed: BF (n = 80), SBM (n = 42) and TE (n = 84). PGD-A was performed on 23 BF-TE pairs; PGD-M was performed on 38 BF-SBM-TE trios, 19 BF-TE pairs and 4 SBM-TE pairs. TE biopsy results were used as control to assess the reliability of BF- and SBM-based diagnosis.

Participants/materials, setting, methods: SBM (25ul) and BF samples were collected from expanded blastocysts, PGD-A on BF was performed by next-generation sequencing (NGS) and compared to qPCR outcomes generated by the corresponding TE. PGD-M analysis included 10 different disorders. Genotyping analysis was performed for the three study groups using 59 TaqMan probes: 48 for informative SNP and 11 for direct mutation analysis. A total of 311 probes were tested for TE, 286 for BF and 242 or SBM.

Main results and the role of chance: PGD-A in BF showed 60.9% amplification failure (AF) rate (n = 14/23;95%IC=38.5–80.3); discordance between BF and TE was 17.4% (n = 4/23;95%IC=4.9–38.8) and concordance 21.7% (n = 5/23; 95%IC=7.5–43.7). Sensitivity per chromosome was 66.7% (n = 4/6;95% IC=11.8–61.6) and specificity 91.1% (n = 185/203;95%IC=95%IC=86.6–94.7). For PGD-M genotyping assays, 69.6% (n = 199/286; 95%IC=63.9–74.9) and 10.7% (n = 26/242; 95%IC=7.1–15.3) of probes showed AF on BF and SBM, respectively. Discordant results in 14.3% of BF (n = 41/286; ,95%IC=10.5–18.9) and 35.5% of SBM (n = 86/242; ,95%IC=29.5–41.9) were due to allele drop-out (ADO) and artifacts/contamination. ADO occurred for 12.2% (n = 35/286; 95% IC=8.7–16.6) of probes in BF; artifacts/contamination occurred in 2.1% (n = 6/286; 95%IC=0.8–4.5). For amplified probes from SBM, ADO occurred in 17.4% (n = 48/242;95%IC=12.8–22.7) while artifacts/contamination occurred in 15.7% (n = 38/242;95%IC=11.4–20.9). TE analysis reported just 0.3% of ADO (n = 1/311;95%IC=0.01–1.8) and no amplification failure (n = 0/311). Overall, concordant results were obtained for 16.0% (n = 46/286;95%IC=12.0–20.9) and 53.7% (n = 130/242;95% IC=47.2–60.1) of probes on BF and SBM, respectively. Haplotype reconstruction for PGD-M has been successful and consistent compared to TE in only 3.5% of BF (n = 2/57;95%IC=0.4%-12.1%) and 21.4% of SBM (n = 9/42; 95% IC=10.3–36.8). SBM showed higher amplification and consistent results compared to BF ($P < 0.05$). Considering probes for direct mutation analysis, AF in SBM was 18.3% (n = 9/49; 95%IC=8.7–32.0) and concordance 51.0% (n = 25/49;95% IC=36.4–65.6); while in BF 70.7% (n = 41/58;95%IC=57.3–81.9) and 15.5% (n = 9/58;95%IC=7.3–27.4) respectively.

Limitations, reasons for caution: It does not exist a standard protocol for BF and SBM samples' retrieval. This may limit the reproducibility and reliability of the procedure across different studies. DNA in BF and SBM may originate from apoptotic cells and contamination and this hypothesis has not been tested in the study.

Wider implications of the findings: BF and SBM cannot be considered reliable sources of eDNA for diagnostic purposes, especially due to significantly lower amplification rates compared to TE biopsy. However, BF and SBM can still be considered important specimens for research purposes, which could contain novel biomarkers of reproductive competence beyond chromosomal constitution.

Trial registration number: None.

O-028 Development and application of a novel strategy to explore blastocoel fluid and spent culture media as a source of embryonic DNA

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Study question: Is blastocoel fluid and/or spent culture media a reliable source of embryonic DNA and could they be potentially used for non-invasive preimplantation genetic testing (PGT)?

Summary answer: Blastocoel fluid and spent culture media contain embryonic DNA. The sex of all the embryos was correctly detected from spent culture media samples.

What is known already: PGT is used in many clinics to identify genetically normal IVF embryos for transfer. Genetic material is obtained via biopsy of blastomeres (cleavage stage) or trophectoderm cells (blastocysts). Embryo biopsy requires highly skilled personnel and expensive equipment, yet even in experienced hands the invasive nature of the method poses a potential risk to viability. To reduce costs associated with biopsy and minimise the possibility of damage, blastocoel fluid and spent culture media are being explored as sources of genetic material for non-invasive PGT. However, the minute quantity and degraded nature of cell free DNA poses problems for conventional amplification methods.

Study design, size, duration: A novel method was developed allowing amplification of minute quantities of DNA present in samples of blastocoel fluid and spent media (i.e. the medium in which the embryo was cultured). After validation, the method was applied to 21 blastocyst stage embryos donated for research by 5 IVF patients undergoing PGT for aneuploidy (PGT-A). A total of 16 blastocoel fluid samples and 21 spent media samples were investigated for the presence of embryonic DNA.

Participants/materials, setting, methods: Cryopreserved blastocysts were warmed and incubated in ~3 µl culture media droplets overnight. Blastocoel fluid samples were aspirated, added to ~2 µl drops of buffer, then placed in microcentrifuge tubes. Thereafter, embryos were transferred to new drops of medium and the spent media was collected. Additionally, control media droplets (no embryo exposure) were collected. The samples were amplified using a novel multiple displacement amplification strategy, optimised for degraded DNA, and subsequently subjected to next generation sequencing.

Main results and the role of chance: Initial validation of the new method was done using a heterogeneous mixture of over 200 short DNA fragments (70-100 bp) at different concentrations ranging from 1 pg to 1000 pg. Following amplification, next generation sequencing (NGS) showed that >90% of the fragments had been successfully amplified.

Moving onto analysis of embryo samples, successful amplification was observed in all the blastocoel fluid and spent culture media samples (100%). To assess the extent of genome amplification in these samples, PCR was used for the detection of 224 distinct sequences, including multiple sites on each chromosome. In general, fewer targets amplified from blastocoel fluid samples. Amplification and analysis of the control media droplets confirmed absence of human DNA.

The embryos were also tested using NGS applied to biopsied trophectoderm cells and the results compared to data from blastocoel fluid and spent media. A PCR-based assay for detecting the presence of the Y-chromosome successfully identified all of the male samples (5/5) in spent media samples. Conversely, Y-chromosome material was only identified in 25% of blastocoel fluid samples (1/4). Preliminary analysis of NGS data from spent media samples showed the chromosomal copy number concordance of 95.65% with the trophectoderm samples of the embryos.

Limitations, reasons for caution: At the time of writing the sample size is relatively modest. Additionally, assessment of concordance between blastocoel fluid/spent culture medium and results following traditional embryo biopsy overestimates discrepancies as the assumption is that none of the embryos are mosaic, which is unlikely to be true.

Wider implications of the findings: Noninvasive PGT would be revolutionary, reducing risks to the embryo and increasing patient access by lowering costs. Spent culture media appears to be a superior source for embryonic DNA as compared to blastocoel fluid. Moreover, it represents the least invasive and least expensive approach for sampling genetic material.

Trial registration number: not applicable.

O-029 Non-Invasive PGS reveals the existence of complementary aneuploidy between DNA obtained from trophectoderm biopsy versus DNA in spent culture medium in the same embryo

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Study question: What are the aneuploidy concordance rates within the same embryo between trophectoderm biopsy (PGS) and DNA analysis of the embryo spent culture medium (NI-PGS).

Summary answer: The chromosomal diagnosis obtained by non-invasive PGS is possible, revealing complementary aneuploidies in gains or losses to the obtained by PGS in a trophectoderm biopsy.

What is known already: Preimplantation genetic screening (PGS) 2.0 is gaining momentum in reproductive care with several randomized studies demonstrating its usefulness. Nevertheless, this technique requires an embryo biopsy, which results in an invasive procedure for the embryo and increases the workload for the clinic. The non-invasive diagnosis methods are the main goal of the current personalized medicine. Non-invasive PGS (NI-PGS) aims to diagnose the chromosomal status of the embryo from the DNA present in the spent culture medium in which the embryo has been cultured. However, the efficiency and reliability of NI-PGS are yet to be determined before its clinical application.

Study design, size, duration: We evaluate aneuploidy concordance rates within the same blastocyst (n = 6) between the PGS diagnosis from trophectoderm biopsy versus the results obtained from DNA in spent culture medium analyzed using NI-PGS. Then, whole blastocysts were disassembled into single cells and analyzed by fluorescent in situ hybridization (FISH) for those aneuploid chromosomes by PGS or NI-PGS, with a total of 8 chromosomes analyzed. This study was performed between September 2016 and January 2017 with IRB approval (#1501-IGX-003-CS).

Participants/materials, setting, methods: Trophectoderm biopsies underwent whole genome amplification, a library was generated from dsDNA and loaded into a PGM (Thermo Fisher Scientific Inc.). For NI-PGS, 20 µl of spent culture medium in which the embryos were cultured were amplified, libraries were generated and sequenced in a S5 (Thermo Fisher Scientific Inc.). Finally, whole blastocysts were fixed and individual cells were analyzed by FISH (7.5 ± 3.1 cells per embryo) using telomeric/centromeric probes (Abbott) for the affected chromosomes.

Main results and the role of chance: From the 6 aneuploid embryos analyzed (Table 1), three (#1, #2, #4) showed complementary aneuploidies between day-5 PGS and NI-PGS. Whole-blastocyst FISH for these embryos confirmed PGS diagnosis and the mirror image of NI-PGS further revealing the mosaic constitution for the indicated chromosomes. Two embryos (#3, #5) were diagnosed with mosaic aneuploidies in the trophectoderm biopsy, but these aneuploidies were not confirmed by NI-PGS. Nevertheless, after FISH analysis, these two blastocysts were shown to be mosaics at low-medium degree (20–50% aneuploid cells) for the chromosomes of interest. Finally, embryo #6 had one extra aneuploidy detected by NI-PGS in comparison to PGS, and the FISH corroborated the information observed by NI-PGS, although with a mosaic pattern.

Table 1 Chromosome results per technique.

Blastocyst	Day-5 PGS	NI-PGS	Chromosome analyzed (FISH)	1 copy (FISH)	2 copies (FISH)	3 copies (FISH)	Result (FISH)
#1	+10+15	-10-15	10	0%	0%	100%	Trisomy
			15	25%	0%	75%	Mosaic
#2	+19	-19mos	19	25%	12.5%	62.5%	Mosaic
#3	+2 mosaic	46 XX	2	0%	80%	20%	Mosaic
#4	+15	-15mos	15	10%	0%	90%	Mosaic
#5	+13q mosaic	46 XX	13q	0%	50%	50%	Mosaic
#6	+16	-1 +16	1	0%	33.3%	66.7%	Mosaic
			16	0%	71.4%	28.6%	Mosaic

Limitations, reasons for caution: This study was limited by the sample size and the number of cells analyzed by FISH. In addition, we cannot discard maternal contamination, especially in those cases in which NI-PGS showed an euploid female pattern, masking the proper analysis of aneuploidy discrepancies and the potential identification of mosaicism.

Wider implications of the findings: Here we show that NI-PGS diagnosis is possible, and can be complementary in term of gains/losses to the obtained by day-5 PGS. Our results from the same whole-blastocyst analysis provides explanation for the discordances and supports that maternal DNA contamination needs to be reduced to provide a consistent NI-PGS diagnosis.

Trial registration number: NA.

O-030 Superiority of array Comparative Genome Hybridization (aCGH) over Next Generation Sequencing (NGS) for PGD for reciprocal translocations

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Study question: Is the telomeric resolution of Next Generation Sequencing (NGS) always superior to aCGH?

Summary answer: Though NGS resolution is superior to aCGH, specific genomic areas are better targeted for one technique; rendering aCGH a better technique in some circumstances.

What is known already: The incorporation of Complete Chromosome Screening (CCS) techniques has revolutionized the PGD/PGS worlds. The ability to identify aneuploidy, partial aneuploidy and mosaicism has helped select normal embryos for transfer, thus increasing pregnancy rates. CCS has also opened the door for a variety of patients carrying structural chromosome abnormalities which previously were not amenable to PGD, even with tailor made FISH protocols (deletions, insertions, inversions, etc). The resolution of these techniques in the telomeric regions of the chromosomes will determine their usefulness in cryptic translocations or translocations with telomeric breakpoints.

Study design, size, duration: This retrospective study compare two groups. aCGH group has 20,720 embryos from 3,733 cycles from centers all over USA. Likely the NGS group has 20,720 embryos from 3,926 cycles from centers all over USA. The span of the study is from May 2015 to June 2016.

Participants/materials, setting, methods: Embryo samples were collected for these two groups and were processed by NGS or aCGH depending on each group. The samples were analyzed for partial aneuploidies and the position of the breakpoint was noted.

Main results and the role of chance: For aCGH group, 1,720 partial aneuploidies were detected. Of those partials only 24 were located in a telomeric band. For the NGS group, 3,358 partials were obtained out of those 52 were located in a telomeric band. This study shows the specificity of each technique for different telomeres (table 1).

Telomere	aCGH	NGS	Telomere	aCGH	NGS
1p	NO	YES	1q	YES	NO
2p	YES	YES	2q	NO	YES
3p	YES	YES	3q	YES	NO
4p	NO	YES	4q	YES	NO
5p	YES	YES	5q	NO	NO
6p	NO	YES	6q	NO	YES
7p	NO	YES	7q	NO	YES
8p	NO	NO	8q	NO	YES
9p	NO	YES	9q	YES	YES
10p	NO	YES	10q	YES	YES
11p	NO	NO	11q	NO	YES

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Continued

Telomere	aCGH	NGS	Telomere	aCGH	NGS
12p	NO	YES	12q	NO	YES
			13q	YES	NO
			14q	NO	YES
			15q	NO	YES
16p	NO	YES	16q	NO	YES
17p	NO	YES	17q	NO	NO
18p	NO	YES	18q	NO	YES
19p	NO	NO	19q	NO	YES
20p	YES	YES	20q	YES	NO
			21q	YES	YES
			22q	YES	NO
Xp	NO	NO	Xq	NO	NO
Yp	NO	NO	Yq	NO	NO

Limitations, reasons for caution: aCGH and NGS were performed following Illumina protocols. Other protocols from other companies may have different results.

Wider implications of the findings: Preparatory tests of the anticipated technique in a PGD case for small terminal translocations are crucial. While new technologies such as NGS allow for more detailed information regarding some embryo characteristics (e.g. mosaicism), the importance of older technologies, e.g aCGH, particularly in structural chromosome aberration cases, should not be discounted.

Trial registration number: N/A.

O-031 Development, validation and first clinical application of a novel ultra-rapid comprehensive chromosome screening technique utilising an array based nano scale quantitative DNA amplification technology

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Study question: Can a low-cost methodology be created, which is capable of simultaneously performing ultra-rapid aneuploidy detection and mitochondrial DNA (mtDNA) quantification in blastocyst biopsy specimens?

Summary answer: The technique developed enables rapid, high-accuracy aneuploidy detection, at comparatively low cost and has the capacity to quantify mtDNA, providing extra information on embryo viability.

What is known already: Morphology-based embryo selection is incapable of revealing aneuploidy, one of the principal causes of implantation failure and miscarriage. Therefore, aneuploidy screening has been proposed to assist in the identification of euploid embryos. However, most widely used techniques typically require 24-hours or more. Given that some blastocysts are not ready for biopsy until late on day-5 or even day-6, cryopreservation is necessary to provide sufficient time for testing. The addition of cryopreservation represents an extra risk to the embryos and a further cost to the patients. High costs, complex cycles and treatment delays associated with testing, combine to restrict patient access.

Study design, size, duration: Prior to clinical implementation the methodology was extensively validated. Initially, a total of 111 3-cell and 5-cell samples isolated from two euploid and 13 different established aneuploid cell lines were tested. The test was then applied to 30 donated surplus embryos. Following embryo biopsy at the blastocyst-stage, trophectoderm samples were tested and the results compared to results obtained from the corresponding embryos which were assessed utilising a well-established highly validated next generation sequencing (NGS)-based technique.

Participants/materials, setting, methods: Following validation, the method was clinically applied to 37 patients referred for chromosome screening due to either previous miscarriage(s), IVF failure(s) or advanced maternal age. Average female age was 38.9 ± 3.4, ranging from 31 to 45 years of age. Blastocyst-stage embryos were biopsied on day-5 post fertilisation. 25 patients had at least one euploid embryo available and received a fresh embryo transfer early in the morning of day-6.

Main results and the role of chance: Of the cell samples tested during the initial validation phase, 99.1% (110/111) were correctly identified as euploid or aneuploid. Among all chromosomes analysed, copy number was assigned correctly to 99.9% (2660/2664). Of the blastocysts analysed during the preclinical validation phase, 96.7% (29/30) gave results consistent with those obtained following testing of a separate biopsy specimen using NGS, while at the level of individual chromosomes agreement was seen for 99.7% (718/720). Clinical implementation, saw the application of the method to 173 blastocysts. No result was obtained from eleven embryos (6.4 %). The aneuploidy rate was 63.6 % (103/169). 25 patients had at least one euploid embryo available and received a fresh embryo transfer in the morning of day-6 post-fertilisation. In total, 28 embryos were transferred (1.12 per cycle), of which 18 (64.3 %) implanted, resulting in a pregnancy rate of 64 % (16 patients). Two patients miscarried, hence ongoing pregnancy rate was 56.0 % (per transfer). Overall ongoing pregnancy rate per cycle was 37.8 % (14/37). Interestingly, a significant correlation ($r=0.3$; $p = 0.0013$) between advancing female age and increasing mtDNA copy number was observed. Moreover, aneuploid embryos carried significantly ($p = 0.0002$) higher mtDNA quantities as compared to euploid embryos, independently of female age.

Limitations, reasons for caution: The reported method is likely to be less sensitive than some others (e.g. NGS) for the detection of segmental abnormalities and chromosomal mosaicism. Comparing results generated using the new method with those obtained using a separate biopsy specimen likely overestimates discrepancies, since it is known that some blastocysts are mosaic.

Wider implications of the findings: We report the first application of a chromosome-screening technique, providing results at relatively low cost and substantially reduced turn-around times (5 h) compared to most methods, potentially improving the logistics of utilisation in IVF clinics. The fact that cryopreservation is not essential further simplifies the procedure and reduces costs for patients.

Trial registration number: Not applicable.

O-032 A novel next generation sequencing (NGS)-based comprehensive chromosome screening (CCS) platform that provides accurate copy number and genotyping in parallel from a single trophectoderm biopsy

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Study question: Can an NGS-based CCS methodology simultaneously provide correct evaluation of aneuploidy and precise single nucleotide polymorphism genotyping in human blastocysts from a single trophectoderm biopsy?

Summary answer: This novel NGS-based CCS platform provided 98.7% concordant aneuploidy diagnoses and 99.8% concordant genotype predictions. Genotypes provided for accurate polyploidy detection and sibling embryo discrimination.

What is known already: Most contemporary NGS-based CCS platforms require whole genome amplification (WGA). As a result, only shallow sequencing is obtained, preventing simultaneous analysis of genotypes. Genotyping data from SNP arrays has been shown to provide an opportunity for detection of contamination, triploidy, uniparental disomy, and parental origins of aneuploidy. In addition, genotyping data has been used to determine siblingship of embryo biopsies allowing for the co-development of new biomarkers of reproductive potential. Targeted amplification of specific locations in the genome, instead of WGA, allows for deep sequencing to obtain accurate genotypes.

Study design, size, duration: Diagnostic test. 128 blinded samples of 12 different cell lines were tested by targeted (t)NGS and compared for consistency

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with their known karyotypes. 32 human embryos diagnosed as aneuploid by qPCR-based CCS were rebiopsied, blinded and processed by tNGS. 6-cell samples from a cell line were compared to purified gDNA from the same cell line to determine genotyping accuracy. Multiple biopsies were performed on each of 10 blastocysts to further validate genotyping precision.

Participants/materials, setting, methods: Samples were processed by a proprietary tNGS platform. Copy number results of all blinded samples were compared to either cell line karyotypes or previous qPCR-based CCS diagnoses. Accurate genotyping of 2690 single nucleotide polymorphisms (SNP) obtained after tNGS was validated by comparing consistency between samples from same cell line or between biopsies from the same blastocysts. Genotyping data was used to identify triploid samples based on altered allele ratios and to perform DNA fingerprinting.

Main results and the role of chance: 99.2% (123/124) of the randomized blinded samples of 12 cell lines presented concordant results across all chromosomes. Overall consistency of chromosome copy number assignment was 99.97 (2975/2976). Consistency of normal vs abnormal diagnosis between qPCR and tNGS-based CCS of rebiopsied blastocysts was 98.7 % (76/77). TNGS-predicted a median allele ratio in known triploid cell line controls of 0.336 (0.329–0.343; 95% CI), while diploid samples was 0.437 (0.428–0.448)(p=8.3e-19). A blastocyst triploidy frequency of 0.43% was observed, with 32 out of 7516 embryos falling within the triploid range. Embryos with available parental DNA were used to confirm triploidy and all were found to be of maternal origin. Genotyping concordance among 12 6-cell-aliquot samples and purified gDNA from the same cell line was 99.839 (99.707–99.907, 95% CI). When genotypes of multiple biopsies from the same embryo where compared, the concordance rate was 99.857 (99.734–99.979, 95% CI). Genotypes of 188 embryo biopsies and gDNA of the delivered baby of 32 single embryo transfers were compared to confirm identity of the transferred embryo among siblings. In all cases the transferred embryo was correctly identified showing a mean genotype similarity of 99.73% (99.70–99.76, 95% CI) and a sibling similarity of 93.34% (93.15–93.52, 95% CI).

Limitations, reasons for caution: Although reduced in costs per diagnosis, the workflow of this novel platform requires cryopreservation of all embryos per cycle after biopsied.

Wider implications of the findings: This novel tNGS approach provides a unique opportunity to use accurate genotyping data in support of accurate copy number data in parallel, allowing for the ability to fingerprint embryos as a tool to develop new biomarkers of reproductive potential, and to identify polyploid embryos.

Trial registration number: None

SELECTED ORAL COMMUNICATIONS
SESSION 07: LONG-TERM CONSEQUENCES IN ART

Monday 3 July 2017 Room C 10:00–11:30

O-033 Weight and waist circumference of IVF children at the age of 9 years still affected by embryo culture medium

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Study question: Do embryo culture media used during an IVF/ICSI treatment have an effect on growth and body composition of 9-year-old singleton IVF children?

Summary answer: Nine-year-old children from the Vitrolife group have a higher bodyweight and a higher waist circumference and waist/hip ratio compared to the Cook group.

What is known already: Children born after IVF/ICSI have a lower birthweight and adverse perinatal outcome compared to children born after natural conception. Previously, we have shown that the culture medium used in an IVF/ICSI treatment affects birthweight (Dumoulin et al. 2010). After alternating assignment to embryo culture in either G1™ Version3 (Vitrolife group) of K-SCICM (Cook group), birthweight of the resulting children was significantly higher in the Vitrolife group. This weight effect persisted during the first 2 years of life (Kleijkers et al. 2014).

Study design, size, duration: In this observational cohort follow-up study (MEDIUM-KIDS), parents of all singletons from the abovementioned study were approached after the ninth birthday of their child to participate in a further follow up. Measurements were performed between March 2014 and December 2016. Of the 294 eligible children included in the original study, 136 children (75 Vitrolife and 61 Cook) participated in the current study.

Participants/materials, setting, methods: Parents were invited to attend our clinic with their child for a single visit lasting approximately 2.5 hours. Two experienced clinicians performed the anthropometric measurements as a part of the MEDIUM-KIDS study for which also several cardiovascular parameters were collected. The following anthropometric measurements were performed by two experienced clinicians in a standardized way: height and weight of the child using calibrated scales, 4-point skinfold thickness measurements in threefold and waist/hip circumference.

Main results and the role of chance: Baseline characteristics between the participating children from the Vitrolife and Cook group were similar (mean age 9.5 years in both groups). Data (mean ± SEM) were compared using the Student's t-test. Height and height corrected for age and gender (SDS scores) were similar for the Vitrolife vs Cook group (139.3 cm ± 6.0 vs 138.8 cm ± 7.8 and -0.21 vs -0.28). Weight and weight SDS scores were higher in the Vitrolife group as compared to the Cook group (34.2 kg ± 6.6 vs 32.1 kg ±5.9 (P = 0.06) and -0.51 vs 0.11 (P = 0.02)). After correction for several potential confounding factors among which age, length and gender of the child and anthropometrics of the parents, the difference in weight attributable to culture medium was 1.7 kg (P = 0.047). Furthermore, waist circumference and waist/hip ratio were significantly higher in the Vitrolife group (61.4 cm vs 58.0 cm, (P = 0.01) and 0.86 vs 0.83, (P = 0.02), respectively) as well as subscapular skinfold (7.9 mm vs 6.6 mm P = 0.02) and truncal adiposity (1.94 cm ±0.99 vs 1.63 cm±0.88 (P = 0.05)). The peripheral skinfolds biceps and triceps were similar. After correction for confounders the difference attributable to culture medium was 2.7 mm (P = 0.044) and 0.030 (P = 0.015) for waist and waist/hip ratio and 0.15 cm (P = 0.08) for truncal adiposity.

Limitations, reasons for caution: The most important limitation of this study is the relatively low participation rate of 46%. However no differences were seen in baseline characteristics between participants and non-participants.

Wider implications of the findings: The results of our study underline the importance of structured follow-up of IVF/ICSI children to further elucidate possible long-term health effects. They should urge embryologists and manufacturers to focus on culture conditions since the early embryo is in a stage where possible long-term health effects can be triggered.

Trial registration number: NL45845.068.13.

O-034 Effect of culture medium on cardiovascular characteristics of 9-year-old IVF/ICSI singletons

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Study question: Do culture media have an effect on cardiovascular characteristics of 9-year old children born after an IVF/ICSI treatment?

Summary answer: No significant differences were observed between the Cook and Vitrolife group for bloodpressure, fasting glucose, insulin, cholesterol and TSH, nor for endothelial or glycocalyx function.

What is known already: Children born after IVF/ICSI are known to have an increased risk of adverse perinatal outcomes such as low birthweight, if compared to children born after natural conception. The Developmental Origin of Health and Disease (DOHaD) hypothesis states that lower birthweight and poor perinatal outcome can affect postnatal growth, glucose metabolism, fat distribution and vascular function. Furthermore, studies have been published in which 12-year-old IVF children already show a significantly higher systolic and diastolic blood pressure and

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higher fasting glucose levels compared to naturally conceived children. Our group has shown that culture media can affect birthweight (Dumoulin et al. 2010).

Study design, size, duration: In this observational cohort study (MEDIUM-KIDS), parents of the singletons from our previous study (Dumoulin et al.) were approached for further follow up after the ninth birthday of their child. The singletons were born after an IVF/ICSI treatment performed between July 2003 and December 2006 in our clinic, when two different culture media were used alternately: either G1™ Version3 (Vitrolife) or K-SICM (Cook). Follow up measurements were performed between March 2014 and December 2016.

Participants/materials, setting, methods: Parents were invited to attend our clinic with their child for a single visit lasting approximately 2.5 hours. Two experienced clinicians performed all measurements as part of the MEDIUM-KIDS study. The following cardiovascular parameters were measured in a standardized way: blood pressure, heart rate, endothelial function by skin laser-Doppler and iontophoresis with vasodilatory drugs and glycocalyx function using Glycocheck™. A blood sample was taken after an overnight fast for insulin, glucose, TSH, and lipid analysis.

Main results and the role of chance: Of the 294 eligible children, 136 children (75 Vitrolife and 61 Cook) participated in the study. Baseline characteristics between the participating children from the Vitrolife and Cook group were similar (mean age 9.5 years in both groups). Data (mean ± SD) were analysed by Student's t-test. Both systolic and diastolic blood pressures (mmHg) were comparable: 101.2 ± 7.2 and 60.3 ± 6.5 for Vitrolife and 99.8 ± 7.6 and 60.0 ± 7.3 for Cook ($P = 0.31$ and $P = 0.81$). After an overnight fast, cholesterol, glucose, insulin, LDL, HDL and triglycerides were normal and similar between the two groups. Also TSH (mU/L) did not differ between the groups (Vitrolife 3.1 ± 1.8 and Cook 2.8 ± 1.2, $P = 0.29$). Endothelial function in the microcirculation was compared with maximum perfusion units corrected for the baseline value as a measure for vasodilation capacity. After correction for baseline, the difference for nitroprusside was -7 for Vitrolife compared to Cook ($P = 0.458$). For acetylcholine, after correction, the difference was only 0.19 for Vitrolife compared to Cook ($P = 0.98$). Also glycocalyx function was tested in a small group of the children, the results for the two groups were comparable as well (PBR 5–25 was 2.0 ± 0.33 for both groups).

Limitations, reasons for caution: The most important limitation of this study is the relatively low participation rate, however no differences were seen in baseline characteristics between participants and non-participants. With the current number of participants, this study had a power of 80% to detect a difference in systolic blood pressure of 3.6 mmHg.

Wider implications of the findings: The normal and comparable cardiovascular results for the two groups of children are reassuring for this moment. However, further research with a larger study population and the addition of a spontaneously conceived control group is necessary to gain more insight and reassurance in the cardiovascular development of IVF children.

Trial registration number: NTR4220.

O-035 Assisted reproductive technology might affect the growth of children: A prospective cohort study on 3,509 babies

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Study question: What is the difference between the growth and development of singleton children conceived after infertility treatments and those conceived naturally at 6 years of age?

Summary answer: At 6 years of age, the weight and height of children conceived through assisted reproductive technology (ART) are higher than those of children conceived naturally.

What is known already: Several large cohort studies have shown a similar or even better outcome for babies conceived after frozen embryo transfer (FET) than those conceived after fresh embryo transfer (FrET) and natural conception (NC). Meanwhile, recent studies have reported that the birth weight of FET children was significantly higher than that of NC children. Some researchers suggest that ART might affect the development of children with respect to gene expression.

Although the safety aspects of different ART techniques are important, few studies have investigated the long-term prognosis of children born through ART.

Study design, size, duration: This was a nationally representative prospective cohort study conducted in Japan, a racially homogeneous country. A total of 4,095 mothers who conceived through ART at the Japanese Institution of Standardizing Assisted Reproductive Technology (JISART), a group of ART clinics in Japan, participated in the study between October 1, 2008 and November 30, 2009. A total of 3,509 babies born at these clinics in 2009 were targeted for follow-up.

Participants/materials, setting, methods: This study was conducted in 26 ART clinics in Japan. Each baby was born after FET (n = 1,223), FrET (n = 1,005), or NC (n = 1,281). Embryo freezing was performed by vitrification. The data on their growth and development (KIDS developmental scale) at 18 months and 6 years of age were obtained using a questionnaire. Weight, height, and KIDS scale score were compared between each treatment group by analysis of variance.

Main results and the role of chance: Finally, 1,830 (52.2%) participants undertook the follow-up survey.

The birth weight of singleton children conceived via FET, FrET, and NC was 3080, 3024, and 2998 g; that after 18 months was 10451, 10517, and 10375 g; that at 6 years of age was 20.3, 20.4, and 19.7 kg, respectively. The birth height of singleton children conceived via FET, FrET, and NC was 49.1, 49.1, and 48.4 cm; that after 18 months was 80.2, 80.5, and 80.4 cm; that at 6 years of age was 114.8, 115.3, and 114.2 cm, respectively.

After adjustment for confounders (gestational weeks at delivery, sex, child's age-in-days from delivery, parity), the statistical analysis between three groups was performed.

Although FET singleton babies had a significantly higher birth weight and height than the NC singleton babies ($P < 0.001$), the average individual weight curves showed that this difference disappeared before 18 months of age and reappeared at 6 years of age. In contrast, there was no significant difference in the KIDS score between each treatment group at 6 years of age.

Limitations, reasons for caution: Slight differences in the ART procedure and the culture medium at each IVF clinic might affect childhood growth. As it was a volunteered survey, the response rate was relatively low; therefore, it is necessary to consider the effect of selection bias on each group.

Wider implications of the findings: Although there was no evidence of adverse effects on health up to 6 years of age, it might be necessary to reconsider the effect on growth and epigenetic implications in children born through ART.

Trial registration number: None.

O-036 Effect of parental and treatment aspects of IVF/ICSI on perinatal outcomes: a nationwide study of IVF/ICSI singletons born in The Netherlands between 2000 and 2011

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Study question: Do parental and treatment aspects of in vitro fertilization with or without intracytoplasmic sperm injection (IVF/ICSI) affect perinatal outcomes in singleton pregnancies?

Summary answer: Both parental characteristics and treatment aspects of IVF/ICSI have effects on perinatal outcomes in singleton pregnancies.

What is known already: Previous studies have shown that singleton pregnancies resulting from IVF/ICSI are at risk of preterm birth, and children born after IVF/ICSI have a lower birth weight and an increased risk for congenital abnormalities. To date, it remains to be fully elucidated, which aspects of IVF/ICSI treatment impact perinatal outcomes and whether these are independent of parental characteristics.

Study design, size, duration: All 13 IVF clinics in The Netherlands provided data on all assisted reproductive technology (ART) treatment cycles performed between January 1st, 2000 and January 1st, 2011 that resulted in an ongoing pregnancy (n = 37.683). Using probabilistic data-linkage, these data were linked to the Perinatal Registry Netherlands which includes all children born in The Netherlands in the same time period (n = 2.548.977). 31.184 treatment cycles were successfully linked to their perinatal outcome (83%).

Participants/materials, setting, methods: Analysis were limited to singletons born after IVF, ICSI or frozen-embryo transfers (FET) (n = 23.588). In order to assess independent effects, multivariable logistic or linear regression models were constructed including parental characteristics (i.e. age, subfertility duration, treatment indication) and treatment characteristics (i.e. treatment type, culture medium, number of embryos transferred). We assessed the impact of parental and treatment aspects of IVF/ICSI on perinatal outcomes (birth weight, occurrence of premature birth, congenital malformation and perinatal death).

Main results and the role of chance: Longer subfertility duration was associated with lower birth weight (adjusted difference [adj β] -7 gram birth weight for each added year of subfertility, 95% CI -11 to -4). "Male factor" as indication for treatment was associated with a higher birth weight compared to "tubal factor" (adj β 36 g, 95% CI 10 to 61) and lower risk of premature birth (adj OR 0.7, 95% CI 0.6 to 0.8). "Female non-tubal factor" as indication for treatment was associated with a higher risk of congenital malformations compared to "tubal factor" (adj OR 1.6, 95% CI 1 to 2.6). Birth weight was higher in children born after FET as compared to children born after fresh embryo transfer (adj β 96 g, 95% CI 79 to 112). ICSI (fresh embryo) babies had higher birth weight than IVF (fresh embryo) babies (adj β 25 g, 95% CI 9 to 41) and were less likely to be born prematurely (adj OR 0.8, 95% CI 0.7 to 0.9). The risk of very premature birth or perinatal death was not affected by duration of subfertility, indication for treatment or any ART treatment characteristics.

Limitations, reasons for caution: Due to limited information on parental characteristics we cannot exclude the possibility of residual confounding by lifestyle factors, and due to the observational nature of our study we cannot assess causality.

Wider implications of the findings: Parental and treatment aspects of IVF/ICSI both affected perinatal outcomes. This highlights the sensitivity of the early embryo for parental conditions and for the in vitro environment. Whether to embark on ART and which procedures to follow, should take chances of ongoing pregnancy aswell as perinatal outcomes into account.

Trial registration number: Not applicable.

O-037 How to compare outcomes of IVF/ICSI patients with different backgrounds and treated by different regimens

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Study question: Is it possible to compare ovarian stimulation outcomes in patient groups with different background characteristics and stimulation regimens?

Summary answer: Total gonadotropin dose per oocyte is associated with the quality of ovarian stimulation and is useful when comparing outcomes of patient groups with different origins.

What is known already: The selection of patients for IVF/ICSI as well as ovarian stimulation protocols vary between clinics and countries, which makes it difficult to compare outcomes of treatments with differing practices. Total gonadotropin dose per number of oocytes retrieved (dose/oocyte) is a measure of ovarian response to stimulation. Previous research from our group has shown that a lower dose/oocyte indicates better odds for live birth than a higher dose/oocyte. In this study, we wanted to examine the usefulness of this parameter in cases where direct comparison of outcomes is impossible.

Study design, size, duration: Retrospective cohort study of 17,728 first-time ovarian stimulation cycles for non-donor IVF/ICSI performed in 2008–2012 in the U.S. (National Assisted Reproductive Technology Surveillance System, n = 14,230) and Finland (LUMI database, n = 3,498). Women aged ≤40 years undergoing stimulation with long gonadotropin-releasing hormone (GnRH) analogue (n = 9,175) or with GnRH antagonist protocols (n = 5,630) were included. All had >3 oocytes retrieved before elective single embryo transfer on day 2/3 or day 5/6, followed by cryopreservation of supernumerary embryos.

Participants/materials, setting, methods: We compared three groups of patients treated in different settings: transfer on day 2/3 in Finland (Group A, n = 3,498), transfer on day 3 in the United States (Group B, n = 1,756), and transfer on day 5/6 in the U.S. (Group C, n = 12,474). Differences in patient and stimulation characteristics, and cumulative live birth rates using embryos from the same stimulation (cLBR) were examined. Total gonadotropin dose/oocyte was analysed using generalized linear equations accounting for confounding factors.

Main results and the role of chance: Group A had the highest proportion of patients aged ≥35 years (35.3% vs. Group B: 23.6% vs. Group C: 22.1%, P < 0.0001). However, subjects in Group A received the lowest gonadotropin doses (1756.7 ± 698.8 (mean ± SD) vs. 2218.8 ± 1167.4 vs. 2314.9 ± 1083.3 IU, respectively, P < 0.0001) and had the lowest numbers of oocytes retrieved (12.4 ± 6.0 vs. 14.6 ± 7.1 vs. 18.8 ± 8.2, respectively, P < 0.0001). Patients in Group C were treated with the highest gonadotropin doses and had the highest numbers of oocytes retrieved. cLBR was lowest in Group A (1639/3498, 46.9%), intermediate in Group B (933/1756, 53.1%) and highest in Group C (8702/12474, 69.8%, P < 0.0001).

Due to multicollinearity, analysis of raw data could not determine whether the high numbers of oocytes in Group C could be explained by higher stimulation doses, by better ovarian reserve or by another factor. Dose/oocyte was different across the groups (Group A: 188.9 ± 142.3; Group B: 201.7 ± 183.4; Group C: 155.9 ± 176.4 IU/oocyte, P < 0.0001). After correction for confounding factors, dose/oocyte was similar in Groups A and B (238.1 ± 286.3 vs. 234.0 ± 275.8, P = 0.6) but was significantly lower in Group C (155.2 ± 130.7, P < 0.0001).

Limitations, reasons for caution: Due to classification differences, infertility diagnoses were not accounted for in the present analysis.

Wider implications of the findings: The lowest dose/oocyte in the blastocyst group indicates that this group had the highest odds for live birth already after oocyte retrieval. Adjusted data show that these patients also had the best ovarian reserve. Dose/oocyte is a useful tool for comparison when the groups examined are not directly comparable.

Trial registration number: not applicable.

O-038 The number of oocytes retrieved during IVF: a balance between efficacy and safety

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Study question: How does the number of oocytes retrieved in one IVF cycle influence live birth rates and safety?

Summary answer: Live birth rates increased up to 20 oocytes retrieved, when assessed cumulatively, including fresh and frozen cycles and severe side effects followed a similar pattern.

What is known already: Previous studies have shown that the number of oocytes retrieved for IVF is a positive predictor of live birth in fresh cycles. With an increasing single embryo transfer (SET) policy in the world more embryos are available for freezing and thawing cycles (FET). Thus, the cumulative pregnancy and LBR after fresh and subsequent FET become more important as an efficacy variable.

Study design, size, duration: Data were collected from The Swedish National Quality Registry of Assisted Reproduction (Q-IVF) and included all fresh cycles, using patients own gametes, performed during 2007–2013 and the accompanying FET cycles. In total, 39 387 women undergoing 77 956 fresh IVF/ICSI cycles and 36 270 FET cycles were included. For investigating the association between number of oocytes and severe complications the data from Q-IVF were cross linked with the National Patients Registry.

Participants/materials, setting, methods: Generalized Estimating Equations analyses were used to explore the association between the number of oocytes retrieved and live birth rates for fresh cycle and cumulatively after fresh and consecutive FET cycles. Both linear and quadratic models were used. Adjustments were made for maternal age, year of treatment, number of previous failed fresh cycles, previous children, and IVF /ICSI. The association between the number of oocytes retrieved and severe complications was investigated in a similar way.

Main results and the role of chance: The median number of oocytes retrieved was 9 (interquartile range 5–12). The median age of women was 34 years The rate of fresh SET in this cohort was 73%. LBR in fresh cycle increased by the number of oocytes retrieved with a plateau from 11 oocytes., where the LBR was 30.3% for a 34 year old woman. Cumulatively, a steady increase in LBR was observed, up to around 20 oocytes, where the cumulative LBR was 45.8%. The observed rates and the predicted, second degree curves fitted well both for fresh cycles and on a cumulative basis. In the linear multivariate model, a significant increase in cumulative LBR was observed per oocyte retrieved (OR 1.064, 95% CI 1.061 to 1.067). Maternal age, previous failed cycles and ICSI (vs IVF) affected cumulative LBR negatively while year of treatment and previous live birth after IVF affected LBR positively, though adjusting for these factors had only a marginal impact on the estimates of the association between the number of retrieved oocytes and LBR. The presence of the severe complications ovarian hyperstimulation syndrome (OHSS) and tromboembolic events were mainly observed when a high number of oocytes were retrieved and the treatment resulted in pregnancy.

Limitations, reasons for caution: Even though most frozen embryos are thawed and transferred within a year from retrieval there is still some cryopreserved embryos left unused giving a possibility that the cumulative LBR for the cycles performed 2010–2013 might be slightly higher.

Wider implications of the findings: The results from this large, national study including all fresh IVF/ICSI cycles and their subsequent FET cycles performed in private and public clinics in Sweden 2007–2013 are of crucial importance for the IVF society as well as for patients undergoing IVF, by taking both efficacy and safety into account.

Trial registration number: NA

INVITED SESSION

SESSION 08: OVARIAN REJUVENATION: FOR REAL OR JUST A DREAM?

Monday 3 July 2017 Plenary 2 11:45–12:45

O-039 Mitochondrial transfer- can it improve oocyte quality?

J. Cohen

Tyho-Galileo Research Laboratories, Livingston- New Jersey, U.S.A.

Abstract text

Changes in genomic expression may occur after assisted reproduction, but such alterations are rarely intentional and may be transitional. One of the mechanisms that could lead to generational changes involves cytoplasmic or mitochondrial donation. The proposition of mitochondrial transfer into human oocytes and early embryos has been contemplated for decades. Yet relatively few births have been reported. Efficacy is complex and often considered inadequate from an evidenced-based medicine point of view. These experimental procedures may have profound consequences as generational changes may be transmitted through the germline. The first clinical experiments followed observations in the mouse during the 1980 s, to alter genomic expression by cytoplasmic transfer between normally dividing and blocked mouse embryos. Four different approaches have been reported resulting in live birth in patients with repeated or suspected implantation failure: (1) Heterologous injection of a small volume of MII donor cytoplasm into a mature oocyte (ooplasmic transplantation), (2) autologous injection of isolated somatic mitochondria from granulosa cells into mature oocytes, (3) autologous injection of Egg Precursor Cell (EggPC) mitochondria into mature oocytes and (4) Reconstitution after spindle transfer or pronuclear transplantation similar to the technique used for mitochondrial replacement therapy (MRT) or cytoplasmic donation. These modalities aim to suppress purportedly poorly functioning mitochondria by supplementing or replacing with presumably normally functioning mitochondria. Artificial gene sequence alterations of deleterious mitochondrial mutations are also being considered for clinical application, but studies remain limited to mouse models. Many questions remain about patient selection, diagnosis of mitochondrial insufficiency, mitochondrial replication, heteroplasmy, safety and follow-up. How should patients be selected? A simple survey follow-up study was only recently published after the use of approach 1, cytoplasmic transplantation. No other follow-up studies have been reported from the other modalities. Based on the follow-up survey study of 13 teenagers, there do not seem to be significant effects on pregnancy, birth, health and academic performance, but sample size is small. Patients rarely disclosed the procedure to their children, possibly indicating the difficulty performing more invasive follow-up of such children as has been advocated by the UK panels advising on MRT. Although early case series show promises, there seem to be considerable limitations. Possible solutions will need to be evaluated and an in-depth debate on the ethics of these approaches must be encouraged.

O-040 Improving ovarian response through ovarian tissue activation

K. Kawamura[1], A. Hsueh[2]

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[2]*Stanford University School of Medicine, Obstetrics and Gynecology, Stanford, U.S.A.*

Abstract text

In patients with primary ovarian insufficiency (POI), early exhaustion of ovarian follicles was evident due to genetic, immunological, iatrogenic, or other causes. POI affects 1% of women and is characterized by high circulating levels of gonadotropins along with amenorrhea before 40 years of age. They are infertile due to a lack of follicle growth and ovulation; oocyte donation is the only effective treatment option, because residual ovarian follicles in these patients are not responsive to traditional gonadotropin treatments. Recently, we developed a method for activation of dormant follicles by using in vitro culture of ovarian fragments treated with PI3K stimulators following disruption of Hippo signaling pathway (IVA, in vitro activation).

Under laparoscopic surgery, ovaries were removed and cut into strips. Ovarian strips from POI patients were vitrified. After thawing, strips were fragmented into 1–2 mm cubes before treatment with PI3K stimulators. Two days later, cubes were autografted under laparoscopic surgery beneath serosa of Fallopian tubes. Follicle growth was monitored via transvaginal ultrasound and serum estrogen levels. After detection of antral follicles, patients were treated with FSH followed by hCG when preovulatory follicles were found. Mature oocytes were then retrieved and fertilized with the husband's sperm in vitro before cyropreservation of four-cell stage embryos. Patients then received hormonal treatments to prepare the endometrium for implantation followed by transferring of thawed embryos. When we published second paper in Human Reproduction (2015 Mar;30(3):-608–15), three pregnancies were achieved based on serum hCG after IVF and embryo transfer. Although one was a miscarriage, two healthy IVA babies have been born with the first one being more than four year of age now. Two other pregnancies by two other centers have also been achieved.

In this presentation, I will introduce our IVA procedure and update clinical outcome of IVA so far. Also, I will show some recent progresses in the IVA approach.

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INVITED SESSION
SESSION 09: DATA REPORTING SESSION
Monday 3 July 2017 Room A 11:45–12:45

O-041 Data from the ESHRE PGD Consortium

M. De Rycke

UZ Brussel, Centre for Medical Genetics, Brussels, Belgium

O-042 First results of the ESHRE study into the evaluation of oocyt euploidy by microarray analysis (ESTEEM), a randomized controlled trial to test preimplantation genetic testing for aneuploidy

K. Sermon[1], P. Bossuyt[2], V. Goossens[3], J. Geraedts[4]

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Abstract text

Preimplantation genetic testing for aneuploidy has been suggested to improve live birth rates in reproductive treatment.

In 2009, the ESHRE PGS Task Force undertook a pilot study to analyse 300 oocytes by polar body (PB) array comparative genomic hybridisation (aCGH) and to transfer embryos developing from euploid zygotes, with the aim to assess the feasibility of an RCT. PB analysis was chosen, because it circumvents the issue of mosaicism occurring later in development, and most meiotic abnormalities are maternal in origin.

In 2011, two back-to-back papers were published on the results of this pilot study, a clinical paper (Joep Geraedts et al., "Polar Body Array CGH for Prediction of the Status of the Corresponding Oocyte. Part I: Clinical Results" Human Reproduction 26: 3173-80) detailing the outcome of transferred embryos, and a technical paper (M Cristina Magli et al., "Polar Body Array CGH for Prediction of the Status of the Corresponding Oocyte. Part II: Technical Aspects" Human Reproduction 26: 3181-85) detailing the optimal techniques for first and second polar body analysis.

The outcome of the pilot study was positive, and an RCT named the ESHRE Study into the Evaluation of oocyte Euploidy by Microarray analysis (ESTEEM) was initiated.

The experimental questions addressed by this trial are: in women aged 36 to 40 years (1) does aCGH in the first and second PB to select euploid embryos for transfer increase the likelihood of a live birth within one year, compared to IVF without aCGH, and (2) is aCGH in the first and second PB in women with no euploid embryos in a first cycle indicative of the probability of having no euploid oocytes in a subsequent cycle?

Importantly, it was decided that ESTEEM was to allow for an intention-to-treat analysis as well as to include data on cumulative pregnancies from cryopreserved embryos. Patients were recruited by IVF centres in seven countries.

All patients underwent conventional ovarian stimulation followed by ICSI. In the study arm simultaneous PB biopsy was performed followed by array comparative genomic (aCGH) hybridization. Subject to availability of embryos, single or double embryo transfer was performed.

The study was performed between June 2012 and December 2016. Two hundred and five patients were allocated to chromosome screening versus 191 patients to the control group. Baseline characteristics were similar between trial arms. In both groups, the mean age was 38.6 and the mean BMI was 23.2. Fifty-eight percent in both groups had not received IVF treatment before. Sixty-one percent (612/994) of the zygotes analysed were aneuploid.

The results of this multicenter RCT will be presented during the meeting. This is the largest RCT to date addressing the potential benefit of PGT-A by aCGH in patients of advanced maternal age.

INVITED SESSION
SESSION 10: FERTILITY SOCIETY OF AUSTRALIA EXCHANGE LECTURE
Monday 3 July 2017 Room B 11:45–12:15

O-043 New strategies for ovarian preservation during cancer treatment: Elimination of PUMA confers protection against chemotherapy-mediated oocyte loss

Q.N. Nguyen[1,2], N. Zerafa[2], S. Liew[2], A. Strasser[3], C. Scott[4], M. Hickey[1,5], J. Findlay[6], K. Hutt[2]

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Abstract text

The rapid rise in cancer survivorship over recent decades has brought with it a pressing need to address the late effects of treatment. DNA-damaging radiotherapy and chemotherapy can cause female infertility and ovarian endocrine failure by depleting the ovarian reserve of primordial follicles, which once destroyed, cannot be replaced. However, the mechanisms by which chemotherapy-induced DNA damage lead to primordial follicle death are still unclear.

Using mouse knockout models, we have previously shown that primordial follicle oocyte apoptosis is mediated by the potent pro-apoptotic BH3-only protein, PUMA. PUMA is transcriptionally activated by TAp63 in response to DNA damage caused by g-irradiation, and *Puma*$^{-/-}$ mice retain a proportion of their primordial follicles following a radiation dose that is sterilizing in wildtype (WT) mice, and are fertile.

In the current study we aimed to examine the role of PUMA and TAp63 in mediating depletion of the ovarian reserve following treatment with cyclophosphamide or cisplatin. Adult female C57BL/6 *Puma*$^{-/-}$ or wild-type (WT) mice received IP injection with saline (control), cisplatin (Cis) 5 mg/kg, or cyclophosphamide (Cy) 300 mg/kg (n = 5/group). Ovaries were harvested 5 days after treatment, follicles quantified by stereology, and follicular morphology assessed. Control WT females contained 4982 ± 760 (mean±SEM) primordial follicles per animal. As expected, treatment with cisplatin or cyclophosphamide caused a dramatic reduction in this number, with 27% surviving cisplatin (control WT: 4982 ± 760 vs Cis WT: 1365 ± 308, $p < 0.05$), and only 4% surviving cyclophosphamide (control WT: 4982 ± 760 vs Cy WT: 212 ± 79, $p < 0.05$). In marked contrast, primordial follicles were completely preserved in cyclophosphamide-treated *Puma*$^{-/-}$ females compared with *Puma*$^{-/-}$ controls (control *Puma*$^{-/-}$: 6294 ± 955 vs Cy *Puma*$^{-/-}$: 7251 ± 995, $p = 0.78$). A similar effect was observed for cisplatin treatment (control *Puma*$^{-/-}$ vs Cis *Puma*$^{-/-}$: 5035 ± 574, $p = 0.66$). By comparison, *TAp63*$^{-/-}$ females were protected from oocyte depletion after cisplatin, but not cyclophosphamide, indicating that cyclophosphamide induces PUMA via an alternative, non-*TAp63*-mediated pathway (control *TAp63*$^{-/-}$: 4060 ± 497 vs Cy *TAp63*$^{-/-}$: 957 ± 171, $p < 0.05$; vs Cis *TAp63*$^{-/-}$: 3918 ± 754, $p = 0.98$). A second cohort of female mice was treated similarly, but held for continuous breeding with proven males. Results of the fertility study showed that cyclophosphamide-treated WT females had a shortened fertile lifespan (-192 ± 6 days; $p < 0.0001$) and fewer litters overall (-4 ± 1 litters; $p < 0.01$). This was ameliorated in cyclophosphamide-treated *Puma*$^{-/-}$ females (age at last litter; control *Puma*$^{-/-}$: 283 ± 20 days vs Cy *Puma*$^{-/-}$: 320 ± 8 days, $p = 0.19$; number of litters; control *Puma*$^{-/-}$: 7 ± 1.1 vs Cy *Puma*$^{-/-}$: 7.33 ± 1.2, $p = 0.85$).

In summary, we show for the first time that *Puma* is the key apoptotic trigger in primordial follicle oocytes in mice following treatment with a single dose of

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cyclophosphamide or cisplatin. Elimination of PUMA rescues 100% of the ovarian reserve following administration of either drug, although the pathways via which *Puma* is transcriptionally activated differ between them, with cyclophosphamide acting via a non-*TAp63*-mediated pathway. Crucially, we have shown that this translates to a complete preservation of fertile potential and the fertile lifespan in these mice, with no obvious ill effects on offspring. Collectively, these data further strengthen the argument that inhibition of oocyte apoptosis is a promising potential means of fertility preservation in females following DNA-damaging cancer therapies.

INVITED SESSION

SESSION 11: PARAMEDICAL INVITED SESSION: SPERM MESSAGING – WHAT'S THE STORY?

Monday 3 July 2017 Room W+X 11:45–12:45

O-044 Genetic screening in cases of extreme poor spermatogenesis

L. Ramos[1], M. Oud[2], L. Vissers[3], M. Noordman[4], J. Veltman[5]

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Abstract text

For the past 25 years we have been successful in treating male infertility with intracytoplasmic sperm injection (ICSI), while diagnosis and aetiology of the underlying problem has not been completely understood. The origin for the poor spermatogenesis can be explained by anatomical (b.v. cryptorchidism), iatrogenic causes (e.g. chemotherapy), lifestyle factors (e.g. smoking/drugs) or genetic anomalies (e.g. Klinefelter, Y-chromosome AZF deletions) but still in almost 60% of cases, infertility is categorized as unexplained. The percentage of genetic causes involved in male infertility increases with the severity of the oligospermia, been highest in azoospermia. The latest publications show that a genetic cause is found in approximately 15% of males tested by karyotyping, Y-chromosome deletions, CFTR point mutations. More rare, but most probably also of genetic origin, are the monomorphic sperm anomalies such as globozoospermia, acepahlic spermatozoa, tail anomalies, etc. Up to the present, only a few causative genes for azoospermia of monomorphic anomalies has been described (e.g. CFTR, DDX3Y, TEX11, DPY19L2, AURKC, SYPCP3 genes); unfortunately these genes are not routinely tested before enrolment of patients for ICSI-treatment.

In the recent years the feasibility of approaches such as targeted disease gene panel sequencing, whole exome- and even whole genome- sequencing has dramatically increased the potential of genetic approaches in male infertility. During the presentation, an overview of the different novel genetic techniques will be given (targeted gene sequencing, WES, WGS), and advantages and disadvantages of each methodology are discussed. Exome and genome sequencing can be used to identify novel disease genes, whereas targeted gene sequencing is useful to follow-up novel candidate genes in large cohorts. An example of this strategy is our ongoing study, an international collaboration, in which a panel of 6 causative and 101 candidate male infertility genes have been sequenced in a cohort of more than 1000 extreme oligospermic/azoospermic males. This validated method will be discussed in both research and diagnostic applications. Also, we will discuss the unexpected finding of pathogenic mutations in the CFTR gene in males which were not previously suspected of CBAVD by physical exploration for the presence/absence of the vas deferens.

Why should we focus on the diagnosis of male infertility when ICSI is still successful even in azoospermia or in cases with 100% abnormal sperm morphology? Increasing the knowledge of the underlying anomalies in the spermatogenesis pathways will be useful (1) to avoid taking unnecessary testicular biopsies in case of sertoli cell only (SCO) or completely maturation arrest (MA), or when sperm produced is unable to produce a healthy/viable embryo, (2) to predict and prevent the risk of passing on the infertility to the next generation, and (3) to learn more about normal and abnormal human reproduction in general, which may aid in developing novel treatment strategies.

O-045 Evaluating oocyte activation potential of sperm using calcium pattern analysis

B. Heindryckx[1], M. Ferrer[2], F. Vanden Meerschaut[2], P. De Sutter[2]

[1]*Ghent University Hospital Ghent Fertility and Stem cell Team, Department for Reproductive Medicine, Gent, Belgium*
[2]*Ghent University Hospital, Department for Reproductive Medicine, Ghent, Belgium*

Abstract text

Fertilization failure, especially after intracytoplasmic sperm injection (ICSI), still occurs in up to 5% of the ICSI cycles. When a normal number of oocytes is retrieved in the presence of motile sperm, the main reason of fertilization failure after ICSI is an oocyte activation deficiency. Oocyte activation is characterised by a series of intracytoplasmic calcium (Ca^{2+}) rises released from the inositol triphosphate receptor (IP3R) present in the oocyte, and induced by the sperm-borne factor phospholipase C zeta (PLCz), which produces inositol triphosphate (IP3). It remains challenging to determine whether a sperm or an oocyte factor is the cause of fertilization failure after ICSI. Heterologous ICSI has been used in the past to determine the activation potential of human sperm, for example by injection into mouse oocytes. Given the higher activation potential of human PLCz compared to mouse PLCz, this test might not be sensitive enough to reveal more subtle abnormalities in PLCz. Importantly, a correct series of Ca^{2+} oscillations has been correlated with the early events of fertilization, but also with later stages such as embryonic developmental potential and even post-implantation events in animal models. Therefore, assessment of the precise Ca^{2+} pattern caused by the sperm could be of added value to indicate more accurately the cause of failed or low fertilization after ICSI or later stage problems in some couples. Using specific fluorochromes, the calcium pattern can be visualised after fertilization. Indeed, recent evidence has shown that although human sperm from some couples can activate mouse oocytes, they cause aberrant calcium patterns after injection into mouse or human oocytes, leading to failed fertilization after ICSI. Especially when mutations are found in PLCz, the generated Ca^{2+} patterns are very abnormal, in contrast to control sperm. So calcium pattern analysis of human sperm provides a more sensitive test than heterologous ICSI to reveal causes of fertilization failure after ICSI. Given the scarcity of human oocytes for research, this calcium analysis is mostly done using animal derived oocytes. It remains to be determined how to extrapolate these results to human oocytes.

INVITED SESSION

SESSION 12: THE SCIENCE AND ETHICS BEHIND GERMLINE GENOME EDITING

Monday 3 July 2017 Plenary 2 14:00–15:00

O-046 Gene editing in the human germ line - risks and possibilities

R. Lovell-Badge

The Francis Crick Institute, London, United Kingdom

Abstract text

The human genome is far from static. It changes with some 40 to 80 base pair substitutions and perhaps 4 or 5 small insertions or deletions each generation due to de novo germline mutations. While this only affects a small part of the genome and many of these mutations will be silent, this has contributed to human variation and to selection for specific traits, as well as to the burden of genetic disease. The possibility that we might be able to deliberately alter our own genes has been debated for decades with each new relevant method, from recombinant DNA, transgenics, IVF, homologous recombination in embryonic stem cells or via cloning or iPS cells. But it has always been possible to dismiss the notion because the methods have been too inefficient, inaccurate, and unsafe. But in the last few years with the development of genome editing methods, notably those involving the CRISPR/Cas9 system, these arguments may no longer hold. They are now ubiquitous in basic research and have proven to be immensely invaluable. They can be used to make precise genetically altered animals with efficiencies approaching 100%. The methods are also already proving to be valuable tools clinically for somatic gene therapies. I will discuss the use of the methods to address questions

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about the biology of human germ cells and of pre- and peri-implantation human embryo development. I will also discuss the possibility of their use clinically, to make heritable changes for the purposes of avoiding disease. There are, of course, other potential uses. For this reason it is critical that genome editing is only employed to make heritable changes in those jurisdictions that have appropriate regulation and oversight, and following acceptance of a core set of principles that include established notions of fairness and of avoiding harm, and with appropriate levels of public support. I will discuss ideas of how this might be achieved.

O-047 Should we edit the genomes of our future children?

S. Wilkinson

Lancaster, United Kingdom,

Abstract text

Should we edit the genomes of our future children?

This lecture reviews some of the main ethical objections to 'editing' the genomes of our future children by intervening to modify early embryos or gametes.

Having noted that this possibility raises various practical concerns, notably concerning safety, it proceeds first to describe some of the many different reasons why people may wish to do this. It is argued that the ethics of genome editing depends, to a large extent, on what ends it is designed to achieve. There may, for example, be a huge difference between seeking to prevent painful and life-threatening diseases in future generations and seeking merely to satisfy prospective parents' aesthetic preferences.

The lecture argues that, while there are genuine moral concerns about using genome editing to avoid disease (such as worries about eugenics and about the effects on existing people with disabilities) these concerns can, for the most part, be assuaged provided that the motivation for the practice is clearly and sensitively communicated. Such measures would however need to be allied with continued or strengthened social and political support for existing people with disabilities.

The position of genome editing for other purposes (such as enhancement or satisfying parents' aesthetic preferences) is more complex. Not all such practices are wrong but they are liable to objections which are less forceful when genome editing is used to improve health outcomes.

INVITED SESSION

SESSION 13: RECENT DEVELOPMENTS IN OVARIAN (HYPER) STIMULATION AND OVULATION INDUCTION

Monday 3 July 2017 Room A 14:00–15:00

O-048 Individualised ovarian stimulation for IVF: balancing cumulative outcome, safety, burden and costs

A.N. Andersen

Copenhagen University Hospital Rigshospitalet, The Fertility Clinic- section 4071, Copenhagen, Denmark

Abstract text

Presently we have fairly good predictors (AMH and AFC) of ovarian response when exposing patients to conventional ovarian stimulation with high FSH doses. The clinical difficulty is how we translate our knowledge on response predictive factors into FSH dosing algorithms.

Individualization is only meaningful, if you accept that for IVF, as for many other health technologies there exist an optimal balance of benefits (pregnancies/births) versus risks (OHSS and burdens). The present status seems to be that individualization of the FSH dose and protocol can maintain, but does not increase, pregnancy rates but diminish the risks and burdens of IVF.

The first choice in treatment naïve patients is which protocol has the best benefit/risk ratio. Meta-analysis shows that the antagonist protocol provide the same pregnancy rates and simultaneously reduce the OHSS risk to around 50% compared to the agonist protocol. A recent large trial of more than 1000 cycles in a broad selection of women under 40, using the same FSH starting doses in the two protocols showed, that a) the cumulative live birth rates (Fresh and FER cycles within 24 months) were the same b) the OHSS risk was reduced to half c) the gonadotropin consumption was reduced by 25% and the psychological burden was also reduced in antagonist cycles. There is thus good evidence that in order to improve the balance between benefit, risk, and burden the first choice to make is to use the antagonist protocol.

Regarding individualization of the FSH dose two huge randomized controlled multicenter trials are now available. The Dutch "OPTIMIST" trial (n = 1503) where FSH dosing was determined by AFC and the "ESTHER" Trial (n = 1329) where FSH dosing was based on AMH and bodyweight. In both studies the patients predicted to be in the lower response segment (either by AFC < 10 or by AMH < 15 pmol/l) had an increased yield of oocytes with 1.1 more in the OPTIMIST trial and 1.0 in the ESTHER trial, after individualized up-dosing of the daily FSH dose. However, the modest increase in number of oocytes was not associated with any increase in delivery rates. Among patients predicted to be in the normal to high response (ESTHER trial) or high response (OPTIMIST) segment down-dosing of the daily FSH dose lowered the number of oocytes by 2–4. Both studies showed that "down-dosing" shift the egg distribution to the left and that a number of patients end up being under-dosed. Overall both studies showed that individualized dosing had no benefits in terms of higher ongoing pregnancy rates (ESTHER and OPTIMIST) and cumulative live-birth rates (OPTIMIST). The number of cryopreserved blastocysts was unchanged by individualized stimulation in the ESTHER trial, so the cumulative outcome will most likely be the same. A possible advantage of the individual dosing was the finding that patients exposed to individual dose achieved the same pregnancy rates following fewer treatment cycles (OPTIMIST). The cost-effectiveness has been studied in the OPTIMIST trial, and the conclusion was that AFC-based dosing offered no advantage over standard dosing. In terms of risks, major risks like OHSS are infrequent, and may be graded and analyzed differently – both OHSS and secondary preventive measures like agonist triggering seem lowered by individualized dosing.

In summary individual dosing models using either AFC or AMH and bodyweight maintain cumulative success rates in terms of live pregnancies, the impact of FSH dose adjustments on the number of oocytes is modest, but sufficient to lower the risks of severe adverse effects as OHSS and presumably the overall burden for the patients – but economic cost-effectiveness seems unchanged.

O-049 The evolution of treatment approaches in PCOS: management of ovulation and metabolism

R. Anderson

University of Edinburgh The Queen's Medical Research Institute, MRC Centre for Reproductive Health, Edinburgh, United Kingdom

Abstract text

Polycystic ovary syndrome is a pleiotropic condition, and as befits the most common endocrinopathy, there is a corresponding range of treatment options, including surgical, medical and lifestyle modification approaches. Treatments address the woman's presenting complaint, ie how it affects her at that stage in her life, rather than addressing the underlying pathology. This reflects our limited understanding of the aetiology and pathogenesis of the condition, although a unifying hypothesis is perhaps doomed to failure given the heterogeneity of the condition. Both the initial surgical approach, the wedge resection of the ovary, and the subsequent development of clomifene citrate were very successful, and clomifene remains the first line treatment for anovulation due to PCOS today. The wedge resection has evolved into laparoscopic ovarian diathermy, with high quality evidence supporting its efficacy, but our understanding of how it works has not changed from 'no idea'. Medical ovulation induction is seeing the increasing use of aromatase inhibitors, which may be more effective than clomifene for very obese women, if not for most. Gonadotropin therapy for mono-ovulation remains very effective and can achieve a cumulative birth rate of around 70% with <10% multiple pregnancies in clomifene non-responders, but it is challenging to both doctor and patient and appears increasingly bypassed in favour of IVF. For those not wishing to conceive, there is a wide range of combined contraceptive pills to regulate menses and perhaps improve hirsutism and acne, although the less androgenic pills have an increased venous thrombosis risk compared to 2nd generation pills, and some women's BMI may preclude this approach. Weight loss remains the mainstay of approaches to the metabolic manifestations of the condition, and when achieved can be very effective in improving fertility as well. Medical therapies designed to improve the well-documented insulin resistance have been largely disappointing in their impact. A novel approach currently in clinical trials is based on slowing GnRH

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secretion, and thus lowering LH and testosterone levels, through administration of neurokinin B antagonists. This does seem to address one of the key abnormalities underlying the reproductive (although not metabolic) aspects of PCOS, so if effective, may have value whether or not a women is wishing to conceive.

INVITED SESSION

SESSION 14: DEBATE: DEEP ENDOMETRIOSIS: TO OPERATE OR NOT IN INFERTILE WOMEN

Monday 3 July 2017 Room B 14:00–15:00

O-050 Pro

H. Roman[1], I. Chanavaz-Lacheray[2]

[1]Rouen University Hosp. Charles Nicolle, Gynecology and Obstetrics, Rouen Cedex, France
[2]Rouen University Hospital, Expert Center in the Diagnosis and Multidisciplinary Management of Endometriosis, Rouen, France

Abstract text

One of the most interesting debates surrounding deep endometriosis concerns the management of patients with colorectal lesions and pregnancy intention, for which no strong first level of evidence data exists to recommend performing surgical excision of deep endometriosis or ART. Worldwide meetings on endometriosis thrive on debates, with conclusions varying significantly from one conference to another according to speaker experience and views. An absence of consensus and inevitable divergent advice leave patients routinely confused with hesitation further delaying accurate management of the disease. Studies assessing the policy of primary IVF in women with colorectal endometriosis have recorded pregnancy rates inferior to 45% and estimated cumulative pregnancy rates after up to 3 cycles or IVF as high as 68%. Other authors have reported pregnancy rates over 60% in patients undergoing primary surgery for colorectal endometriosis, with spontaneous conception representing up to 60% of pregnancies. Recent studies suggest that surgical management of deep endometriosis would increase cumulative pregnancy rate up to 78% in patients undergoing 3 postoperative IVF cycles. In addition, questions remain as to whether delaying surgery for months or years impairs health. Delaying surgery may lead to bowel occlusion, dysuria, higher rates of radical colorectal procedures, increased postoperative morbidity and postoperative complications and prolonged painful complaints. As data available in the literature suggest that the current policy of systematic IVF prior to surgery in patients with deep colorectal endometriosis is questionable, we propose a custom made management of pregnancy wish, based on complete assessment of both fertility and deep endometriosis characteristics.

O-051 Con

F. Golfier

Hotel-Dieu De Lyon, Gynecology Obstetrics, Lyon, France

SELECTED ORAL COMMUNICATIONS

SESSION 15: PARAMEDICAL SESSION - LISTENING TO THE PATIENT

Monday 3 July 2017 Room W+X 14:00–15:00

O-052 Endometriosis: Why am I feeling pain?

M. Uny[1], J.M. Wenger[2], S. Baudry[3], S. Campione[2], S. Constantino[2], N. Pluchino[2]

[1]University Hospital of Geneva, Gynécologie / obstétrique, Geneva 14, Switzerland
[2]University Hospital of Geneva, Gynécologie/ Obstétrique, Genève, Switzerland
[3]University Hospital of Geneva, Gynécologie /Obstétrique, Genève, Switzerland

Study question: Can we improve the quality of endometriosis care by systematically offering a validated information and support?

Summary answer: Information sessions led by a nurse specialist help patients to better understand the cause of pain, to cope with disease and increase compliance to treatment.

What is known already: Endometriosis is a complex disease often diagnosed in advanced stages and with unclear etiologies. Patients may have long medical history with important repercussions on the quality of life. During medical consultations, patients receive specific medical information and a detailed treatment plan that often requires surgery, IVF procedures and hormonal treatment. Their care requires also answers to specific needs in terms of listening, understanding and support. Moreover, it is essential for patients to understand the heterogeneity of the disease and symptoms in order to improve the sense of frustration caused by a chronic disease and better adhere to treatment.

Study design, size, duration: Since the end of 2014 at the University Hospital of Geneva endometriosis center, a specialized nurse manages monthly sessions of information dedicated to patients and to their close relatives. Clear simple language is used to explain the physiopathology of endometriosis, symptoms, diagnosis tools and treatments. The nurse also explains the impact of the disease on fertility and quality of life. The quality this activity has been evaluated over a period of 6 months

Participants/materials, setting, methods: Patients are invited to participate by the medical team. Participation is free. Patients can be accompanied by their close relatives. The registration is made by e-mail or phone. At the end of each meeting an anonymous questionnaire is given to every participant. It evaluates the relevance of the session, the quality of the information and if sessions meet patient needs. The role of such sessions in the therapeutic course has been also evaluated.

Main results and the role of chance: 110 patient have attended the sessions. Results of questionnaires show that informative sessions offer a clear advantage for a better understanding of medical condition and proposed treatments.

57% of patients declared that the information is adequate and useful, 7 % of them too complicated abd 7% simplistic. The patients also express their wish of having additional information concerning management of the pain (28 %), complementary medicine (28 %), infertility (26 %) and also practical strategies for improving daily activities (35 %). The organization of sessions is considered adequate in term of schedule and duration. All patients have defined the session helpful for a better understanding of treatment options.

Limitations, reasons for caution: Patients included are heterogeneous and they differ in terms of disease characteristics and treatment plan. Each participant has also a different level of knowledge of the disease.

Wider implications of the findings: Validated and consistent informative sessions may meet crucial needs of endometriosis patients and their family members, representing an additional instrument for improving quality of care.

Trial registration number: none.

O-053 Follow-up of oocyte donors ten years after donation: what we are doing well and what we could improve

M. Perul[1], M. Corral[1], M. Caballero[1], C. Conti[1], J.M. Gonzalo[1], S. Brazal[1], D. García[2], R. Vassena[1], A. Rodríguez[1]

[1]Clínica EUGIN, EUGIN, Barcelona, Spain
[2]Fundación EUGIN, EUGIN, Barcelona, Spain

Study question: How do oocyte donors regard their experience in a long term follow up, a decade after the donation?

Summary answer: Most oocyte donors regard their donation as a positive experience a decade later, and would recommend being a donor to other women.

What is known already: Oocyte donation in Spain is altruistic and anonymous and can be performed by healthy women between the ages of 18 and 35. Few reports investigated the psychological impact of donating oocytes and motivation to donate in potential donors, or in donors immediately after the donation. Treatment management, healthcare staff involvement, and personal satisfaction were considered positive by most women at the time of donation. However, whether this experience remains positive in the long-term, or

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whether contradictory and painful feelings emerge, has not been evaluated yet. Reports of negative feelings should especially be investigated and could help adjust pre-donation counselling.

Study design, size, duration: Anonymous telephone survey of 121 women in their 40 s having donated their oocytes in their 20 s-30 s. Surveys consisted of 20 structured questions and were carried out by 3 ART nurses with extensive experience interacting with oocyte donors, in the period between June and November 2016. One-hundred and twenty-one former oocyte donors out of 141 (85.5%) were contacted by phone and agreed to participate in the survey.

Participants/materials, setting, methods: Participants were between 40–49 years old (average 41.6) at the time of the survey and between 26–35 years (average 32.2) at the time of donation. Most of them were Spanish (99.2%), had high school (50.4%) or university (27.3%) education and had children (69.4%) at the time of interview. Inclusion criteria were to be ≥40 to encompass most of the donor reproductive life, and speaking Spanish as mother tongue to ensure questions understanding.

Main results and the role of chance: Most former donors highlighted positive aspects and feelings about their donation (113, 93.4%), while less than half (53, 43.8%) mentioned some negative ones. Former donors reported feelings of altruism (84, 69.4%) and personal satisfaction (13, 10.7%) in relation to the donation. Economical compensation was also reported as a positive aspect of donation by some (18, 14.9%). Negative aspects reported were mainly related to the physical discomfort at the time of donation: injections (20, 16.5%), pain (17, 14.1%) and hormones (10, 8.3%). Participants also valued the reproductive care received; for instance, one woman reported being grateful for the identification and treatment of a cervical dysplasia during the donor screening process. In general, former donors have shared their donation experience with friends (76, 62.8%), family (55, 45.5%), partner (24, 19.8%), and colleagues (13, 10.7%). Most participants (56.2%) have performed more than one donation cycle and 96.7% would recommend the donation to other women. About one third of donors reported physical discomfort during the treatment (43, 35.5%), and, some, concerns about the destiny of their oocytes (13, 10.7%). For these same reasons, 8 women (6.6%) reported to regret having donated. Finally, 101 participants (83.5%) reported that oocyte donation increased their awareness about infertility.

Limitations, reasons for caution: A limitation of the current study is that all women performed their donation after the age of 25; experiences of women having donated their oocytes at a younger age may be different due, for instance, to unfinished education trajectories, unstable work or incomplete family composition.

Wider implications of the findings: The great majority of former donors regard their donation as positive with the passing of the years, while few negative aspects, related mostly to the treatment physical discomfort, emerge. More user friendly and comfortable stimulation protocols should be developed, in order to increase both short and long term donor experience.

Trial registration number: NA.

O-054 Patient-friendly insemination with homologous and donor sperm positively influences clinical pregnancy rates

H. Bijnens[1], A. Thijssen[2], P. Jacobs[3], S. Theys[3], C. Pascale[3], V. Vandewal[3], W. Cools[4], G. Mestdagh[3], N. Dhont[3], R. Campo[3], W. Ombelet[3]

[1]GIFT VZW - Dr. W. Ombelet, Fertility, Genk, Belgium
[2]Hasselt University, Department of Physiology, Hasselt, Belgium
[3]Ziekenhuis Oost-Limburg, Department of Obstetrics and Gynecology, Genk, Belgium
[4]Hasselt University, Center for Statistics CENSTAT, Hasselt, Belgium

Study question: Does a patient friendly approach increase the clinical pregnancy rate (CPR) after intrauterine insemination with homologous or donor semen?

Summary answer: CPR increased from 9.5% to 14.6% per cycle with homologous and 16.7% to 21.1% per cycle with donor semen when insemination was performed by midwives.

What is known already: In many studies, it has been proven that fertility patients want a sensitive fertility staff and need sufficient emotional support. Patients also appreciate continuity of care and do not want to be treated by too many different fertility clinic staff members. They prefer an individualised approach. Most studies performed focus on the psychological aspect, but not on success rates.

Nurses/midwives are in a potentially unique position in the assisted reproductive technology environment in that, unlike other professionals, they maintain a more direct contact with the patient.

Study design, size, duration: The study was performed as a prospective cohort study. During the period of July 2011 until July 2016, data from 1711 insemination cycles with homologous semen in 688 women and 1579 insemination cycles with donor semen in 473 women were collected prospectively in a tertiary referral infertility centre. As from February 2016, the insemination procedure at our centre was performed by midwives instead of medical doctors.

Participants/materials, setting, methods: Homologous insemination was performed in couples with unexplained or moderate male factor infertility. As from February 2016, the insemination procedure at our centre was performed by the midwife instead of medical doctors. This resulted in a slower injection of the inseminate and a more relaxed contact with the patient or the couple. Above this, more time was spent on the procedure. Results were analysed with Chi-square analysis.

Main results and the role of chance: Clinical pregnancy rates (i.e. presence of fetal heart beat at 6-7 weeks of gestation) following homologous insemination increased significantly from 9.5% to 14.6% ($p = 0.038$) in the study group. In case of donor inseminations, clinical pregnancy rates increased from 16.7% to 21.1%, ($p = 0.088$), a non-significant increase.

Limitations, reasons for caution: Results were analysed in a prospective cohort study; however, a prospective randomised trial is needed to confirm our findings.

Wider implications of the findings: According to our preliminary results, it seems that a more patient-friendly approach might increase the clinical pregnancy rates after intrauterine insemination. Future studies should investigate the importance of slow versus rapid intrauterine injection of the inseminate and the possible value of patient-centred measurements to achieve better success-rates after insemination.

Trial registration number: NA.

O-055 Are nurse-led consultations of benefit to patients? Evidence of effectiveness from a UK clinic specialising in IUI

B. Woodward, D. Littlehales, N. Cotgreave-Keates, S. Deb, M. Tomlinson

Nottingham University Hospital, Fertility Unit, Nottingham, United Kingdom

Study question: Are nurse-led consultations a cost-effective way to improve the standard of patient care by expediting access to lower technology fertility treatments such as IUI?

Summary answer: The majority of patients were satisfied with nurse-led consultations, stating that this approach improved continuity of care and that their needs were adequately met.

What is known already: UK state-funding for IUI was reduced following the NICE guideline recommendation that IVF should be offered as an initial treatment for unexplained infertility. This has led to less physician time allocation for initial consultations in our IUI clinic, with resources preferentially allocated to IVF. Fertility nurse practitioners spend significant time interacting with patients, yet there is limited research on this interaction, and in particular patient perception. Nurse-led consultations increase clinic capacity and patient choice. Physician to nurse task-shifting has gained increasing interest in health policy but little is known about how patients perceive its effectiveness, particularly with regard to consultations.

Study design, size, duration: Patient perception of nurse-led consultations was evaluated via a questionnaire sent to a random sample of treated couples, whether successful or not, to seek their views on the nurse-led consultation for our IUI service.

Participants/materials, setting, methods: This study included couples ($n = 50$) with unexplained infertility, anovulation problems and/or borderline male factor who received a nurse-led consultation for IUI treatment, at a fertility clinic specialising in IUI with partner sperm. The questionnaire was

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posted to couples who had previously attended the clinic regardless of the outcome. No identifying information was requested to assure anonymity of the respondents.

Main results and the role of chance: The response rate to the survey was 54.0% (27/50). 85.2% couples stated they were informed in advance that their consultation would be with a nurse rather than a physician, and 11.5% stated they would have preferred at consultation with a doctor. 96.3% felt sufficient information was provided and were happy with the nurse's medical knowledge. 88.9% felt their needs adequately met and 96.2% felt the nurse-led consultations improved their continuity of care. 92.3% graded that the explanation and organization of investigations and treatment by the nurse as either excellent or very good. 96.2% stated that they would recommend nurse-led consultations to others. When asked about the preferred timing of consultation, a high proportion preferred the consultations to be held at either end of the standard working day, with 32.2% preferring before 9 am and 19.8% after 6 pm, or at the weekend (21.5%).

Limitations, reasons for caution: Only 27 patients from 50 responded to the survey. A wider survey might reveal different responses, although from the data provided, there was an overwhelming trend for patients supporting access to nurse-led consultations. Our setting of a small fertility clinic specialising in IUI may have influenced responses.

Wider implications of the findings: Provided patients expectations are met, then nurse–led consultations appear to be an effective resource allocation in terms of both costs and time, allowing physicians' skills to be utilised elsewhere. This action must be supported by detailed accounts of fertility nurses' competencies and training in this area.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS
SESSION 16: OPTIMIZING THE EMBRYO ENVIRONMENT

Monday 3 July 2017 Plenary 1 15:15–16:30

O-056 Ultra-low (2%) oxygen tension significantly improves human blastocyst development and quality

A. Ferrieres-Hoa[1], K. Roman[2], T. Mullet[2], A. Gala[2], S. Hamamah[2]

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[2]*CHU Montpellier, Biologie de la Reproduction, Montpellier, France*

Study question: The present study investigated the effect of ultra-low (2%) O_2 tension on blastocyst development and on the rate of useful blastocysts (UBs).

Summary answer: This study shows that ultra-low O_2 tension is a successful technique to improve both blastocyst development and the rate of useful blastocysts (UBs).

What is known already: Improving the ex-vivo culture conditions of early embryos is still the main challenge in ART. The oxygen concentration is one of the criteria that influence protonated culture. In the uterus, early embryos are exposed to an ultra-low (1.5%) O_2 atmosphere. Moreover, embryo damage caused by reactive oxygen species (ROS) is related to the O_2 tension during in vitro culture. Currently, the traditional 6%/20% of CO_2/O_2 or 6%/5% CO_2/O_2 conditions are the most currently culture parameters used for early human embryo culture. However, few studies investigated the in vitro culture conditions under more physiological O_2 tension.

Study design, size, duration: This is a single-center observational study, performed from January to December 2016.

The endpoint for the study was whether O2 tension could enhance blastocyst development. The relationship between the oxygen tension and standard morphological evaluation according to the Gardner grading system was also examined: at day 5/6, full (grade 3), expanded (grade 4), hatching (grade 5) or fully hatched (grade 6) blastocysts with at least a grade B trophectoderm quality were considered as UBs.

Participants/materials, setting, methods: Day 1 embryos were cultured in 6% CO_2 and 5% O_2 for three days; and then in 6% CO_2 and 2% O_2 atmosphere from day 3 to day 5/6 (ultra-low O_2 group, n = 238 embryos, n = 42 patients, age 33.09 ± 4.71) or in 6% CO_2 and 5% O_2 (controls, n = 169 embryos, n = 42 patients, age 33.10 ± 4.66).

Main results and the role of chance: Blastocyst formation rate was 60.1% (143/238) in the ultra-low O_2 group and 52.1% (88/169) in the control group ($p = 0.1078$). The UB rate was significantly higher in the ultra-low O_2 group (103/143; 72.0%) than in the control group (40/88; 45.5%) ($p < 0.0001$). There is no significant different in patient's age between groups.

Limitations, reasons for caution: This preliminary results need to be completed with a prospective randomized controlled trial including the living birth rates to ensure the efficiency of these in vitro culture conditions.

Wider implications of the findings: Our study demonstrates that ultra-low O_2 tension is associated with higher blastocyst formation and better blastocyst quality compared to low O_2 tension, supporting the hypothesis that more physiological culture conditions improve early embryo development.

Trial registration number: .

O-057 The composition of human preimplantation embryo culture media and their stability during storage and culture

M. Tarahomi[1,2], J.P. Van Straalen[3], F.M. Vaz[4], F.M.P. Schrauwen[3], G. Hamer[1], M. Van Wely[1], S. Repping[1], S. Mastenbroek[1]

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[2]*Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR*
[3]*Department of Clinical Chemistry, Academic Medical Center, University of Amsterdam*
[4]*Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam*

Study question: To analyze the concentration of thirty seven components of seventeen commercially available human preimplantation embryo culture media and their stability during storage and culture.

Summary answer: The composition was different for each culture medium. There is an effect of storage and culture on the concentration of some of the components.

What is known already: In vitro fertilization (IVF) is currently the most commonly used intervention in subfertile couples. During IVF, embryos are cultured for several days in embryo culture medium. It is has recently been established that the type of embryo culture medium used in IVF can affect treatment success as well as the health of the future child. The exact composition of specific media are often not disclosed by the manufacturers. Furthermore, it is unknown whether the composition of these media changes during storage or during culture.

Study design, size, duration: Between October 2014 and October 2015, all ready-to-use human preimplantation embryo culture media (n = 17) that were commercially available at that time were purchased from eight suppliers in the Netherlands. Osmolarity and the concentration of thirty seven components were tested upon arrival in the IVF laboratory, after three days of culture without embryos (sham culture) starting from the arrival day, at the expiry date, and after three days of sham culture just before the expiry date.

Participants/materials, setting, methods: Osmolarity was analysed by Advanced 3320 Micro-Osmometer [Advanced Instruments inc., Norwood, Massachusetts,USA]. Ions, metabolites, immunoglobulins, albumin and total protein were quantified using Roche Cobas chemistry analyzer (Cobas 8000) [Roche Diagnostics, GmbH, Mannheim, Germany], and pyruvate and 21 amino acids were analysed by Ultra- Performance Liquid Chromatography Mass Spectrometry [Acquity-Quattro Premier XE, Waters, Milford, Massachusetts, USA]. Statistical analysis was performed using general linear models.

Main results and the role of chance: The composition varied between media, no two media had the same concentration of components. Storage led to significant changes in 17 of the 37 analyzed components, i.e. magnesium chloride, phosphate, albumin, total protein, tyrosine, tryptophan, alanine, methionine, glycine, leucine, glutamine, asparagine, arginine, serine, proline, and threonine. Storage also significantly affected the osmolarity in 3 of the 17 media,

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but for all media combined this change in osmolarity was not significant (p = 0.051). Sham culture of the analyzed media had a significant effect on the concentrations of 15 of the 37 analyzed components, i.e. phosphate, iron, albumin, total protein, tyrosine, tryptophan, alanine, methionine, glycine, isoleucine, glutamine, asparagine, arginine, proline and histidine. Sham culture significantly affected the osmolarity in 10/17 culture media (overall p-value <0.001).

Limitations, reasons for caution: Embryo culture media could contain components that were not analyzed in this study, and the clinical relevance of the varying concentrations are yet to be determined.

Wider implications of the findings: The wide variation in composition between the culture media indicates the optimal composition is still unknown. Companies of culture media should fully disclose the components and their concentrations. The clinical relevance of the observed differences as well as the effect of storage and culture needs to be evaluated.

Trial registration number: Not applicable.

O-058 Potential contamination of fresh embryo cell culture medium with cell free DNA

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[3]*Inserm U1203- Hôpital Arnaud de Villeneuve- CHU Montpellier, Département de Médecine et Biologie de la Reproduction/DPI, Montpellier, France*

Study question: Is there a potential contamination of embryo culture medium with cell free DNA (cfDNA) by the in embryo cell culture medium of human serum albumin?

Summary answer: Contamination of the embryo culture medium by cfDNA is not excluded.

Thus, the possibility to contaminate human embryos under ex vivo culture conditions still asked.

What is known already: Free extracellular nucleic acids are now highly scrutinized with regards to their potential as a molecular tool in the diagnostic field. In particular, clinical validation of circulating cell free DNA was recently shown in oncology or prenatal diagnosis. They are present in circulating fluids, non-circulating and in cell culture media. We are investigating the value of cell free DNA analysis towards improving embryo culture and pre-implantation diagnosis. We used an ultrasensitive Q-PCR based assay. The aim of this study was to determine if the embryo culture media, G-1 PLUS and G-2 PLUS contains detectable level of nuclear/mitochondrial derived cfDNA.

Study design, size, duration: Nuclear and mitochondrial derived cfDNA were extracted from drops with the QIAamp kit and quantified by RT-qPCR (IntPlex). 89 drop samples from 3 patients underwent IVF/ICSI and 10 controls were included in the analysis. Data are expressed in mean +/− SD.

Participants/materials, setting, methods: We used, G-1 PLUS and G-2 PLUS embryo culture media (Vitrolife, Sweden). Drops performed during the ICSI procedure were obtained from patients (n = 6). Then, all drops of embryos culture media were frozen until the cfDNA and mitochondrial derived DNA analysis.

Main results and the role of chance: We observed that embryo culture media, G-1 PLUS and G-2 PLUS (Vitrolife), of freshly open flasks under laminar flow hood, consistently contains detectable level of mitochondrial derived DNA (Mean = 0.215 ng/ml; SD=0.09). Nuclear DNA was detected in one out of ten G2 culture medium with a concentration of 3.65 ng/ml. Note, cfDNA was not detectable in all our negative controls. This observation point out the contamination of circulating cfDNA from either the serum used for preparing albumin or from the manufacturer manipulator. These medium contain in addition to bicarbonate buffer, human serum albumin, hyaluronan and gentamicin. This implies the need of using a detection threshold when analyzing cfDNA in series of embryo cell cultures. No contamination due to the manipulator was detected in the control drops for nuclear DNA media (3 different patients; 89 samples and 10 controls), whereas a possible manipulator contamination was found in one control drop for mitochondrial DNA; p-value is near the statistical significance (P value = 0.058).

Limitations, reasons for caution: Further investigations with a larger number of culture media drops are in progress to confirm theses results.

Wider implications of the findings: Our results seriously impact the determination of cfDNA level and might raise concerns with regards to the foreign cfDNA potential biological effect on embryo culture and growth.

Our data strongly open the possibility to develop a new quick and low-cost test for embryo culture safety.

Trial registration number: Not applicable.

O-059 A novel three-dimensional environment for human blastocyst development up to 10 days post-fertilization

F. Arjmand[1], S. Kazemnejad[2]

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[2]*Avicenna Research institute, -Director of Tissue Engineering Department -Head of Reproductive Biotechnology Research Center in Avicenna Research Institute- ACECR, Tehran, Iran*

Study question: The objective was development of an in vitro system to culture human embryo beyond blastocyst stage to future study of factors regulate post-implantation embryo upgrowth.

Summary answer: Co-culturing of embryos with endometrial cells in alginate hydrogel and media supplementation with melatonin creates an environment for blastocyst development up to 10 days post-fertilization.

What is known already: In comparison with 2D culture system, three-dimensional (3D) culture provides structural integrity of embryos and offers direct physical and chemical interactions of embryos with their surrounding environment, similar to that experienced in vivo. 3D culture system has been extensively used for the development of follicles or oocytes, and a number of studies have examined the culture of bovine and porcine embryos in vitro using agarose gel tube system or alginate hydrogel encapsulation device. To the extent of our knowledge, there is no report on human embryo development beyond the blastocyst stage.

Study design, size, duration: Ethical approval was granted by the Avicenna Research Institute Bioethics Committee. A luteal phase endometrial biopsy was performed in five women with regular menstrual cycle and no pathologic disorder. Sixty human preimplantation embryos in blastocyst stage were included in the study. The embryos were nondemanding ones with opposite sex provided from the couples referred for sex selection after signing the informed consent. Seven different culture systems were considered for blastocyst culture.

Participants/materials, setting, methods: The EnSCs were mitotically inactivated and then decidualized by 8-Br-cAMP. Blastocysts were co-cultured with EnSCs or encapsulated in alginate (ALG) beads. The embryos were treated with conventional culture media (CCM) with or without melatonin (Mela). The embryos were monitored post-encapsulation. To find out the proportion of cells originated from inner cell mass (ICM) or trophectoderm (TE), the embryos were stained using PI/Hoechst. The secretion levels of of 17b- oestradiol (E2) and hCG were measured.

Main results and the role of chance: - The alginate beads were stable and did not rupture during the study period. At 5 days post-encapsulation, 85 ± 3 % of the alginate-encapsulated EnSCs remained viable.

- 23 % (2 embryos out of 9) of the encapsulated blastocysts survived until day 4 in CCM/ALG culture condition.

- Melatonin fortification of CCM (CCM/ALG/Mela) remarkably improved the maintenance rate (44 %) and survival time (day 5) of expanded embryos in alginate hydrogel. Moreover, co-encapsulation of EnSCs increased the maintenance rate of blastocysts by 44 %.

- Based on this differential staining, embryos cultured in CCM/ALG/EnSCs/Mela or CCM/ALG/Mela were composed of live cells originated from both TE and ICM cells at day 5.

- The gastrula-like morphology was marked on the fourth day post-encapsulation especially in CCM/ALG/EnSCs/Mela group.

- E2 hormone levels in CCM/ALG/Mela group and CCM/ALG/EnSCs/Mela groups at day 4 post- encapsulation were averagely two folds more than those at day 2 ($P \leq 0.025$ and$P \leq 0.003$, respectively). Levels of hCG hormone in CCM/ALG/Mela group and CCM/ALG/EnSCs/Mela group at day 4 were 14.43 ($P \leq 0.024$) and 48.01 ($P \leq 0.004$) respectively.

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- The levels of the hCG hormones were significantly greater in CCM/ALG/EnSCs/Mela group in contrast to CCM/ALG/Mela culture system in the same day (P≤0.028).

Limitations, reasons for caution: More techniqual assessments like molecular analysis could be helpful to get more insights about molecular properties of developed embryos. However, problem with providing enough human embryos (no big sample size to analysis) caused to withdraw the molecular evaluation.

Wider implications of the findings: In vitro culture of human embryos would facilitate monitoring of human embryo development and consequently understanding the mechanisms such as molecular and hormonal ones, underlying human embryo growth.

Trial registration number: It was not a trial, it was a basic science research.

O-060 Cell allocation patterns during pre-implantation embryo development are not perturbed by blastomere removal in mouse and bovine embryos

L.P. Sepulveda-Rincon[1], P. Marsters[1], B.K. Campbell[1], N. Beaujean[2], W.E. Maalouf[1]

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Study question: Are embryo cell-allocation patterns disturbed by single blastomere removal at cleavage stage in mouse and bovine embryos?

Summary answer: Cell allocation patterns are not disturbed by embryo biopsy in mouse and bovine embryos. However, embryos following different cell allocation patterns present different compensatory mechanisms.

What is known already: It has been suggested that cell fate might be predisposed in early embryos. The first cleavage plane in mouse embryos has been related with the embryonic-abembryonic (Em-Ab) axis at blastocyst stage, hence the first cell lineage specification. Cell allocation patterns during pre-implantation embryo development have been related with further developmental potential. Thus, cell removal at cleavage stage might shift these cell allocation patterns; perturbing development.

Study design, size, duration: Two different species (mouse and bovine) were used during more than 5 repetitions of the experiment. Embryos were injected with a lipophilic tracer at the 2-cell stage and then divided into two groups: control and biopsied group. A single blastomere was removed around the 8-cell stage on the biopsied group. Embryos were cultured up to the blastocyst stage where cell allocation patterns were noted as well as total cell counts (TCC).

Participants/materials, setting, methods: A single blastomere of the 2-cell embryos was injected with Dil tracer. Within the biopsied groups a single blastomere was removed around the 8-cell stage. Blastocysts were classified into: orthogonal, if the borderline of two clusters of cells (stained and non-stained) was orthogonal ±30° to the Em-Ab axis; deviant, if the borderline was parallel ±30°; and random, if more than two clusters were observed and stained/non-stained cells were intermingled. TCC was performed by DAPI staining.

Main results and the role of chance: A total of 287 and 214 blastocysts were analysed on mouse control and biopsied group respectively, whereas 346 and 107 blastocysts for bovine embryos. Incidence of random cell allocation pattern was predominant in both species (>58%, $p < 0.05$). Whereas deviant and orthogonal pattern incidence was around 20% in both species. Incidence of cell allocation patterns was not disturbed by embryo biopsy ($p>0.05$). Nonetheless, TCC at blastocyst stage decreased significantly after biopsy in embryos following the deviant pattern in mouse (55 vs 42 cells respectively) and bovine (140 vs 95 cells respectively) embryos ($p < 0.05$). This tendency was not observed in orthogonal embryos (45 vs 41 cells and 91 vs 136 cells for mouse and bovine embryos respectively) or random embryos (47 vs 43 cells and 108 vs 90 cells for mouse and bovine embryos respectively). It is worth noting that deviant embryos in control groups present significantly higher TCC ($p < 0.05$) when compared with orthogonal or random embryos. In conclusion, cell allocation pattern incidence is comparable in mouse and bovine embryos, thus it might represent a conserved mechanism among mammals and they should be established before 8-cell stage. Also, deviant group might have a different coping mechanism(s) against cell removal at cleavage stage.

Limitations, reasons for caution: Even though the results suggest that incidence of cell allocation patterns might be a conserved mammalian process, this theory has not yet been studied in human embryos. Also, the mechanism(s) driving cell allocation patterns in mammalian embryos still remain to be discovered.

Wider implications of the findings: Embryos following the deviant cell allocation pattern might present different coping mechanisms against *in vitro* manipulations, which in turn might affect further embryo development. New embryo de-selection strategies could be developed in order to avoid deviant embryos, and therefore improve clinical success after ART.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 17: LIFE-STYLE AND PREPARATION FOR IVF

Monday 3 July 2017 Plenary 2 15:15–16:30

O-061 Effect of a lifestyle intervention in obese infertile women on cardiometabolic health and quality of life: results of a randomised controlled trial

V. Wekker[1], L. Van Dammen[2], A.M. Van Oers[3], M.A.Q. Mutsaerts[4], R.C. Painter[1], A.H. Zwinderman[5], H. Groen[2], C. Van de Beek[1], A.C. Muller Kobold[6], J.A. Land[3], W.K. Kuchenbecker[7], R. Van Golde[8], B.W.J. Mol[9], T.J. Roseboom[5], A. Hoek- on behalf of the LIFEstyle study group[3]

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[2]*University of Groningen- University Medical Centre Groningen, Department of Epidemiology, Groningen, The Netherlands*
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[8]*Maastricht University Medical Centre- Maastricht University, Department of gynaecology and obstetrics, Maastricht, The Netherlands*
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Study question: Does a lifestyle intervention prior to infertility treatment compared to prompt infertility treatment lead to better cardiometabolic health and quality of life in obese infertile women?

Summary answer: A lifestyle intervention among obese infertile women has immediate beneficial effects on cardiometabolic health and physical quality of life.

What is known already: The prevalence of obesity, an important cardiometabolic disease risk factor, is rising in women. Obesity reduces fertility, regardless of ovulatory status. Lifestyle improvements are the first step in the prevention and treatment of obesity. The success of improving lifestyle depends on factors like timing and motivation. Women are especially receptive to advice about lifestyle before and during pregnancy. Therefore, the prepregnancy period may be a window of opportunity to improve health and quality of life (QoL) of obese infertile women by means of a lifestyle intervention.

Study design, size, duration: The LIFEstyle study, a multicenter RCT was conducted between 2009 and 2012 in 23 medical centers in the Netherlands. 577 participants were randomised 1:1 to a 6 month lifestyle intervention prior

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to infertility treatment (intervention group; n = 290) or to prompt infertility treatment (control group; n = 287). Randomisation was stratified according to trial center and ovulation status with an online program. The intervention consisted of dietary counselling, increasing physical activity and an individualised behavioral modification plan.

Participants/materials, setting, methods: Participants were infertile women aged between 18 and 39 years with a Body Mass Index (BMI) of ≥ 29 kg/m^2. The goal of the intervention was 5–10% weight loss or a BMI < 29 kg/m^2. Cardiometabolic outcomes were measured by research nurses at randomisation, 3 and 6 months. QoL was assessed by the short form 36 questionnaire and was also measured at 12 months. Mixed effects regression models analyses were performed.

Main results and the role of chance: Based on the data at 3, 6 and 12 months, results are given as estimated mean differences between the intervention and control group. Weight (-3.1 kg 95% CI: -4.0 - -2.2 kg; P < .001), waist circumference (-2.4 cm 95% CI: -3.6 - -1.1 cm; P < .001), hip circumference (-3.0 95% CI: -4.2 - -1.9 cm; P < .001), BMI (-1.2 kg/m^2 95% CI: -1.5 - -0.8 kg/m^2; P < .001), systolic blood pressure (-2.8 mmHg 95% CI: -5.0 - -0.7 mmHg; P = .01) and HOMA-IR (-0.5 95% CI: -0.8 - -0.1; P = .01) were lower in the intervention group as compared to controls. Hs-CRP and lipids did not differ between groups. Based on the 2001 revised criteria of the National Cholesterol Education Programme ATP III, after the intervention, the odds ratio for metabolic syndrome (MetS) in the intervention group was 0.53 (95% CI: 0.33–0.85; P < .01) compared to controls. The lifestyle intervention led to a relative risk reduction of 29.4% for MetS. Physical QoL scores were higher in the lifestyle intervention group (2.2 95% CI: 0.9 – 3.5; P = .001) while mental QoL scores did not differ.

Limitations, reasons for caution: No power calculation was performed for the reported outcomes. However, based on comparable studies, the sample size was sufficient to find relevant effects. Prompt start of infertility treatment after randomisation in the control group could have affected cardiometabolic health. However, adjustment for receiving infertility treatment did not change the results.

Wider implications of the findings: A lifestyle intervention among obese infertile women has immediate beneficial effects on cardiometabolic health and physical quality of life. Optimizing preconceptional lifestyle could potentially lead to a healthier intrauterine environment. Based on the principles of fetal programming, improving lifestyle before conception might lead to improved long-term health of the offspring.

Trial registration number: Dutch Trial Register NTR1530.

O-062 Weight reduction intervention for obese infertile women prior to In vitro fertilisation; a randomised controlled trial

S. Einarsson[1,2], C. Bergh[3,4], B. Friberg[5], A. Pinborg[6], A. Klajnbard[7], P.O. Karlström[8], L. Kluge[4], I. Larsson[9], A. Loft[10], A.L. Mikkelsen-Englund[11], K. Stenlöf[12], A. Wistrand[13], A. Thurin-Kjellberg[1,4]

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[12]*Institute of Medicine- Sahlgrenska Academy- Gothenburg University, Department of Molecular and Clinical Medicine, Gothenburg, Sweden*
[13]*Örebro University Hospital, Reproductive medicine, Örebro, Sweden*

Study question: Does an intensive weight reduction program prior to IVF increase live birth rates for infertile obese women?

Summary answer: An intensive weight reduction program prior to IVF, resulted in a substantial weight loss, but did not improve live birth rates in obese women.

What is known already: Among obese women fertility and obstetric outcomes are influenced negatively with increased risk of miscarriage and a higher risk of maternal and neonatal complications. A recent large randomized controlled trial found no effect of life style intervention on live birth in infertile obese women.

Obese women performing in vitro fertilization (IVF) have lower live birth rate than normal weight women. Despite the fact that only a few small trials, not powered for pregnancy and live birth rates, have been performed it has often been suggested that weight reduction interventions should be considered for obese infertile women before IVF treatment.

Study design, size, duration: A prospective, multicenter, randomized controlled trial was performed between 2010 and 2016 in the Nordic countries. In total, 962 women were assessed for eligibility and 317 women were randomized. Computerised randomization with concealed allocation was performed in the proportions 1:1 to one of two groups; weight reduction intervention followed by IVF-treatment or to IVF-treatment only. One cycle per patient was included.

Participants/materials, setting, methods: Nine infertility clinics in Sweden, Denmark and Iceland participated. Women under 38 years of age planning for IVF, and having a body mass index (BMI) ≥30 and <35 kg/m^2 were randomized to two groups. An intervention group (160 patients) with weight reduction before IVF, starting with 12 weeks of a low calorie liquid formula diet (LCD) of 880 kcal/day and thereafter weight stabilization for 2–5 weeks, or a control group (157 patients) with IVF only.

Main results and the role of chance: In the full analysis set (FAS) the live birth rate was 29.6% (45/152) in the weight reduction and IVF group and 27.5% (42/153) in the IVF only group. The difference was not statistically significant (difference 2.2, 95% confidence interval 12.9 to -8.6, p = 0.77). The mean weight change was -9.44 (6.57) kg in the weight reduction and IVF group as compared to +1.19(1.95) kg in the IVF only group being highly significant (p < 0.0001). Significantly more live births were achieved through spontaneous pregnancies in the weight reduction and IVF group, 10.5% (16) as compared to 2.6% (4) (p = 0.009). Miscarriage rates and gonadotropin dose used for IVF stimulation did not differ between groups. In two subgroup analyses, comparing women with PCOS in the two randomized groups and women in the weight reduction group reaching BMI ≤25 kg/m^2 or reaching a weight loss of 5 BMI units to the IVF only group, no statistical differences in live birth rates between groups were found.

Limitations, reasons for caution: The study was not powered to detect small increase in live births by weight reduction and was not blinded for patients or physician. Further, the intervention group had longer time to achieve a spontaneous pregnancy, but was also slightly older than the control group at IVF.

Wider implications of the findings: The study does not support that obesity (BMI 30–35) in women has a detrimental effect on IVF outcome, nor does it show that an intensive weight reduction with LCD treatment negatively effects the results.

Trial registration number: ClinicalTrials.gov number, NCT01566929.

O-063 Impact of dramatic weight loss linked to bariatric surgery on ovarian response and pregnancy rates after in vitro fertilization (IVF): a case-control study

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[6]Centre Hospitalier Intercommunal Poissy Saint Germain, ART, Poissy, France

Study question: Is there any difference in IVF results in women having undergone bariatric surgery as compared to women of identical age and body mass index (BMI)?

Summary answer: Ovarian response to stimulation and pregnancy rates are comparable between the two groups, even after adjustment for confounding variables.

What is known already: Morbid obesity is associated with higher gonadotrophin consumption, lower embryo implantation, and higher miscarriage rates in IVF. Bariatric surgery leads to long term significant weight loss and improves spontaneous fertility and obstetrical and neonatal prognosis in obese women. So far, the few articles concerning IVF women after bariatric surgery included small cohorts and were unable to establish the surgery's effect on IVF outcome among infertile women. To our knowledge, no previous study has compared IVF results between women having undergone bariatric surgery to matched women of identical BMI.

Study design, size, duration: This case-control study was performed from retrospective analysis of three IVF centre databases. Data from 10,287 IVF/ICSI cycles between January 1st, 2012 and September 30th, 2016 were extracted and retrospectively analysed.

Participants/materials, setting, methods: All cases with previous history of bariatric surgery undergoing their first IVF cycle within three IVF centres were included and matched with two controls of similar BMI and age. Main outcome measures were cumulative pregnancy rates, average number of mature oocytes and embryos obtained in the first IVF cycle. Ovarian response was compared between groups for possible confounding variables. Univariate and multivariate conditional logistic regression analysis were carried out using R statistical software.

Main results and the role of chance: Data analysis included 249 women (83 cases, 166 controls) of similar BMI (28.60 ± 5.54 vs. 28.78 ± 4.52; p: NS) and age (33.08 ± 4.44 vs. 32.95 ± 4.36; p: NS). No significant difference in cumulative pregnancy rates per transfer was found between cases and controls (29.90% vs. 26.61%; p: NS), nor were differences observed in average number of mature oocytes (6.88 ± 4.85 vs 7.37 ± 4.85; p: NS) nor in average number of embryos obtained (4.43 ± 4.18 vs. 4.85 ± 3.79; p: NS). Among measured confounding variables, cases were observed to have significantly higher anti-müllerian hormone (AMH) levels (4.56 ± 5.38 vs. 3.29 ± 2.43; p = 0.02), antral follicle count (AFC) (17.06 ± 11.53 vs.13.9 ± 9.85; p = 0.001), and end-of-cycle estradiol (E2) levels (1662 ± 973 vs 1930 ± 1084; p = 0.00004) as compared to controls. Multivariate conditional logistic regression analysis found no significant difference in cumulative pregnancy rates between both groups after adjustment for AMH, AFC, E2, number of stimulation days, polycystic ovary syndrome status, and number of top embryos. Additionally, miscarriage rates were found to be 37.9% in cases and 25.0% in controls, but this difference was not significant (p = 0.31).

Limitations, reasons for caution: This is the largest study matching IVF patients having undergone bariatric surgery to patients of identical BMI and age; however, further analysis including cumulative live birth rates is underway. Cases included consulted for infertility, and results cannot be extended to all women candidates for bariatric surgery, regardless of fertility status.

Wider implications of the findings: Infertile obese women can be reassured as to effects of dramatic weight loss on IVF outcomes. Yet, the question of fertility preservation before bariatric surgery should be raised in light of negative ovarian reserve impacts due to the time delay needed to achieve weight stabilization before a pregnancy is allowed.

Trial registration number: not applicable.

O-064 A concise infertility work-up reduces pregnancy chances: a historical cohort study

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Study question: Is the outcome after a concise infertility work-up (IW) the same as after an extensive IW?

Summary answer: The ongoing pregnancy rate within a follow-up of one year after a concise IW is significantly lower than after an extensive IW.

What is known already: Based on cost-effectiveness studies, which have mainly focused on diagnosis, IWs have become less comprehensive. Many centers have even adopted a onestop approach to the IW.

Study design, size, duration: We performed a historically controlled cohort study. In 2012 and 2013 all new infertility couples (n = 795) underwent an extensive IW (Group A). In 2014 and 2015 (n = 752) all new couples underwent a concise IW (Group B). The follow-up period was one year for both groups.

Participants/materials, setting, methods: The concise IW was mainly based on history taking, a gynecological ultrasound and semen analysis. A hysterosalpingography (HSG) was only performed if tubal pathology was suspected or before starting therapy. Laparoscopy and hormonal tests were only performed if indicated. The extensive IW also included ultrasonongraphic cycle monitoring, timed postcoital testing, timed progesterone and chlamydia antibody titer. A HSG was done routinely. Ongoing pregnancy rates in both groups were calculated.

Main results and the role of chance: The descriptive data like age, duration of infertility, type of infertility, lifestyle habits in both groups were comparable. In Group A many more infertility investigations were done. HSG was performed less frequently in Group B (29,8% versus 41,6%) and at a later stage. The diagnosis cervical infertility was only made in group A (9,3%). The definition of unexplained infertility 23,6% versus 31,9% (P < 0,001), therefore, was not the same in both groups.

Couples were treated according to the diagnosis with either expectant management (Hunault prognostic score > 30% or ovulation disorders, IUI in natural (cervical factor) or stimulated cycles (Hunault <30%) or IVF/ICSI (tubal factor, advanced female age, severe male factor).

Ovulation induction was more often started in Group A (35,8% versus 31,6% p < 0.05). Expected management was less often started in Group A (18,9% versus 24,8% p < 0.01), because in Group A more couples conceived during the IW (17,5% versus 9,7% p < 0.001). A significantly higher ongoing pregnancy rate within a follow-up of one year was found in Group A (60,2% versus 49,3% in Group B (p < 0.001)).

Limitations, reasons for caution: This was a controlled cohort study, introduction of bias cannot be ruled out. The drop-out rate differed 2,5% (A: 1,5 versus B: 5%) but this was not sufficient to explain the differences in pregnancy rate.

Wider implications of the findings: (Re-)introduction of an extensive IW should be considered as it may lead to higher ongoing pregnancy rates within a year, as the therapeutical effects of HSG and postcoital test have to be taken into account. This finding should be verified in an RCT.

Trial registration number: none.

O-065 Maternal obesity programs transgenerational mitochondrial dysfunction via the original, maternal oocytes, not due to exposure during gestation

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Study question: Are the effects of maternal high fat/high sucrose diet on offspring predisposition to cardiometabolic diseases due to oocyte specific changes or exposure during gestation?

Summary answer: Mitochondrial metabolic changes in the F0 oocytes are carried over to the next generations as evident by IVF and embryo transfer into control females.

What is known already: Maternal obesity impairs offspring health, but the responsible mechanisms are not fully established. Previously, we have shown that feeding a high fat/high sugar diet to C57Bl/6 mice results in dysfunctional and misshapen oocyte mitochondria which appear for 3 generations of offspring conceived via normal mating. To confirm that this was an oocyte event and not due to the diet throughout gestation, we retrieved the oocytes from 10 week old female mice fed the diet for 6 weeks, performed IVF with sperm from males fed a regular diet and transferred the blastocysts into control ICR females on a regular chow diet.

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Study design, size, duration: This was an observational, basic science experiment with inbred mice.

Participants/materials, setting, methods: Mice were placed on a normal chow diet (22% fat, 3% sucrose) or a high fat/high sucrose diet (59% fat, 23% sucrose) for 6 weeks after which time the oocytes were retrieved, fertilized and the embryos were transferred into control mice on a normal chow diet. At least 5 mice per group were used for the experiments. All experiments were repeated 3 times.The skeletal muscle and cardiac muscles were removed and subject to electron microcopy.

Main results and the role of chance: Remarkably the same abnormal pattern of mitochondrial dysfunction and misshapen mitochondria was seen in the offspring from HF/HS moms that underwent IVF and embryo transfer into mice on a regular diet, as had been seen in the offspring of mice fed the HF/HS diet from prior to and throughout gestation. In both cardiac and skeletal muscle which have high mitochondrial demands, mitochondria with severely disarrayed cristae, bloated abnormal shapes and decreased mitochondrial matrix density were seen in the IVF pups from HF/HS moms as compared to control moms. Mitochondrial respiration in isolated cardiac and skeletal was abnormal in the same group of mice using an Oxygraph 2 K (OROBOROS Instruments). ECHO of the hearts of the IVF pups from the HF/HS moms showed fractional shortening of the muscle of the left ventricle indicating early heart failure. None of these changes were seen the IVF pups from oocytes of mothers fed the normal chow diet.

Limitations, reasons for caution: This is a mouse study, not a human study but the epidemiological data strongly suggests that children of obese mothers are predisposed to developing cardiometabolic diseases and obesity much earlier than the children from lean mothers.

Wider implications of the findings: Our results suggest that maternal programming of metabolic disease can be passed through the maternal oocyte and that the transfer of aberrant oocyte mitochondria to subsequent generations may contribute to the increased risk for developing obesity and cardiometabolic disease in their offspring.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 18: UPDATES ON PCOS

Monday 3 July 2017 Room A 15:15–16:30

O-066 Follicular ADAMTS-1 and aggrecan in polycystic ovary syndrome (PCOS)

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Study question: Do follicular ADAMTS-1 and aggrecan have any possible role in anovulation and predictor effect on in vitro fertilization (IVF) success rate in women with PCOS?

Summary answer: This study revealed the role of ADAMTS-1 and aggrecan in the etiopathogenesis of PCOS and the predictive effect of ADAMTS-1 level on implantation.

What is known already: Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility. Proteoglycan degradation by a disintegrin- like and metalloproteinase with thrombospondin type motifs (ADAMTS-1) is essential for ovulation and fertilization. Matrix metalloproteinases are involved in follicular fluid (FF) and the cumulus oocyte complex (COC) and regulate extracellular matrix (ECM) remodeling. Proteoglycans, the basic components of the ECM and FF proteoglycans—such as versican, aggrecan- and hyaluronan are important during folliculogenesis. An essential role of versican cleavage by ADAMTS-1 during ovulation, as well as abnormal morphogenesis of versican in ADAMTS-1 null mice, has been demonstrated.

Study design, size, duration: The present study was a prospective cross-sectional clinical trial performed in the assisted reproduction clinic of a tertiary center between August, 2016 and December, 2016. A total of 43 patients undergoing IVF treatment—21 diagnosed as PCOS according to the Rotterdam consensus (*the PCOS group*) and 22 diagnosed as normal ovulatory women (*the control group*)—were recruited.

Participants/materials, setting, methods: The control group received IVF for tubal factor and/or male infertility, except azoospermia and severe oligoasthenospermia. Patients were prepared using antagonist protocol. Oocytes were considered fertilized when two pronuclei were observed. The fertilization rate (FR) was calculated as the number of fertilized oocytes divided by the number of metaphase (MII) oocytes. Implantation was determined by serum HCG levels. Aggrecan and ADAMTS-1 levels were analyzed using human aggrecan and ADAMTS-1 ELISA kit, respectively.

Main results and the role of chance: Age, body mass index, hormone levels at the 3rd day of the cycle (except estradiol) and the percentage of MII were distributed homogeneously. E_2 levels, oocyte, MII, and MI numbers were significantly higher in the PCOS group ($p = 0.003$, $P < 0.001$, $p = 0.003$ and $p = 0.011$, respectively). ADAMTS-1 levels were higher in the PCOS group (33.84 ± 17.7 ng/ml) than in controls (23.58 ± 5.67 ng/ml, $p = 0.013$). Aggrecan levels were higher in women with PCOS (8.05 ± 4.78 ng/ml) than in controls (3.63 ± 2.18 ng/ml, $p < 0.001$). For the prediction of the diagnosis of PCOS, when the cut-off level of aggrecan was taken as 4.10 ng/ml, the sensitivity was 72.7%, and the specificity was 76.1% (p = 0.001). For ADAMTS-1, when the cut-off level was taken as 27.50 ng/ml, the sensitivity was 72.2%, and the specificity was 68.0% (p = 0.013). Our study failed to identify any predictor effect of aggrecan or ADAMTS-1 on FR in the PCOS group or among all patients (p>0.5). When implantation was evaluated, there was a statistically significant positive predictor effect of ADAMTS-1 ($p = 0.036$, $\beta = 0,331$) levels. However, there was no statistically significant effect of aggrecan on implantation. Age, BMI, and the percentage of MII were not predictive of implantation.

Limitations, reasons for caution: The lack of evaluation of oocyte quality, which is closely associated with fertilization and the small sample size are the limitations of this study.

Wider implications of the findings: The increase of ADAMTS-1 and aggrecan in the FF could be a pathogenic mechanism of anovulation in PCOS patients. Follicular ADAMTS-1 is a potential predictor marker for implantation capacity. Further studies are needed to investigate the underlying causes of anovulation and to improve IVF success rates in PCOS patients.

Trial registration number: none.

O-067 Randomized controlled trial of combined lifestyle and herbal medicine in women with polycystic ovary syndrome

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Study question: Does combined lifestyle intervention and a novel herbal formulation improve oligomenorrhoea compared to lifestyle alone in overweight community based women with polycystic ovary syndrome (PCOS)?

Summary answer: At three months, women in the combination group recorded a reduction in oligomenorrhoea of 32.9% (95% CI,23.3–42.6, p < 0.01) compared to controls, estimated as a moderate-large effect.

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What is known already: Lifestyle intervention is first line treatment for overweight women with polycystic ovary syndrome (PCOS) and may regulate menstruation, reduce hyperandrogenism, treat hyperinsulinaemia and improve quality of life. However, the strength of evidence for lifestyle is limited by high attrition in RCTs and clinical uptake remains impeded by the lack of evidence for optimal dietary and exercise practices. In addition, physical and psycho social barriers are commonly observed in overweight women, particularly those with established obesity. Many women use additional complementary medicine including herbal medicines, however despite potential positive endocrine effects in PCOS, there is no evidence of effectiveness or safety.

Study design, size, duration: This study examined the relative effectiveness of combined lifestyle intervention and herbal medicine compared to lifestyle alone for oligomenorrhoea in overweight women with PCOS in a prospective, randomized controlled trial conducted between in August 2012 to June 2014. We consented and randomized 122 women to three months of lifestyle plus herbal medicine (n = 60) or lifestyle intervention alone (n = 62).

Participants/materials, setting, methods: The interventions were delivered in urban and regional communities in Australia. Participants were overweight women with PCOS (Rotterdam criteria), not taking contraceptive pills or antidepressants. Energy deficit, personalized lifestyle plans were revised fortnightly. The herbs included daily dosed Cinnamomum verum, Glycyrrhiza glabra, Hypericum perforatum, and Paeonia lactiflora with Tribulus terrestris on menstrual cycle days 5–14. The primary outcome was oligomenorrhoea. Secondary outcomes were serum hormones, anthropometry, quality of life, psychology, pregnancy, birth and blood pressure.

Main results and the role of chance: At three months the mean menstrual cycle length was 43 days (95% CI, 21 to 65, $p < 0.001$) lower for women in the combination group compared to those in the lifestyle only group.Secondary outcomes were significantly improved for women taking combined herbal medicine and lifestyle compared to controls for BMI (MD -1.0 95% CI -1.6 to -0.5, $p < 0.01$), increased oestradiol (MD 68.9 pmol/L, 95% CI 5.5 to 132.3, $p = 0.03$); lowered LH (MD -1.82IU/L, 95% CI -3.5 to -0.1, $p = 0.04$), fasting insulin (MD -5.9mU/l, 95% CI -10.9 to -1.0, $p = 0.02$; systolic (MD -3.6 mmHg, 95%CI-6.3 to -0.9, $p = 0.01$) and diastolic (-5.1 mmHg, 95%CI -7.8 to - 2.4, $p < 0.01$) blood pressure; PCOSQ score (MD -31.1, 95% CI -41.4 to -20.7, $p < 0.01$), depression (-4.3, (95% CI -5.9 to -2.7, $p < 0.01$), anxiety (-4.0, 95% CI -5.4 to -2.6, $p < 0.01$) and stress (-5.0, 95% CI -6.5 to -3.5, $p < 0.01$) scores and increased conception rates (RR 3.9, 95% CI 1.1 to 13.1, $p = 0.01$). The residual effect of loss of body weight on the primary outcome (number of days in the menstrual cycle) after controlling for baseline menstrual variation was not significant ($p = 0.07$). Two women in in the herbal medicine group were withdrawn due to non-serious adverse effects.

Limitations, reasons for caution: The lack of a placebo group prevents identification of the active component of this herbal and lifestyle intervention that has generated these outcomes. The lack of blinding could have influenced the estimated treatment effect size and confounded the outcomes particularly for subjective outcomes such as self-reported QoL, psychology and compliance.

Wider implications of the findings: In light of the limitations for lifestyle intervention for overweight women with PCOS, changes demonstrated in this study, with both specific gynaecological, physical and mental health improvements are an important finding.There were no serious adverse events during the trial, and non-serious adverse effects were fewer compared to pharmaceutical interventions.

Trial registration number: Australia and New Zealand Clinical Trial Registry (ANZCTR) 126 12000 122 853.

O-068 Comparative effectiveness of 9 ovulation-induction therapies on patients with clomiphene citrate-resistant polycystic ovary syndrome: a network meta- analysis

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Study question: How are the comparative efficacies of ovulation-induction treatments in clomiphene citrate-resistance (CCR) PCOS and which is the most efficacious one?

Summary answer: The co-treatment of metformin with letrozole (metformin+letrozole) is proved to be the most efficacious therapy among the included treatments.

What is known already: Between 15 and 40% of PCOS patients are termed as CCR because of their failure to ovulate with CC. Gonadotropins, including follitropin (FSH) and human menopausal gonadotropin (hMG), and laparoscopic ovarian diathermy (LOD) are recommended as efficacious options for CCR PCOS. Apart from that, letrozole, metformin, metformin+letrozole and metformin+CC are reported to be efficacious on ovulation induction in PCOS who are CCR. However, there has been no network meta-analysis to compare and rank the above therapies.

Study design, size, duration: It is a network meta-analysis. The Cochrane Library, PubMed, and EMBASE was searched to identify randomized clinical studies for the treatment of clomiphene citrate-resistance PCOS. After electronic and manual searching and screening, we ultimately included 26 randomized clinical trials including 2722 participants and investigating 9 types of therapies: CC, metformin, letrozole, FSH, hMG, unilateral LOD (ULOD), bilateral LOD (BLOD), metformin+letrozole, and metformin+CC. The treatment duration of included trials ranged between 3 to 6 months.

Participants/materials, setting, methods: The network meta-analysis was performed using the Markov Chains Monte Carlo method based on the Bayesian framework and using the non-programming software of the Aggregate Data Drug Information System version 1.16.7. The endpoints we focused on included reproductive outcomes (pregnancy rates per intention to treat (ITT), live birth rates per ITT, abortion rates per pregnancy, ovulation rates per cycle, and multiple pregnancy rates per pregnancy) and adverse events (side effects and the occurrence of OHSS).

Main results and the role of chance: The FSH and metformin+letrozole therapies were all identified as more efficacious than CC in improving pregnancy rates and live birth rates. The pregnancy rates in the hMG groups were significantly higher than those of the BLOD, ULOD and CC groups. The ovulation rates were significantly higher in the metformin+letrozole groups than BLOD, CC, letrozole, metformin and metformin+CC groups. The abortion rates in the metformin+letrozole groups were significantly lower than metformin+CC groups. The occurrence of OHSS was mainly observed during gonadotropins therapies. Based on the ranking probability results, the hMG and metformin+letrozole therapies had the highest probabilities of ranking the first in the comparisons of pregnancy rates (0.71, 0.17, respectively) and live birth rates (0.26, 0.64, respectively) and the highest probability of ranking the last in the comparisons of abortion rates (0.27, 0.42, respectively). The metformin +letrozole and FSH therapies had the highest probability of ranking first in the comparisons of ovulation rates per cycle (0.89, 0.1, respectively). In conclusion, gonadotropins (FSH, hMG) and metformin+letrozole are the most effective therapies with regard to reproductive outcomes.

Limitations, reasons for caution: The majority of the trials were of low to moderate quality. The exclusion of conference abstracts and inclusion of only English articles in this analysis might account for the observed publication bias. Conclusions should be strengthened by large multicenter clinical trials under the restriction of specific PCOS phenotypes.

Wider implications of the findings: It is the first time that the efficacy of metformin+letrozole on ovulation induction is verified. The treatment of metformin+letrozole is a reasonable choice for CCR PCOS before implementing IVF.

Trial registration number: N/A.

O-069 Factors Associated with Gestational Weight Gain and Birth Weight in Women with PCOS: A Controlled Study

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Study question: Is there an association of pre-gestational obesity and gestational weight gain(GWG) with birth weight and what factors predict GWG in an infertile(PCOS vs. Unexplained) population?

Summary answer: Matched for weight category, only normal/overweight (and not obese) PCOS women experienced increased rates of GWG compared to controls, without differences in mean birth weight.

What is known already: Women with PCOS have more perinatal complications, including abnormal birth weight than other women, though it is difficult to separate out the roles of subfertility per se, pre-existing obesity and excessive GWG. Many infertility studies fail to capture pregnancy outcomes, both maternal and fetal, up to birth, so there are few data in this area. Similarly, GWG studies often initiate in the late first/early second trimester and therefore do not capture all GWG from conception onwards.

Study design, size, duration: Case/Control study of pregnancy and singleton live births in Polycystic Ovary Syndrome(PCOS) women and Controls (unexplained infertility) from two large multicenter randomized controlled trials (RCTs), the Pregnancy in Polycystic Ovary Syndrome II (PPCOS II) trial where patients conceived with either letrozole or clomiphene and the Assessment of Multiple Ovarian Gestations from Ovarian Stimulation (AMIGOS) trial where patients conceived with either letrozole, clomiphene or gonadotropin and intrauterine insemination.

Participants/materials, setting, methods: Singleton pregnancies with live birth(>20 weeks) were compared(PCOS: N = 164, Controls: N = 176) from 14 participating academic health centers. Data were abstracted from clinical trial records from baseline, through participation and conception, and review of obstetric records of study participants. We compared groups(PCOS/Controls) using the Institute of Medicine BMI categories(normal, overweight, and obese) given varying recommendations for GWG by BMI group, modeled GWG by study and BMI group, and developed predictive models for GWG by study group.

Main results and the role of chance: From pre-conception baseline, normal weight(BMI<25) PCOS women gained 2.3lbs more during the first trimester(95%CI 0.3, 4.2, P = 0.02) and by the end of the second trimester gained a total of 4.2lbs more than Controls(95% CI 0.7, 7.7, P = 0.02). Overweight PCOS women(25 < =BMI<30) gained significantly more weight only by the end of the second trimester(5.2lbs, 95%CI 0.2, 10.2, P = 0.04) and obese PCOS women(BMI≥30) had similar weight gains as Controls throughout pregnancy. Mean birthweights were similar between PCOS and Controls for each weight group. The prevalence of small, appropriate, and large for gestational age babies in PCOS women(4.9%, 80.3% and 14.8%, respectively) was not significantly different than Controls(10.3%, 75.9% and 13.8%, respectively) (P = 0.21). Examining baseline GWG factors using a multivariate logistic regression model pre-pregnancy weight was inversely and significantly predictive for GWG in both PCOS(each 1 unit BMI increase correlated with a -0.54 lb GWG, 95% CI -0.83, -0.28 lb, P < 0.001) and Controls(each 1 unit BMI increase associated with a -0.57 lb GWG, 95% CI -0.95, -0.18 lb, P = 0.004). In Controls for every 1 ng/dL increase in baseline testosterone levels, there was a -0.13 lb GWG decrease(95% CI -0.25, -0.01, P = 0.03), with no additional significant predictors for PCOS women, although insulin and AMH levels approached significance.

Limitations, reasons for caution: We examined only singleton and not multiple pregnancies. These data are from prospective RCTs of patients treated for infertility and may not be generalizable to other obstetric populations. There were more missing data from the 3rd trimester due to early delivery or incomplete office obstetric records.

Wider implications of the findings: Excessive GWG in normal/overweight women with PCOS may protect against small for gestation age(SGA) without an increase in large for gestational age(LGA) babies. Increasing BMI is associated with less GWG independent of diagnosis, therefore pre-existing obesity should be incorporated into studies of pregnancy outcomes in women with PCOS.

Trial registration number: Clinicaltrials.gov: NCT00719186(PPCOS II) and NCT01044862(AMIGOS).

O-070 Healthcare providers' knowledge, diagnosis and management of polycystic ovary syndrome (PCOS) in Europe, North America and internationally

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Study question: What are the key evidence-practice gaps in PCOS care and do these vary across world regions and between disciplines?

Summary answer: These findings suggest significant PCOS evidence-practice gaps that differ in North America, Europe and other world regions, and between gynaecologists and endocrinologists.

What is known already: Polycystic ovary syndrome (PCOS) affects 6–21% of reproductive-aged women, depending on diagnostic criteria and population studied. Women around the world report suboptimal diagnosis experiences and dissatisfaction with care and they desire high-quality information from their doctors about the full range of PCOS features. Multidisciplinary care for PCOS is recommended yet little is known about the knowledge and practices of different medical professions around the world.

With development of the first international evidence-based guideline for PCOS underway, it is timely to investigate health professionals' knowledge and practices regarding PCOS to identify evidence practice gaps and guide translation activities.

Study design, size, duration: An online, anonymous questionnaire completed by 1654 clinicians involved in PCOS care in North America (39%), Europe (43%) and other world regions (18%). Respondents were recruited through professional societies, including ESHRE, in 2015-2016.

Participants/materials, setting, methods: Almost all clinicians (92%) were obstetrician/gynaecologists (60%), reproductive endocrinologists (27%) or medical endocrinologists (5.4%). Approximately half the clinicians reported seeing over 50 women with PCOS in the last year (52%). Multivariable logistic regression analyses generated adjusted odds ratios (OR) and 95% confidence intervals (CI) for associations between clinicians' knowledge or practices regarding PCOS, and world region of residence [North America (reference category), Europe, other] and profession [gynaecologist (reference category), reproductive endocrinologist, medical endocrinologist].

Main results and the role of chance: Generally, the reproductive features of PCOS were well recognised (e.g. irregular menses=98%), followed by metabolic features (e.g. increased risk of diabetes=90%), but psychosocial features were under-recognised (e.g. depression = 49%). European clinicians were less likely to be aware of the broader features than North American clinicians (e.g. anxiety OR:0.7, 95%CI:0.5–0.9, p = 0.003), with endocrinologists reporting more awareness than gynaecologists (e.g. anxiety OR for reproductive endocrinologists:1.8, 95%CI:1.4–2.2, p < 0.001; anxiety OR for medical endocrinologists:2.0, 95%CI:1.2–3.2, p = 0.004).

The internationally-accepted Rotterdam/ESHRE diagnostic criteria were most commonly used (65%), although 17% reported not knowing what criteria they use. European clinicians were more likely to use Rotterdam criteria than North American clinicians (OR:4.0 95%CI:3.1–5.2, p < 0.001). Reproductive, but not medical, endocrinologists were more likely to use these criteria than gynaecologists (OR:3.1, 95%CI:2.3–4.1, p < 0.001).

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The non-fertility treatments most commonly prescribed were oral contraceptives (76%) and lifestyle management (73%). Compared to the reference categories, European clinicians were less likely to prescribe lifestyle management (OR:0.7, 95%CI:0.6–0.9, p = 0.009) and endocrinologists were more likely (OR for reproductive endocrinologists:2.1, 95%CI:1.6–2.8, p < 0.001; OR for medical endocrinologists:3.1, 95%CI:1.7–5.7, p < 0.001).

Lifestyle management (59%) and clomiphene citrate (45%) were most commonly prescribed for fertility. Reproductive, but not medical, endocrinologists were more likely to prescribe lifestyle management than gynaecologists (OR:1.6, 95%CI:1.2–2.0, p < 0.001).

Limitations, reasons for caution: Selection bias is possible as clinicians who are more confident in their knowledge of PCOS, or who see more women with PCOS, may have been more likely to complete the survey. The findings may also be subject to recall bias as they rely on self-report rather than direct observation.

Wider implications of the findings: Variation in clinical care may be reduced by increasing clinician awareness of the broader features of PCOS and increasing the application of accepted diagnostic criteria. These findings have informed how the translation program of the first international evidence-based PCOS guideline should be tailored to different locations and professions.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 19: CRYOPRESERVATION OF OOCYTES AND EMBRYOS

Monday 3 July 2017 Room B 15:15–16:30

O-071 Laser assisted hatching before embryo transfer improves the clinical outcome in cases with vitrified oocytes from an egg donor cryobank: a prospective, control, randomized study

A. Papatheodorou[1], Y. Panagiotidis[1], P. Vanderzwalmen[2], S. Petousis[1], M. Goudakou[1], T. Pavlidou[1], A. Gountis[1], K. Ravanos[1], N. Prapas[1], Y. Prapas[3]

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[3]Institut Médical de Champel, IVF, Geneve, Switzerland

Study question: Is beneficial the performance of laser assisted hatching prior transfer in cases with embryos deriving from vitrified/ warmed oocytes in an oocyte donation program?

Summary answer: Clinical results are significantly higher when laser assisted hatching is being performed before transfer in embryos which derive from donated vitrified/ warmed oocytes.

What is known already: Lately, studies show very promising results after vitrification/warming of donated oocytes. However, a recent review on oocyte vitrification (Potdar et al, 2014) shows great heterogeneity in the reported clinical results after oocyte vitrification/warming and thus highlights that there is space for improving the clinical outcome after the use of donated vitrified oocytes. It is hypothesized that oocyte vitrification/warming procedure induces zona pellucida hardening which eventually will result to embryo hatching failure, compromising the implantation process and the clinical result. There are not enough studies which evaluate the clinical impact of assisted hatching in human embryos deriving from donated vitrified oocytes.

Study design, size, duration: A control randomized study was performed from February 2015 to June 2016. Based on a pilot study that showed 23% higher pregnancy rate (51%vs74%) when laser hatching was used, a power analysis was performed in order to determine that 176 cases were needed in order to detect the significance of laser assisting hatching on clinical outcome. A randomization list was used in order to allocate the recruited cases in group1 or group2.

Participants/materials, setting, methods: Oocyte donation cases that used vitrified oocytes from IAKENTRO egg cryobank were included in the study. Oocytes were vitrified using a closed system vitrification. After warming, oocytes were fertilized and cultured to day 5. All transfers performed on day 5. Two groups were formed. Group 1 embryos were transferred without assisted hatching. Group 2 embryos were hatched with the use of laser pulses two hours before transfer. Eighty eight cases were included in each group.

Main results and the role of chance: Analyzing the results of our study we showed that the mean number of embryos between Group 1 (no laser) and Group 2 (laser) was similar (1.88 ± 0.31 vs 1.85 ± 0.35, p = 0.69). The pregnancy rate was significantly higher in Group 2 (52.3% vs. 68.2%, p=.031, 95% CI: 1.06–3.61, 1.95-fold increase when laser applied, number of patients need to treat:6,28). Clinical pregnancy rate was significantly higher in Group 2 (44.3% vs. 66.3%,p=.01, 95% CI: 1.20–4.02, 2.2-fold increase when laser applied, number of patients need to treat:4,54). Accordingly, in group 2 cases we observed significantly higher ongoing pregnancy rate (37.5% vs. 59.1%, p=.004, 95% CI: 1.31–4.41, 2.4-fold increase when laser applied, number of patients need to treat:4,62) and higher delivery rate (37.5% vs. 59.1%, p=.004, 95% CI: 1.31–4.41, 2.4-fold increase when laser applied, number of patients need to treat:4,62). The mean number of gestational sacs observed in group1 was statistically lower than the one observed in group2 (0.57 ± 0.72 vs 0.97 ± 0.84, p = 0.003). Therefore the implantation rate was significantly higher in the laser group (0.31 ± 0.37 vs 0.52 ± 0.44, p = 0.0003, 95% CI: 0.351 to 0.478).

Limitations, reasons for caution: In power analysis, although it was based on a pilot study, we used a relatively big difference (23%) between control and intervention group in order to determine the sample size.

This study includes only oocyte donation cases. Our findings should not be extrapolated to any other female group.

Wider implications of the findings: Laser assisted hatching of embryos deriving from vitrified oocytes, significantly improves the clinical outcome and therefore is highly recommended when oocytes from egg donor cryobanks are used. This study shows that oocyte vitrification/warming procedure affects the implantation capacity of the resulting embryo probably due to hardening of the zona pellucida.

Trial registration number: ISRCTN21836222.

O-072 Laser-assisted hatching improves clinical outcomes of top grade, vitrified–warmed blastocysts developed in high prognosis oocyte donation cycles: a prospective randomized study

Y. Panagiotidis[1], A. Papatheodorou[1], S. Petousis[1], M. Goudakou[1], D. Pavlidou[1], A. Gkountis[1], K. Ravanos[1], P. Vanderzwalmen[2], N. Prapas[1], Y. Prapas[1,3]

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Study question: How does partial zona-pellucida (ZP) opening by laser-assisted hatching (LAH) affects the implantation potential of top quality vitrified–warmed blastocysts?

Summary answer: The implantation rate of vitrified-warmed top grade blastocysts is significantly increased if they undergo post warming a partial (15%-20%) ZP opening.

What is known already: Embryos undergo ZP hardening due to both prolonged in vitro culture, as well as a consequence of cryopreservation. The inefficiency of blastocysts to hatch from the ZP post-warming, within the optimal window of endometrial receptivity, may lead to low implantation rates. It is accepted that LAH generally improves the implantation potential of lower quality embryos when transferred in fresh or cryopreserved cycles or when it is applied in cases with poor prognosis, repeated implantation failures and in older women. Little information exists regarding the role of LAH in vitrified–warmed transfer (FET) of top quality blastocysts in high prognosis cycles.

Study design, size, duration: A prospective randomized study, performed in a private IVF clinic from September 2015 to August 2016, including 365 FETs of top quality blastocysts, developed in non-synchronized donor/recipient oocyte donation cycles. Embryos of each FET were randomized according to a computer-generated randomization list to either a group that had 15–20% of the ZP ablated using LAH (Group 1, n = 186) or no LAH (Group 2, n = 177). The clinicians and the patients were blinded to the assigned group.

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Participants/materials, setting, methods: Embryos were cultured in single step culture medium and the developed blastocysts were vitrified using a closed vitrification protocol. If at least 2 top quality blastocysts (2BB and higher) were not developed, cycle was reinitiated. During warming, while blastocysts were still collapsed in the final washing step, LAH was applied in allocated embryos. A 20% opening was created as distant as possible from the trophectoderm cells. All blastocysts were cultured for 3 hours until ET.

Main results and the role of chance: Both groups had equal demographic and clinical characteristics (donor age, sperm quality, number of fertilizing oocytes, 2pn and vitrified blastocysts).

There was no difference between the numbers of warmed (1.90 ± 0.3 vs. 1.93 ± 0.3, p=.31) and transferred (1.83 ± 0.4 vs. 1.79 ± 0.4, p=.30) blastocysts between the two groups (LAH vs. no-LAH) respectively. Embryo implantation rate was set as primary outcome. LAH significantly increases the implantation potential of top quality blastocysts (LAH vs. no-LAH), 44.3% (151/341) vs. 33.1 % (105/317), P=.003, 95% CI: 1.17–2.2, 1.6- fold increase when LAH was applied. Similarly, β-hCG positive rates, 71.5% (133/186) vs. 57.6% (102/177), p=.006 and clinical pregnancy rates 61.2% (114/186) vs. 45.2% (80/177), p=.002 were significantly higher in LAH vs. no-LAH group, respectively. Minimizing the selection threshold to those FETs with absolutely top quality, 4AA blastocysts, produces an even higher positive impact of LAH on all the above clinical parameters.

Limitations, reasons for caution: LAH is suggested to be beneficial in low prognosis cycles. This study included FETs of good prognosis oocyte donation cycles. Live birth rate was not recorded. Moreover, findings have not been verified in fresh or homologous cycles. It is premature to recommend AH in all patients.

Wider implications of the findings: We postulate that the LAH embryos save energy to complete the hatching process, avoiding multiple expansions, ensuring that the energy stores are not depleted prior to implantation. Additionally, embryos that hatch on time could achieve better synchronization to the optimal time range of endometrial receptivity.

Trial registration number: Pending.

O-073 Clinical outcomes of blastocysts vitrified-warmed using a new semi-automated vitrification system are comparable to the gold standard open vitrification method

U. Tumurbaatar[1], N. Hobson[1], T. Roy[2], R. Woolcott[2], M. Bowman[3], S. Arthur[4], S. Brandi[2]

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Study question: Are clinical outcomes from the transfer of human blastocysts vitrified using the semi-automated closed Gavi™ system equivalent to that of the manual open Cryotop™ system?

Summary answer: Clinical outcomes of blastocysts vitrified using the Gavi system are comparable to the Cryotop system including embryo recovery, embryo survival and clinical pregnancy.

What is known already: Vitrification of embryos is an essential component of an effective assisted conception program. When successfully executed, it provides excellent embryo survival and pregnancy rates, with the gold-standard method arguably being the open Cryotop system. However, vitrification is a manual, high-skill and labor intensive procedure that is difficult to standardize, and results can vary greatly between embryologists and clinics. To address these issues, the Gavi system was designed to mechanically automate key steps in vitrification. In vitro studies using mouse and human research blastocysts have shown equivalent results to the Cryotop system (Roy et al. Hum Reprod, 2014; 29: 2431–8).

Study design, size, duration: This is an ongoing study in which preimplantation genetic screening (PGS) patients were randomly allocated to have either their best embryo vitrified using the Gavi system and their second best using the Cryotop system, or vice versa (no additional embryos included in the study). This study, which commenced in August 2015, assessed outcomes of single embryo transfers of Gavi vitrified-warmed blastocysts in comparison to Cryotop controls. Results presented are interim results.

Participants/materials, setting, methods: This study was performed at the private assisted reproductive technology (ART) clinic Genea (Sydney, Australia), which has proven expertise in Cryotop vitrification (Roy et al. Fert Steril, 2014; 101: 1294–301). Participants included infertile couples undergoing assisted conception and PGS with at least one blastocyst for vitrification. Vitrification was performed using either Gavi, as described by the manufacturer Genea Biomedx, or the open Cryotop protocol as described previously (Roy et al. Fert Steril, 2014; 101: 1294–301).

Main results and the role of chance: 665 blastocysts have been vitrified using the Gavi system and 669 using the Cryotop system. Of these, 170 euploid embryos vitrified using the Gavi system and 199 using the Cryotop system have been warmed, resulting in 168 embryos from the Gavi and 196 embryos from the Cryotop being transferred with a single embryo transfer policy. The patient and embryo demographics were similar for Gavi and Cryotop vitrified-warmed transfers; the average maternal age was 36.2 and 36.3 years, respectively, and the proportion of good quality embryos was 95.8% and 94.9%, respectively (ICM and TE grade excellent or good). Recovery rate of embryos from the Gavi system was 99.4% and the Cryotop system 100% (p = 0.28). Of those embryos that were recovered survival was 99.4% and 98.5% respectively (p = 0.39). Clinical use of Gavi vitrified-warmed embryos resulted in 65.5% biochemical pregnancy rate as compared with 66.8% for Cryotop vitrified-warmed embryos (p = 0.78). The fetal heart pregnancy rate per embryo transfer (presence of a fetal heart at 7 weeks by ultrasound) was 57.1% from Gavi system pregnancies as compared with 59.2% of Cryotop system pregnancies (p = 0.69). There are now 26 live births from Gavi vitrified-warmed blastocysts.

Limitations, reasons for caution: Clinical outcomes of Gavi-vitrified cleavage stage embryos or oocytes were not assessed.

Wider implications of the findings: The Gavi system has the potential to revolutionize embryo vitrification; standardizing the process, increasing laboratory efficiencies and improving clinical outcomes for laboratories struggling with cryopreservation. This study demonstrates it is possible to semi-automate complicated ART procedures and opens up the possibility for further improvements in efficiencies and clinical outcomes.

Trial registration number: Not applicable.

O-074 Impact of post-warming culture duration on clinical outcomes of vitrified good-quality blastocyst transfers: a prospective randomized study

C. Herbemont[1], S. Chekroune[2], S. Bonan[2], N. Sermondade[2], J. Boujenah[3], I. Cedrin-Durnerin[3], A. Seroka[3], C. Vinolas[3], C. Sonigo[3], M. Grynberg[3], C. Sifer[2]

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Study question: Does post-warming culture duration (1 hour versus (vs.) 18 hours) influence implantation rates (IR) of good-quality blastocysts in a good-prognosis population?

Summary answer: IR were similar whatever the duration of post-warming culture of good-quality blastocysts. Thawing blastocysts on the day before could enable an adaptation of transfer strategy.

What is known already: Blastocyst survival after warming is difficult to evaluate. Several authors have suggested that re-expansion degree could be useful to predict survival and implantation potential of warmed blastocysts. A recent study reported that more than 75% of blastocysts re-expanded within 5 hours after warming. Nevertheless, 30% of shrunken blastocysts turned out to be viable within 24 hours post-warming. However, methodology, and especially the duration reported between warming and transfer are variable among studies (from 1–5 hours up to 20 hours). To date, a short duration between warming and transfer seems to be insufficient to properly evaluate implantation potential of blastocysts.

Study design, size, duration: This prospective randomized study was conducted between January 2015 and December 2016 in a university hospital. One hundred sixty two good-quality blastocyst transfers were included, and

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randomly allocated to group A: warming on the day before transfer (n = 81); or group B: warming on the day of transfer (n = 81).

Participants/materials, setting, methods: Patients were included according to the following criteria: (i) female age <38 years on the day of oocyte retrieval; (ii) first/second IVF/ICSI attempt; (iii) <5 fresh/frozen embryos previously transferred; (iv) good-quality blastocyst(s) frozen at day 5 (expansion degree ≥B3, inner cell mass (ICM) ≥B, trophectoderm (TE) ≥B according to Gardner and Schoolcraft's classification). Re-expansion degree and blastocyst quality were evaluated after warming and immediately before transfer. Clinical outcomes were compared between both groups.

Main results and the role of chance: Patients' and cycles' characteristics were similar between groups A and B: briefly, women's mean age was 31.0 and 31.4 years, respectively, mean attempt rank was 1.1, and an average of 1.2 blastocysts was transferred for both. Quality of the warmed blastocysts at the time of transfer was comparable (39.1% of top-blastocyst [≥B4AA/AB/BA] in group A vs. 41.7% in group B, respectively; p = 0.69). After an overnight period post-warming (group A), 14/102 blastocysts (12.2%) appeared unsuitable for transfer, whereas only 1/103 (0.9%) in group B (p = 0.001), thus leading to an additional warming. As expected, re-expansion degree just before transfer was higher in group A (93.9% vs. 68.0% respectively; p < 0.0001). Likewise, the proportion of hatched blastocysts before transfer was higher after a longer culture period (37.6% in group A vs. 12.7% in group B; p < 0.0001). IR were similar in both groups (38.0% in group A vs. 36.4% in group B; p = 0.83), as well as clinical pregnancy rates (55.5% in group A vs. 50.6% in group B; p = 0.53). It may be noted that among the 9 cycles in group A requiring an additional warming, 2 patients finally got pregnant.

Limitations, reasons for caution: These findings need to confirmed and completed with live birth rates.

Wider implications of the findings: A long culture period after good-quality blastocyst warming does not seem detrimental on their implantation potential. This strategy could rather enable a better evaluation of blastocyst survival, and, if needed, an adaptation of transfer strategy in case of poor quality on the day of transfer.

Trial registration number: N° ID RCB: 2015-A00562-47

O-075 Live birth after frozen-thawed blastocyst transfer is correlated with the day of blastocyst expansion

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Study question: The aim of this study was to evaluate the live birth rate (LBR) after frozen-thawed day 5 (D5) and day 6 (D6) blastocyst transfer.

Summary answer: LBR following frozen-thawed blastocyst transfer is significantly lower with D6 than D5 blastocyst whatever its quality.

What is known already: During fresh embryo transfer cycles, pregnancy rates (PR) are significantly higher when transferring blastocysts expanded on D5 compared with those developing on D6. According to this result, in programmed thawed blastocyst transfer (TBT) cycles, the same clinical outcomes would be expected when transferring D5 or D6 blastocysts because of the tightly controlled synchronization between endometrium and embryonic stage. However, the impact of delayed blastocyst expansion at D6 on clinical outcomes remains unclear. Some reports have shown higher PR after D5 TBT than those of D6 while others rhave shown equivalent TBT outcomes after D5 and D6 cryopreserved blastocysts transfers.

Study design, size, duration: Cohort study including 1347 frozen - thawed blastocysts transfer from non-donor cycles performed between January 2012 and December 2015 at a tertiary care center.

Participants/materials, setting, methods: All patients were scheduled for TBT and compared between 2 groups: blastocysts vitrified on D5 (n = 994) or on D6 (n = 353). The primary outcome was LBR per embryo transferred in the first warming cycle. Secondary outcomes were clinical pregnancy rate (cPR), early miscarriage rate and neonatal outcomes following TBT at D5 or D6. Statistical analyses were conducted using univariate and multivariate logistic regression model.

Main results and the role of chance: The LBR was significantly increased in the D5 group compared to the D6 group [294/994 (29.6%) vs. 60/353 (17.0%); p < 0.001]. The cPR was also higher when blastocysts were vitrified on D5 than those on D6 [429/994 (43.2%) vs. 95/353 (26.9%); p < 0.001]. No significant differences were found between groups in term of early miscarriage rate (p = 0.862). However, more good quality embryos (defined as an B3-B4 or B5 embryo ≥BB according to the grading scale proposed by Gardner) were transferred in the D5 group than in the D6 group [807 (81.2%) vs. 214 (60.6%); p < 0.001]. Concerning neonatal outcomes, the D5 group infants had a lower mean birth weight compared to those of the D6 group (p = 0.001). In addition, a significantly shorter gestational age at birth is reported in the D5 blastocyst group as compared to D6 group (p = 0.004). After multivariate logistic regression taking into account potential confounders such as women age, number of previous IVF/ICSI procedures, the day of the blastocyst vitrification (D5 or D6) and embryo quality, blastocyst expansion at D6 was independently associated with a significant decrease in LBR compared to D5 expanded-blastocyst (OR 0.52; 95% CI 0.38–0.72; p < 0.001).

Limitations, reasons for caution: The poor predictive value of the morphological approach in embryo selection could constitute a limitation in this study. However, blastocyst quality was evaluated similarly in both groups.

Wider implications of the findings: The delay in reaching the blastocyst expansion in D6 could reflect a poor intrinsic embryo quality, followed by a delayed hatching not synchronized with the endometrium during TBT cycles. These results could influence the cryopreservation policies by prioritizing D5-expanded blastocysts during TBT in order to optimize the chances to conceive.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 20: DIFFERENT TYPES OF PLURIPOTENT STEM CELLS

Monday 3 July 2017 Room W+X 15:15–16:30

O-076 Comprehensive cell-surface protein profiling identifies novel markers of human naïve and primed pluripotent states

S. Panula[1], A. Collier[2], P. Rugg-Gunn[2], F. Lanner[1]

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[2]The Babraham Institute, Epigenetics Programme, Cambridge, United Kingdom

Study question: Are cell-surface markers differentially expressed between naïve and primed human pluripotent stem cells (PSCs) and thus, can be used to isolate naïve PSCs during primed-to-naïve resetting?

Summary answer: We developed an antibody panel targeting multiple cell-surface proteins that can distinguish between naïve and primed PSCs and isolate emerging naïve PSCs.

What is known already: PSCs exist in naïve and primed states, and provide important models to investigate the earliest stages of human development. Naïve cells can be obtained through primed-to-naïve resetting, however, there are no reliable methods to prospectively isolate unmodified naïve cells during this process.

Study design, size, duration: The cell-surface marker expression was compared between established primed and naïve PSCs, and further analysed during 10-day primed-to-naïve resetting.

Participants/materials, setting, methods: Three different PSC lines were used: H9, WIBR3, and H9-FiPS, with primed culture conditions and two different naïve culture conditions: 5i/L/A and t2i/L+PKCi. Human blastocyst stage

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embryos were used to validate the expression of several cell-surface markers by immunocytochemistry. A comprehensive profiling of cell-surface proteins was done by flow cytometry with over 400 antibodies screened. A multicolor antibody panel for FACS was developed to isolate cells for colony formation assay, quantitative PCR and RNA-sequencing.

Main results and the role of chance: Our cell-surface marker screen identified 58 primed-specific markers, 8 naïve-specific markers, and 40 markers positive for both primed and naïve PSCs. We validated a cohort of antibodies in multiple naïve and primed PSC lines and culture conditions, and also found that several naïve-specific, but not primed-specific, proteins were expressed in the pluripotent cells of the human preimplantation embryo. We developed an antibody panel targeting multiple cell-surface proteins, and demonstrated that the panel could distinguish between naïve and primed PSCs, track the dynamics of naïve – primed interconversion, and isolate emerging naïve PSCs from a heterogeneous cell population. Molecular characterisation showed that the transcriptome of the emerging naïve cells was more similar to naïve cells than to primed cells, but was not identical to established naïve PSCs. Furthermore, our analysis revealed that X-chromosome reactivation occured primarily during the late-stage maturation of naïve cells.

Limitations, reasons for caution: We identified naïve-specific and primed-specific markers using PSCs *in vitro*. Although most of the tested naïve-specific markers were expressed in human blastocysts, not all were exclusively expressed in the epiblast. Therefore, the markers by themselves should not be considered as pluripotent-specific markers in the human blastocyst.

Wider implications of the findings: Identification of state-specific proteins provides a robust set of molecular markers to unambiguously define human PSC state, and allows new insights into the molecular events leading to naïve cell resetting.

Trial registration number: not applicable.

O-077 Wnt inhibition during hESC culture provides an improved pluripotent state starting point for directed differentiation

J. Taelman[1], L. Tilleman[2], F. Van Nieuwerburgh[2], S. Warrier[1], M. Popovic[1], M. Van der Jeught[1], D. Deforce[2], P. De Sutter[1], B. Heindryckx[1], S.M. Chuva de Sousa Lopes[3]

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[2]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences-Ghent University, Ghent, Belgium
[3]Leiden University Medical Centre, Department of Anatomy and Embryology, Leiden, The Netherlands

Study question: Does Wnt inhibition by JP-Inh enhance hESC characteristics and their functional differentiation potential?

Summary answer: JP-Inh supplemented hESC are in a distinct pluripotent state, which appears to be more homogenous, while showing similar neuronal differentiation potential to conventional hESCs.

What is known already: Human embryonic stem cells (hESCs) and their ability to differentiate to specific cell types holds great promise for future therapeutic applications. Standard culture conditions maintain hESCs in a primed state of pluripotency, which harbors disadvantageous heterogeneity and spontaneous differentiation propensity. Efforts to adapt hESC to a more (mouse-like) naive pluripotent state restrict the efficiency of existing directed differentiation protocols. An alternative state has recently been described in mouse epiblast stem cells using the Wnt inhibitor JP-Inh, which seemingly induce the primed pluripotent state to one with less heterogeneity and less spontaneous differentiation.

Study design, size, duration: Two independent in-house derived hESC lines, were cultured in standard primed pluripotency conditions, our in-house naive pluripotency medium and JP-Inh supplemented medium. Subsequently, samples were collected at three consecutive passages and used for next-generation sequencing. Cells from all conditions were further differentiated towards the neuronal lineage and characterized.

Participants/materials, setting, methods: Comparative transcriptomic analysis was performed on primed, JP-Inh and naive pluripotency conditions. All three conditions were analyzed to compare differentially expressed gene profiles.

Following directed differentiation, gene expression was evaluated using quantitative PCR (qPCR), while the presence and localization of neuronal proteins was assessed by immunocytochemistry.

Main results and the role of chance: JP-Inh cultured hESCs displayed a more homogenous morphology with larger colonies and distinct colony borders compared to the primed pluripotency state. Principle component analysis clearly separated JP-Inh from primed and naive samples in both hESC lines. As such, JP-Inh hESCs harbor a distinct state of pluripotency compared to primed and naive pluripotency conditions. Our results further confirmed the downregulation of Wnt-associated genes in JP-Inh hESCs. Expression of pluripotency markers was not significantly different between primed and JP-Inh hESCs. Markers of all three germ lineages were elevated in primed pluripotency conditions, compared to JP-Inh hESCs, suggesting that JP-Inh hESC are less prone to lineage priming. Both primed and JP-Inh hESCs were able to differentiate into $TUBB3^{+}/SOX9^{+}$ neuronal cells, which showed close resemblance to neuronal networks. By contrast, naive hESC-generated neurons failed to form radial outgrowths from the central embryoid body after plating, but contained some sparse and irregularly spaced $TUBB3^{+}/SOX9^{+}$ neuronal cells.

Limitations, reasons for caution: While more homogenous cultures were observed in the JP-Inh conditions, a comparative transcriptomic analysis on the single cell level is necessary to validate this. To establish the clinical relevance of the JP-Inh alternative pluripotent state, differentiation into other cell lineages will further reveal their functional value.

Wider implications of the findings: Directed neuronal differentiation of both primed and JP-Inh hESCs appeared to be morphologically similar. As the JP-Inh condition seems to result in an intermediate state between naive and primed pluripotency, retaining advantages of both stages, it may prove a more beneficial starting point for directed differentiation towards therapeutically relevant lineages.

Trial registration number: not applicable.

O-078 Origins of Human Embryonic Stem Cell Fate: Role of the Post Inner Cell Mass Intermediate

S. Warrier[1], M. Popovic[1], J. Taelman[1], L. Tilleman[2], S. Lierman[1], M. Ferrer-Buitrago[1], S. Chuva De Sousa Lopes[3], A. Van Soom[4], L. Peelman[5], F. Van Nieuwerburgh[2], D. Deforce[2], P. De Sutter[1], B. Heindryckx[1]

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Study question: What is the role of the post inner cell mass intermediate (PICMI) during the process of human embryonic stem cell (hESC) derivation?

Summary answer: Although transcriptionally similar to hESCs, the PICMI is distinct from the ICM and shows upregulation of sex-specification markers, demonstrating similarities to primordial germ cells (PGCs).

What is known already: The in vitro derivation of conventional hESCs from the ICM is preceded by an epiblast-like intermediate stage depicted as the PICMI. Studies have shown that the formation of the PICMI is predictive of hESC derivation success and shows a mixed expression profile of both early- and late-stage epiblast markers. The presence of late epiblast markers in the PICMI, similar to mouse pluripotent stem cells harvested from the post-implantation stage embryo, renders the hESCs in a primed state of pluripotency, already showing some predisposition to differentiation. The transcriptional changes occurring during its transition to hESCs are yet to be fully defined.

Study design, size, duration: To obtain more insight in the molecular signaling network in the transition of the ICM towards hESC, we analyzed genome-wide transcription profiles of 3 preimplantation ICMs, 3 well defined PICMIs

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and 3 biological replicates of primed female hESC using next generation sequencing.

Participants/materials, setting, methods: Approximately 50 cells were collected from a female primed hESC cell line cultured for two feeder-free passages on Matrigel. Pure ICMs were isolated using laser assisted micromanipulation from day 5 good quality blastocysts from frozen thawed donated day 3 embryos. The PICMIs were manually dissected from outgrowths of day 6 expanded blastocysts on mouse embryonic fibroblasts (MEFs) between day 6–10 post plating. Samples were sequenced on the NextSeq500. Statistical analysis was done in edgeR (FDR<0.05).

Main results and the role of chance: Principal component analysis confirmed a transcriptionally different state of ICM compared to PICMI and hESC. The PICMI clustered together with hESC further elucidating change in pluripotency state during in vitro derivation of hESC.

Differential gene expression analysis for protein coding genes revealed 634 and 560 genes were up- and downregulated respectively in hESC when compared to ICM (hESC/ICM). When comparing the PICMI/ICM, 471 and 296 genes were up- and downregulated in PICMI. Naïve pluripotency markers were upregulated in ICM including SOAT1 and KLF4 when compared to PICMI.

Additionally, 326 and 493 long non-coding RNA (lncRNA) were differentially expressed between the ICM/PICMI and ICM/hESC respectively. Only one lncRNA, a pseudogene to DPPA3 (Stella), a marker of early germ cell specification, was differentially expressed between PICMI/hESC.

Gene Ontology (GO) analysis for biological processes showed upregulation of terms related to neural morphogenesis in hESC and downregulation of processes related to transcription and translation when compared to ICM. When comparing PICMI/ICM, processes related to tissue morphogenesis, sexual differentiation, cell-cell adhesion and Wnt receptor signaling pathway were upregulated whereas RNA processing, oxidative phosphorylation and mitochondrial activity were downregulated. GO terms related to embryonic and tubal development were downregulated in hESC when compared to PICMI.

Limitations, reasons for caution: Since only three replicates were used for the study, the sex of the ICM and PICMI was not taken into consideration during the analysis.

Wider implications of the findings: These findings provide further insight into the underlying transcriptome-dependent transition from ICM to established hESC. This will help in gaining insight into the different states of pluripotency that exist in human, the derivation of PGCs and naïve hESCs in vitro which is still inefficient.

Trial registration number: NA.

O-079 Mitochondrial protein acylglycerol kinase orchasdrates stem cell pluripotency

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Study question: What is the potential function of Acylglycerol kinase (AGK) in regulating embryonic stem cell (ESC) self-renewal and somatic cell reprogramming?

Summary answer: AGK played a vital role on maintaining the pluripotency of ESCs and improving the efficiency of iPS reprogramming.

What is known already: Embryonic stem cell (ESC) self-renewal and somatic cell reprogramming require precise coordination of transcription factors, chromatin regulators and RNA modifiers. Many efforts have been devoted to characterizing the underlying mechanism, but it is still remained poorly understood. Acylglycerol kinase (AGK), a lipid kinase related to mitochrondria oxidative respiratory, has been recently reported as a molecular marker in cancer generation. Our preliminary data shows that it was also enriched in ESC and iPS, however, there is no report about its role in ESC. It will be interesting to find out its potential function in ESC and somatic cell reprogramming.

Study design, size, duration: Lentivirus systems were used to generate gain or loss of function ES cell models or cell reprogramming model to study the role of AGK in maintaining stem cell self-renewal, proliferation and pluripodtency.

Participants/materials, setting, methods: Human ESC lines were used to generated AGK gain and loss of function cell models. Human foreskin fibroblasts were transfected with OCT4, SOX2, KLF4, c-MYC (OSKM) with or without AGK by lentiviral system. AP staining, QPCR and immunostaining were used to check the reprogramming efficiency. Flow cytometry and MTT was used to examine the cell proliferation. RNA-seq was used to examine the gene expression panel which might be regulated by AGK.

Main results and the role of chance: In this study, we demonstrated that AGK was significantly enriched in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) compared with HFFs. We also showed that the expression level of AGK gradually decreased in embryonic bodies (EBs), along the course of differentiation, which correlated with the decrease of NANOG and OCT4. Loss-of-function assays by AGK shRNAs exhibited about 75% knockdown of AGK in ESCs, which dramatically decreased the cell proliferation. And it also presented diverse differentiate fate compared to WT ES cells. Meanwhile, HFFs OSKM co-transfected experiment results showed that up regulation of AGK during HFFs reprogramming enhanced the number of iPSC colonies. RNA-seq results revealed that the expression of a couple of mitochrondria oxidative related genes were affected by variation of AGK.

Limitations, reasons for caution: Although we show that AGK is very important in keeping hESCs normal characteristic, the study of working mechanism of AGK in ESCs is still very limited. More studies will be performed to explore its underline mechanism.

Wider implications of the findings: This study was the first time to explore the role of AGK in human ESC. It will help us to better understand the mechanism of ESC self-renewal and somatic cell reprogramming, which might be helpful in future application of ESC and iPS.

Trial registration number: this study is not a clinical trial.

O-080 Two live birth after Stem cell ovarian auto-transplantation in Poor Responder women

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Study question: To assess bone marrow derived stem cell ovarian autologous transplantation (SCOT) competence to optimize ovarian reserve in poor responders (PR).

Summary answer: SCOT improved ovarian function in 69% of patients. Three pregnancies were achieved and 2 healthy babies born in PR whose only option was oocyte donation.

What is known already: Advanced maternal age is the main cause of infertility nowadays; in fact both oocyte quantity and quality are seriously impaired in aged patients. These women are known as PR to controlled ovarian stimulation (COS) and oocyte donation is their only realistic option. Previous studies suggest regenerative effects of bone marrow transplant in ovarian niche of damaged ovaries and raise the possibility that dormant follicles or somatic cells may benefit from the influence of bone marrow derived cells or soluble factors.

Study design, size, duration: A prospective pilot study with 13 PR women (Age:38 yr[37,39] with 4 yr[3,5] infertility) was developed at La Fe University Hospital from 2014 to 2016. Patients were considered as their own control as cells were injected in just one ovary.

Participants/materials, setting, methods: Bone marrow derived stem cells were mobilized to peripheral blood with G-CSF and isolated by aphaeresis. A volume of aphaeresis containing $50x10^6$ non-selected CD133+ cells was delivered into one utero-ovarian artery by catheterism. Serum AMH and AFC were monitored up to 5 months and compared to basal levels. COS was induced when AFC rose following standard procedures.

Main results and the role of chance: Ovarian reserve markers improved in 69.2% of the PR patients and 38.4% increased both AFC (≥3foll) and AMH

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(>2 SD). Higher AFCs were seen 15–21 days after SCOT when compared to the basal AFCs($p = 0.04$).

Two of the recruited patients were withdrawn from the study after SCOT. A total of 22 COS were initiated in 11 patients, starting 78 [31,109] days after SCOT (18.2% cancellation). Oocyte pick-up was successfully performed in 81.8% initiated COS and cancellation rate was 18.2%. A total of 36MII were retrieved and fertilization rate was 69.4% after ICSI. From 23 obtained embryos, 20 reached the blastocyst stage and underwent CGH analysis. Two of them were euploid and therefore transferred in single embryo tranfer (ET). A total of 3 pregnancies were achieved, 2 after ET and one spontaneous pregnancy, during the follow-up period after SCOT. Two healthy babies have been born (1 miscarriage) by the use of this procedure.

Limitations, reasons for caution: Pilot study. Confirmation of our preliminary data on a large population should be required.

Wider implications of the findings: SCOT improved ovarian function and oocyte quantity allowing pregnancy in aged and PR women whose only clinical option was oocyte donation. Nevertheless, SCOT does not modify embryo quality.

Trial registration number: NCT02240342

SELECTED ORAL COMMUNICATIONS

SESSION 21: REPRODUCTION AFTER CANCER TREATMENT

Monday 3 July 2017 Room C 15:15–16:30

O-081 Long-term effect of childhood cancer treatment on ovarian function markers: final results of the DCOG-LATER VEVO study

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Study question: Which dose-related effects of different chemotherapeutic agents and radiation fields on various ovarian function parameters can be determined in a nation-wide cohort of female long-term childhood cancer survivors (CCSs)?

Summary answer: CCSs treated with procarbazine, alkylating therapy, abdominal/pelvic radiation, or TBI were at highest risk of a reduced ovarian function, but not necessarily dose-dependently.

What is known already: Cancer treatment in childhood can reduce fertile life span and induce premature ovarian insufficiency (POI). Risk of gonadal damage in female CCSs has shown to vary according to cancer type, age at treatment, and type and dose of treatment. More specifically, risk factors identified so far include: an older age at treatment, a diagnosis of Hodgkin lymphoma, treatment with alkylating chemotherapy (CT) including procarbazine, and exposure to radiotherapy (RT) involving the pelvis and abdomen

Study design, size, duration: The DCOG LATER-VEVO study is a nation-wide retrospective cohort study on female fertility of Dutch CCSs. The control group consisted of sisters of survivors and females from the general population. Data collection took place between January 2008 and May 2014.

Participants/materials, setting, methods: The study population consisted of female 5-year CCSs treated between 1963 and 2002, and at least 18 years of age at inclusion. Of 1,106 CCSs and 819 controls who participated, 552 and 387, respectively, provided blood samples and/or underwent transvaginal ultrasound. Both multivariate linear and logistic regression analyses were performed.

Main results and the role of chance: CCSs had a 6·5-fold increased risk (95%CI 1·3 to 32·5) of low AMH levels (>2 SD below age-specific cut-off level) and a 14·3-fold increased risk (95%CI 2·4 to 86·4) of high FSH levels (>10 U/l), while risks of low AFC (>2 SD below age-specific cut-off level) and low inhibin B (<20 ng/l) levels were not significantly increased. The following types of treatment were associated with one or more markers: cyclophosphamide (AMH), procarbazine (all four markers), composite group of busulfan, melphalan, chlorambucil and lomustine (AMH, AFC, and FSH), dactinomycin (AMH), doxorubicine (AFC), mitoxantrone (FSH), spinal RT (AMH), abdominal/pelvic RT (all four markers), TBI (all four markers). For the effect on AMH and FSH dose-effect relationships was found for procarbazine, while for AFC and inhibin B such a relationship was found for abdominal/pelvic RT.

Limitations, reasons for caution: AMH, AFC, FSH and inhibinB were used as a proxy for ovarian function, however, the translation to fertility and POI in CCSs has yet to be made. Not all results were consistent across the four markers, thus we discourage use of only one marker to assess ovarian function in CCSs.

Wider implications of the findings: The results of the study can help physicians counselling CCSs, as well as future patients who are about to undergo anti-cancer treatment, regarding their future reproductive potential and the need for fertility preservation interventions.

Trial registration number: NTR2922 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC = 2922.

O-082 Pregnancy after cancer in girls and women in Scotland:: a population-based analysis

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Study question: We assessed the proportion of women having a pregnancy after cancer diagnosis compared with population-based controls to provide an unbiased analysis.

Summary answer: Cancer survivors were 38% less likely to achieve a pregnancy compared to women in the general population; almost all diagnostic groups showed an impact.

What is known already: Many treatment regimens for cancer are known to impact negatively on female fertility, but most studies address the prevalence of surrogates for fertility such as ovarian function. The most detailed analyses, from the US Childhood Cancer Survivor Study, only includes women up to the age of 21 at diagnosis, and is not population based.

Study design, size, duration: We performed a retrospective cohort study relating first incident cancer diagnosed between 1981 and 2012 to subsequent

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pregnancy in female patients in Scotland aged 39 years or less at cancer diagnosis. Pregnancies were included up to end of 2014.

Participants/materials, setting, methods: Scottish Cancer Registry records were linked to hospital discharge records to calculate standardised incidence ratios (SIR) for pregnancy, standardised for age and year of diagnosis. We also selected women from the exposed group who had not been pregnant prior to their cancer diagnosis who were compared with a matched control group from the general population. Additional analyses were performed for breast cancer, Hodgkin lymphoma and leukaemia.

Main results and the role of chance: Cancer survivors (n = 23,201) achieved fewer pregnancies: 6,627 observed compared to 10,736 expected pregnancies, SIR 0.62 (95% CI 0.60–0.63). The reduced SIR was observed for all age groups, falling progressively with age at diagnosis, was reduced for women across almost all cancers (ranging from 0.34 (0.31–0.37) for cervical cancer to 0.87 (0.84–0.90) for skin cancers), and varied by deprivation, being lowest in women from the most deprived quintile. SIR varied strongly by period of cancer diagnosis, being lower with earlier period of diagnosis ie 1981–1988, SIR 0.47 (0.44 – 0.50); 2005–2012, SIR 0.64 (0.60 – 0.69) (skin cancer excluded). 20.6% of cancer survivors achieved a first pregnancy after cancer diagnosis (2,114 first pregnancies in 10,271 women), compared to 38.7% in controls (ratio 0.53, 0.51–0.56), with significant reductions in women with breast cancer (ratio 0.44), leukaemia (0.30) and Hodgkin lymphoma (0.65). The effect was less with more recent treatment period: this was particularly notable for Hodgkin lymphoma, but not leukaemia or breast cancer. The proportion of first pregnancies that ended in termination was lower among women with previous cancer (11.2% vs 14.7% of pregnancies, CI of difference -5.0, -2.0%) but there was no difference in risk of miscarriage or stillbirth.

Limitations, reasons for caution: Details of treatments received were not available, so the impact of specific treatment regimens on fertility could not be assessed. Limited duration of followup was available for women diagnosed in the most recent time period.

Wider implications of the findings: This analysis provides robust, population-based evidence for the extent of the effect of cancer and its treatment on subsequent pregnancy across the reproductive age range. The major impact on subsequent pregnancy in some common cancers highlights the need for enhanced strategies to preserve fertility in girls and young women.

Trial registration number: not applicable.

O-083 Fertility preservation by oocyte vitrification or ovarian cortex cryopreservation. A prospective cohort study

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Study question: What is the best technique for fertility preservation (FP) for medical reasons, oocyte vitrification (OV) or ovarian cortex cryopreservation (OCC)?

Summary answer: OV and OCC yielded similar clinical and ongoing pregnancy rates (CPR, OPR) than OCC, although there is a trend to higher rates with the former.

What is known already: The increasing survival rates and success of oncological treatments make FP procedures a key step in the holistic management of the oncologic patient. FP for oncological reasons should be considered since the moment of diagnosis and it has become a major issue in young women. Counseling should be individualized based on the risk of gonadal failure that depends on patient´s age, ovarian reserve, chemotherapy drug/regimen and time prior to treatment. Different strategies have been proposed for FP, being OV and OCC the most recommended procedures as both provide excellent clinical outcomes.

Study design, size, duration: The aim of the present study was to compare the efficacy of OCC as compared to that of OV in two prospective large cohorts of patients undergoing FP: 1024 patients undergoing OV and 735 OOC were recruited in between 2005 and 2015 in our FP program at IVI clinics and La Fe University Hospital; the program grants free access to OV and OCC and use the same decision-making algorithm to chose the FP technique.

Participants/materials, setting, methods: In the OV cohort, 49 patients came back to use their oocytes and 44 patients came to have their ovarian tissue reimplanted in the OCC cohort. OV was carried out using the cryotop device and OCC was done using a slow freezing protocol. Reimplantations took place orthotopically. Patients were followed up until they used all the oocytes, the lack of function of the reimplanted tissue or the achievement of livebriths.

Main results and the role of chance: No difference was found between groups regarding AMH levels at FP (OV: 11.6 [5.4–24.7] vs OCC: 11.8 [6.4-21.9]; n.s.). The most prevalent pathologies motivating FP were breast cancer (OV: 60.3%, OCT: 58.6%), Hodgkin lymphoma (OV: 14.2%, OCT: 20.7%; $p < 0.001$) and non-Hodgkin lymphoma (OV: 6.0%, OCT: 3.2%; $p < 0.001$). In the OV cohort, patients used the vitrified oocytes after a mean storage time of 3.9 years. In the OCT cohort, after a mean storage time of 5.5 years. The age at utilization of the cryopreserved material was also similar between groups (OV: 39.0 (3.8) vs OOC: 38.9 (4.1); n.s.). When clinical pregnancy rates (CPR) and live birth rates (LBR) (per patient) were compared between groups, the OV group yielded higher, but not significantly different, CPR (40.8% vs 27.3%) and LBR (32.6% vs 18.2%) than the OCT group.

Limitations, reasons for caution: In some clinical scenarios OV is not feasible and OCC offers a different profile of advantages (mainly endocrine function resumption and the possibility of spontaneous pregnancy). Therefore, recommendations on the choice of these techniques have to be based in individualized criteria, oncologist decision and time prior to treatment.

Wider implications of the findings: Both OV and OCC can be recommended as effective FP techniques.

Trial registration number: Not applicable.

O-084 Severely decreased spermatogonial quantity after chemotherapy with alkylating agents in prepubertal boys

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Study question: What is the effect of alkylating and non-alkylating chemotherapy agents on the numbers of spermatogonia in prepubertal human testes?

Summary answer: Malignancy treatment with alkylating agents was associated with significantly decreased numbers of spermatogonia in prepubertal testes. This effect was higher with cumulative CED above 4000 mg/m^2.

What is known already: At present, over 80% of childhood cancer patients become long term survivors, yet the majority experience long lasting side effects of the malignancy treatment including persistent sub-/infertility. Previous studies have demonstrated, that male infertility after cancer treatment depends on the extent of damage to spermatogonia. In prepuberty, the constant turnover of early germ cells in testes makes them highly susceptible to the adverse effects of chemotherapy, thereby increasing the risk of depletion. It is known, that the fertility in childhood cancer survivors can remain poor for over 20 years if germ cell pool is affected during the treatment.

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Study design, size, duration: In this study, we gathered data on cancer treatment regimen in 37 prepubertal boys with acute lymphoblastic leukemia (ALL), examined histology of testicular biopsies acquired at the end of the cancer treatment and testicular function as recorded by semen analysis from those that reached adulthood.

Participants/materials, setting, methods: The patient data were identified through the records of the University Central Hospitals in Helsinki and Turku and included patient age at the end of the treatment, treatment characteristics, and semen parameters in adulthood. Testicular biopsies from each patient were fixed in formalin and H&E stained for analysis under the bright-field microscope. The numbers of spermatogonia per tubular cross-section (S/T) were counted blind by an experienced researcher. Statistical analysis was performed using polynomial regression.

Main results and the role of chance: In the analyzed patient cohort of 37 prepubertal boys with ALL, we found that the mean S/T value in patients exposed to alkylating agents (n = 16) was significantly lower ($p < 0.001$) than in a group exposed to non-alkylating agents, whose mean S/T (n = 19) was within a range of normative reference values. We also found a non-linear association between S/T and cumulative cyclophosphamide equivalent dose (CED), with cumulative CED above 4000 mg/m^2 associated with S/T values close to zero. Testicular function as recorded by semen analysis measurements in adulthood could be retrieved in 17 childhood cancer survivors. We did not find a statistically significant association between S/T and spermatogenic recovery. No significant differences were found between the total sperm count in patients treated with and without alkylating agents (n = 7 and n = 10, respectively) at the mean of 12.9 ± 6.7 years follow-up.

Limitations, reasons for caution: These results should be interpreted considering the small sample size, including the limited number of childhood cancer survivors who performed the follow-up measurements on testicular function.

Wider implications of the findings: Our data suggest that exposure to alkylating agents results in depletion of spermatogonial pool in prepubertal human testes. Patients with higher risk of spermatogonial depletion might be offered fertility preservation by cryopreserving a testicular biopsy before initiation of alkylating agents to avoid potential persistent sub-/infertility.

Trial registration number: Not applicable.

O-085 Assessment of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) in couples with post chemotherapy non-obstructive azoospermia (NOA)

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Reproduction Clinic Osaka, Department of Reproductive Medicine, Osaka, Japan

Study question: What are sperm retrieval rate (SRR) by microdissection TESE (micro TESE), fertilization rate, and pre- and post- implantation development in post chemotherapy NOA couples?

Summary answer: In patients with successful sperm retrieved, age at chemotherapy was older than failure group and their embryonic development was better than unexplained NOA patients.

What is known already: Advances in chemotherapeutic treatments can achieve high remission rates in pediatric and adolescent patients with cancer, but cytotoxic chemotherapy may lead to irreversible spermatogenic dysfunction. Patients with such diseases are often not concerned with reproductive issues at diagnosis, however, recent advances combining TESE and ICSI in patients with post chemotherapy NOA allow these males father their own genetic offspring.

Study design, size, duration: A retrospective study was conducted in 34 patients with post chemotherapy NOA (including 7 patients with bone marrow transplantation (BMT)), 330 NOA patients with 46,XY without past history (unexplained NOA; not including after orchidopexy, Klinefelter's syndrome, cryptozoospermia, mumps orchitis, etc) who who underwent micro TESE, and 107 OA patients with simple TESE between September 2013 to December 2016.

Participants/materials, setting, methods: The cancer types included testicular cancer, leukemia, malignant lymphoma, osteosarcoma, myelodysplastic syndrome, rectal cancer, Wegener's granulomatosis, rhabdomyosarcoma, neuroblastoma, renal carcinoma, and mediastinal malignancy tumor. The age at chemotherapy and micro TESE was 15.7 ± 8.9 and 33.9 ± 5.4 years, respectively, and the mean maternal age at ICSI was 33.7 ± 3.9 years. Two pronuclear (2PN) oocytes, blastocyst development, good-quality blastocysts (Grade 3BB and more on day 5 by the Gardner scoring), and clinical pregnancy rates were examined.

Main results and the role of chance: SRR of micro TESE in post chemotherapy NOA (17/34 = 50.0%) was higher than unexplained NOA (92/330 = 27.9%) patients ($p < 0.01$). In males with successful sperm recovery, age at chemotherapy was older (21.0 ± 7.8 years) than failure group (10.5 ± 7.0 years) ($p < 0.01$). With respect to the type of cancer, there was no predictor for SRR and no significant differences in the pregnancy and live birth rates. In 3 of 7 post BMT patients spermatozoa were successfully retrieved. Two of 3 patients even showed a 46,XX karyotype (transplantation from female donor). The 17 patients who failed to obtain sperm could not find any germ cells in their testicular samples in wet preparations and histopathological sections (Sertoli cell only syndrome). Rates of 2PN oocytes, blastocysts, and good-quality blastocysts were 67.9%, 51.4%, and 45.2% in post chemotherapy NOA, 58.3%, 45.2%, and 37.8% in unexplained NOA, and 66.0%, 51.6%, and 42.8% in OA, respectively. Post chemotherapy NOA tended to be higher in clinical pregnancy rate per ET (14/32 = 43.8%) than unexplained NOA (50/177 = 28.2%) ($p = 0.08$). Ten children have been born and 2 patients are on going pregnancy in post chemotherapy NOA couples.

Limitations, reasons for caution: There was a lack of stratification with respect to the chemotherapeutic regimen. The neonatal outcome and development of these children has not been fully investigated.

Wider implications of the findings: Age at chemotherapy was important predictive factor for successful sperm recovery. Once sperm were obtained their reproductive performance was satisfactory. These findings provides hope for men of reproductive age who have not undergone sperm cryopreservation before chemotherapy.

Trial registration number: N/A

INVITED SESSION

SESSION 22: HAS TRANSCRIPTOME ANALYSIS OF OOCYTES AND EMBRYOS RUN ITS COURSE? SINGLE CELL OMICS

Monday 3 July 2017 Plenary 2 17:00–18:00

O-086 Single-cell proteomics of human oocytes

I. Virant-Klun[1], K. Ferk[1,2], J. Krijgsveld[2]

[1]University Medical Centre Ljubljana, Reproductive Unit- Department of Obstetrics and Gynaecology, Ljubljana, Slovenia

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Abstract text

Single-cell proteomics of human oocytes

In vitro maturation of oocytes is a crucial technology to generate mature oocytes that are utilized for IVF. Importantly, many efforts have been made to improve the efficiency of the in vitro maturation process, yet their competence is lower than of oocytes matured in vivo. Elucidating the molecular mechanisms that regulate oocyte maturation will be important to develop procedures to improve the quality of in vitro-matured oocytes, while potentially providing molecular markers that predict oocyte quality or maturation stage.

Global gene expression analysis by micro-arrays and later by next-generation sequencing have started to reveal the composition of transcriptomes of maturing oocytes, however this has not resulted in a gene signature indicating oocyte competence. One explanation is that mRNA levels often poorly correlate with protein expression, which eventually are the key determinants of cellular

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function. Therefore it will be of crucial importance to use proteomic approaches to monitor and understand protein expression profiles over the course of oocyte maturation. The main challenge is to provide a sufficiently large amount of sample input (i.e number of cells) to obtain insightful information from a proteomic (e.g. mass spectrometric) experiment. This has been attempted for mouse, pig and bovine oocytes, requiring hundreds or even thousands of oocytes as an input, immediately explaining why that this cannot be applied to human cells without a drastic improvement of technologies to reach better sensitivity.

Although mass spectrometers have become much more sensitive over the last few years, further gains can be made in the preceding sample preparation step. With this in mind, we have recently designed a novel strategy to extract, purify and digest proteins in a novel magnetic bead-based procedure, minimizing sample losses and thereby increasing overall sensitivity of detection. As a result, this now allows us to routinely detect, identify and quantify ~600 proteins from single human oocytes. In this presentation we will show that quantitative, single-oocyte proteomics clearly distinguishes GV from MII oocytes by the expression of a distinct set of proteins. Furthermore, principle component analysis of proteomic data shows that oocytes that are in vitro-matured in the presence of gonadotropins FSH and HCG, and in a co-culture with cumulus cells from mature oocytes are more similar to in vivo matured cells than cells matured with FSH and HCG only thus indicating the importance of ovarian niche during the maturation process. Co-culture with cumulus cells can be efficiently replaced by addition of the protein vimentin which is an important component of mesenchymal cells including granulosa cells.

Collectively, these results demonstrate that technological advances now permit proteomic analysis of single human oocytes, which can be used as a powerful readout to optimize in vitro maturation procedures, while giving insight in underlying molecular events. Eventually, this may lead to the identification of maturation-specific markers that may be developed into an assay scoring for oocyte quality in daily clinical practice.

O-087 Deciphering developmental processes from single-cell transcriptomes

P. Robson

Single Cell Biology Laboratory, The Jackson Laboratory for Genomic Medicine, Farmington, CT, U.S.A.

INVITED SESSION

SESSION 23: ARE MICROWAVE FOOD CONTAINERS, WATER BOTTLES AND SOFT DRINK CANS MAKING YOU INFERTILE?

Monday 3 July 2017 Room A 17:00–18:00

O-088 Plasticizers and male fertility

R. Sharpe[1], S. Van den Driesche[2], R. Mitchell[2]

[1]*University of Edinburgh The Queen's Medical Research Institute, Centre for Reproductive Health, Edinburgh, United Kingdom*
[2]*University of Edinburgh, MRC Centre for Reproductive Health, Edinburgh, United Kingdom*

Abstract text

Numerous experimental animal studies have shown that exposure to high levels of plasticizers, in particular certain phthalate esters, but in some studies also bisphenol A, can result in adverse effects on aspects of male reproductive development and function. In keeping with this, epidemiological studies have found significant associations between exposure to these compounds and changes in reproductive functions in human males similar to those found in animal studies. At face value, this concordance raises obvious human health concerns, but as this talk will demonstrate a close animal-human comparison identifies several fundamental differences that together do not add up, and which cast doubt on these particular health concerns about plasticizers. Most notably, the levels of exposure required to induce significant adverse effects in animal studies are several orders of magnitude higher than those to which humans are exposed, which either means that humans are inordinately more sensitive to plasticizers than rats or that the human epidemiological data is confounded in some way.

This talk will review the experimental animal and human epidemiological data, focussing mainly on fetal exposure to phthalate esters (diethylhexyl phthalate, dibutyl phthalate), as it is exposures during this critical period that there is most concern about. In rats, exposure during pregnancy to high doses (>100 mg/kg/day) of these phthalates causes suppression of testosterone production by the fetal testis which can result in impairment of masculinization, manifest, for example, as increased incidence of cryptorchidism and hypospadias and decreased adult size of all male reproductive organs; in addition there is a decrease in anogenital distance (AGD), which is an established marker of fetal androgen exposure during the critical masculinization programming window (MPW). Epidemiological studies in humans, which have measured maternal exposure to these phthalates (in the range 1-10ug/kg/day) and then measured AGD in boys shortly after birth have shown mixed results, some showing an association between exposure and reduced AGD, other studies finding no association.

Arguably the most conclusive (and direct) evidence comes from experimental studies that have used human fetal testis tissue in culture or in a mouse xenograft model and then exposed to phthalate levels (eg 500 mg/kg/day) comparable to those that induce adverse effects in vivo in fetal male rats. These studies have shown absolutely no effect of phthalate exposure on testosterone production by the fetal human testis. Three further pieces of evidence back this up. First, similar culture/xenograft studies with rat testes shows phthalate inhibition of testosterone production, so the model systems work okay. Second, in vivo exposure of pregnant marmoset monkeys to high levels of phthalate does not result in any adverse effects on reproductive development in resulting male offspring. Third, studies that have monitored congenital abnormalities in offspring born to mothers who were exposed to phthalate levels 150–300 times higher (350-900ug/kg/day) than population level exposure, as a result of using phthalate-containing medicines, have not revealed any evidence for reproductive abnormalities in the exposed boys. The strengths and weaknesses of these various data forms will be discussed. In addition, attempts will be made to reconcile human data that is in disagreement, by considering a possible confounding effect of maternal western diet on AGD in sons, which might also result in increased exposure to the phthalate most commonly monitored in human epidemiological studies. Finally, it will be pointed out that studies which have compared phthalate 'effects' after birth (mainly in adulthood), whether in experimental or epidemiological studies, have shown more consistent evidence that phthalate exposure may be associated with inhibition of testosterone production by the human and marmoset testis.

O-089 Plastics and miscarriage

R. Lathi

Sunnyvale- CA, U.S.A.,

Abstract text

Environmental Toxins and miscarriage

Establishment of early pregnancy is the result of complex biochemical interactions between the decidua and blastocyst. Any alteration in this chemical dialogue has the potential to result in adverse pregnancy outcomes including miscarriage. Sporadic miscarriage is the most common complication of pregnancy and can be caused by multiple factors. While the most common cause of miscarriage is genetic abnormalities in the fetus, other contributing factors certainly can play a role in early loss. One such factor is environmental exposure, in particular to endocrine-disrupting chemicals, which has the potential to interfere with endogenous hormone action. These effects can be deleterious, especially in early pregnancy when the hormonal milieu surrounding implantation is in delicate balance. The purpose of this presentation is to review the current evidence on the role of environmental toxins in reproduction.

Ref.

Krieg SA1, Shahine LK2, Lathi RB3. Environmental exposure to endocrine-disrupting chemicals and miscarriage. Fertil Steril. 2016 Sep 15;106(4):941–7

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INVITED SESSION

SESSION 24: CHANGING THE LIFE COURSE FOR PCOS

Monday 3 July 2017 Room B 17:00–18:00

O-090 PCOS in adolescence-to diagnose or not and future fate

K. Hoeger

University of Rochester, Obstetrics and Gynecology, Rochester- NY, U.S.A.

Abstract text

PCOS in Adolescence-to diagnose or not and future fate

PCOS is a disease that presents clinically with abnormalities evident in adolescence. However adolescence is a period of reproductive maturation and many reproductive abnormalities that in adulthood would indicate pathology are part of the maturation process in adolescence. This complicates the diagnosis of PCOS in this age group. Consensus in multiple guidelines is growing to allow for a longer period of time of observation after menarche before formal diagnosis. The Endocrine Society consensus statement of PCOS in adolescents states that both hyperandrogenism and oligomenorrhea need to persist for at least 2 years to consider the diagnosis. Ultrasound criteria are particularly challenging in adolescence due to the overlap with normal ovarian morphology in this age group. Antimullerian hormone (AMH) concentrations are known to be increased in women with PCOS. There are some data to suggest that AMH may distinguish PCOS from normal girls in obese adolescents however there is still considerable overlap to use this as a diagnostic marker. While there is rationale to delay the diagnosis and consider early features of the disease in adolescents to represent those at risk, there is clear evidence of early metabolic dysfunction in adolescents, particularly in those with elevated androgen concentrations. Therefore there is motivation to diagnose and manage the condition as early as possible to minimize long term effects. In the presence of overweight and obesity there is evidence to suggest lifestyle modification with weight loss results in improvement in metabolic features but limited impact on reproductive features. Additionally this is difficult to maintain over the long term. Prevention of weight gain in adolescence may be of most importance to minimize long term metabolic impact. Traditional treatment with combination hormonal contraceptive remains first line treatment but questions remain on the long term metabolic impact. The use of insulin sensitizing therapy may be beneficial in adolescents but controlled large scale long term data in adolescents is lacking. Importantly there is evidence of increased psychological morbidity in adolescents with PCOS. Given the development of self-mage during adolescents, disturbance in mood and behaviors may have particularly long reaching impact in this population. While significant questions remain about the best diagnostic measures for PCOS in adolescence, there is enough concern regarding long term implications that timely interventions are recommended. Further research on the long term outcome of treatments in adolescence is needed.

O-091 Is there a need to increase PCOS awareness?

H. Teede

Monash University/Monash Health, Executive Director Monash Partners Academic Health Sciences CentreDirector Monash Centre for Health Research and Implementation - MCHRI, Clayton- Victoria, Australia

Abstract text

Affecting 12–18% of reproductive aged women, Polycystic Ovary Syndrome (PCOS) is a common, under-recognised, and prevalent condition, with significant health and psychosocial impacts. PCOS is the primary cause of anovulatory infertility; increases the risk of pregnancy complications (such as miscarriage, fetal anomalies, and gestational diabetes); increases obesity and quadruples type II diabetes, with younger onset; increases cardiovascular risk factors, psychological disturbance, and adversely impacts quality of life. PCOS is highly heritable and health burden extends to the next generation, with adverse impacts on children of affected mothers. Importantly, PCOS identifies women from puberty with high reproductive and metabolic risks, who appear prone to weight gain (further exacerbating their condition), and who are in need of targeted prevention and management.

We have engaged with health professionals and affected women internationally through focus groups, surveys and structured Delphi processes internationally. Primary care providers report feeling ill-equipped to manage PCOS, and report it as a high priority for education in women's health. We have also demonstrated key gaps in diagnosis, education, prevention and management among specialist health professionals. Due to the diversity of PCOS health impacts, affected women report feeling marginalised and falling between the gaps in specialty-focused health systems, reporting stigmatised and PCOS related distress. We have established key care and education gaps and priorities for women across diverse age ranges and across Europe, USA and internationally. The experiences of women and knowledge and perceptions of health professionals around PCOS ultimately leaves women feeling isolated, disempowered and underserviced, leaving a high personal and ultimately societal cost.

We have built on the activity of our National Australian PCOS Alliance and informed by gaps identified through international research with women and health professionals, we are expanding rigorous evidence based guidelines internationally and across all features of PCOS. This is supported by international collaboration with 24 professional societies and women's advocacy groups. The Australian government has provided $3 M for research, education and translation including guideline development and translation through a National Centre of Excellence in PCOS. ESHRE and ASRM have joined as leading and co-funding collaborators and 21 other societies and advocacy groups have joined in an international initiative to raise knowledge and awareness in PCOS. This will be underpinned by the development and translation of evidence based guidelines into practice for women and their health professionals.

Translation outputs include a multifaceted program of guideline outputs for women and health professionals. This is being delivered in collaboration with "Making Grade the Irresistible Choice (MAGIC) and includes a website, multifaceted resources translated into 30 different languages, podcasts, patient experience material, M- health apps, lifestyle programs and new, fully evaluated models of evidence based care for scale-up. Collaborations, progress, results of engagement with women and health professionals, evaluation and gaps in currently available education resources and research on translation and women's and health professionals education preferences will be presented, along with timelines and outputs of the international collaborative evidence based PCOS guidelines and translation program.

SELECTED ORAL COMMUNICATIONS

SESSION 25: PARAMEDICAL SESSION - LABORATORY - INCREASING PERFORMANCE

Monday 3 July 2017 Room W+X 17:00–18:00

O-092 A time-lapse incubator is a superior incubator for excellent quality blastocyst embryo development compared to a conventional incubator

S. Ellegiers, K. Tilleman, E. Van den Abbeel, P. De Sutter

Ghent University Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Ghent, Belgium

Study question: Is there a difference in blastocyst formation rate and blastocyst quality in a time-lapse imaging system (TLIS) as compared to a standard incubator (SI)?

Summary answer: There is a significant higher percentage of excellent quality (category A) blastocysts in the TLIS Embryoscope^{TM} as compared to the SI.

What is known already: TLIS is considered an important research tool for learning about embryo morphokinetics. Furthermore, TLIS is considered to be a unique incubator making undisturbed embryo development possible. Although a systematic review (Armstrong *et al.*, 2015) showed no significant difference in clinical outcome between TLIS and SI, there is still debate on whether the undisturbed development of the TLIS could be beneficial for embryo

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quality. A recent RCT (Park *et al.*, 2014) reported no significant difference in embryo quality on day 2 however, limited data exists on the potential benefit of undisturbed culture for blastocyst formation and quality.

Study design, size, duration: For the analysis, ICSI cycles with embryo transfer on day 5 were selected (February 2013 - January 2017). Sequential extended culture (Cook) was performed in either the TLIS Embryoscope^{TM} (n = 96 cycles) or the SI (Binder CB210) (n = 801 cycles). Matching of cycles was performed automatically using SPSS v.23.0 according to patient age (±1 year), treatment type (exact), sperm collection method (exact) and number of normal fertilized oocytes (±1). This resulted in 85,4% paired matched cycles (82/96).

Participants/materials, setting, methods: Blastocysts were scored according to Gardner *et al.* (1998; 2001) and categorized: cat A: minimum blastocyst 4, grade A, B TE or ICM; cat B: blastocyst 3, grade A, B TE or ICM; cat C: minimum blastocyst 4 with grade C in either TE or ICM; cat D: blastocyst 3 with grade C in either TE or ICM; cat blastocyst 2; cat blastocyst 1; cat compaction. Statistical analysis was performed by Fisher´s exact-test (significance p≤0.0500).

Main results and the role of chance: The cycle characteristics of the TLIS-group (82 cycles) showed 1010 cumulus oocyte complexes (COC), 745 MII oocytes underwent ICSI resulting in 570 2PN. The SI-group (82 cycles) consisted of 1021 COC, 779 MII and 545 2PN. Blastocyst outcome parameters were calculated relative to the amount of 2PN. Total blastocyst formation was 52.98% (302/570) in the TLIS-group as compared to 58.72% (320/545) in the SI-group (p = 0.0615). There was a significant difference in percentage cat A blastocysts in the TLIS-group 15.44% (88/570) versus 10.46% (57/545) in the SI-group (p = 0.0160). There was a lower percentage of cat B blastocysts in the TLIS-group 4.91% (28/570) versus 8.07% (44/545) in the SI-group (p = 0.0378). Furthermore, a significant higher percentage of cat blastocysts 1 was shown in the SI-group 15.78% (86/545) versus 9.65% (55/570) in the TLIS-group (p = 0.0022). All other blastocysts categories showed no significant differences between the TLIS and SI. Finally, no significant difference was observed in implantation rate (HCG+/cycle) in the TLIS-group 34.15% (28/82) as compared to the SI-group 40.24% (33/82) (p = 0.5183).

Limitations, reasons for caution: There was no randomization for the choice of incubator for the ART and the indications for culturing embryos in the TLIS incubator were often based on a medical history with poor or impaired implantation in previous cycles using day 3 embryo transfer, which rather strengthens the results than limiting them.

Wider implications of the findings: Although there are no differences in outcome or in early cleavage embryo quality on day 2, this study shows a difference in blastocyst quality for embryos cultured in the TLIS. This indicates that there is a potential benefit for undisturbed extended blastocyst culture.

Trial registration number: EC/2017/0086.

O-093 Effect of accurate temperature regulation during incubation on embryo quality after ICSI: a prospective sibling oocyte study on two incubation temperatures (36.6°C or 37.1°C)

R. Janssens, N. De Munck, H. Tournaye, I. Mateizel, I. Segers, G. Verheyen

UZ Brussel, Centre for Reproductive Medicine, Brussel, Belgium

Study question: Does accurate and stable incubation temperature at 36.6°C or 37.1°C affect the embryo quality after ICSI?

Summary answer: Fertilization rates and embryo quality were comparable between the 36.6°C and 37.1°C arm but ongoing pregnancy and implantation rates were higher after incubation at 37.1°C

What is known already: The primary function of an IVF incubator is to maintain an appropriate microenvironment (temperature and gas composition) for gamete function and embryo development. Although the developmental plasticity of embryos allows them to develop over a wide temperature range, the pre-implantation development is significantly affected in incubators that show a low micro-environment stability. Since most commercial IVF incubators show important temperature variations (up to 1.0°C), reliable studies that accurately determine optimal incubation temperature are scarce. So far, contradicting results on pregnancy data have been obtained when comparing culture temperatures.

Study design, size, duration: A single-centre prospective randomized controlled trial (100 cycles) was performed between May and November 2016, after approval by the local ethical committee of the university hospital. An electronically generated randomisation list was used to allocate half of the mature oocytes to the 36.6°C or the 37.1°C arm. The primary endpoint was embryo quality on day 3 and day 5/6; secondary endpoints were fertilization and embryo utilization rates. Additionally, pregnancy outcomes were recorded.

Participants/materials, setting, methods: Ninety-nine ICSI cycles which fulfilled the following criteria were included: at least six mature oocytes, use of ejaculated sperm and extended culture to day 5/6. Sibling oocytes were cultured in the same G210 incubator (K-Systems, Denmark) at 36.6 ± 0.05°C (chamber 1 to 5) or 37.1 ± 0.05°C (chamber 6 to 10) from the time of ICSI up to day 5/6.

Main results and the role of chance: A total of 1432 cumulus-oocyte-complexes were retrieved of which 1153 were mature and randomised for culture at 36.6°C (n = 572) or 37.1°C (n = 581) after ICSI. Fertilization was comparable in the two groups (80.4% at 36.6°C and 78.3% at 37.1°C) as well as the number of good quality embryos on day 3 (78.3% and 79.6%) and the number of good quality blastocysts on day 5 (53.8% and 55.8%). Embryo utilization rates were also similar (40.0% versus 40.4%).

A fresh embryo transfer (ET) on day 5 was scheduled for 72 patients (two without ET due to insufficient embryo quality) and a freeze all strategy was scheduled for 27 patients (one without freezing). Fifty-nine single ET (SET) (28 from the 36.6°C group and 31 from the 37.1°C group) and 11 double ET were performed. The clinical pregnancy rate after SET was significantly higher when culture was performed at 37.1°C (77.4% versus 46.4%; p = 0.029, Chi Square test).

Limitations, reasons for caution: This trial was designed to detect a possible effect of incubation temperature on embryo quality and not on the treatment outcome. An RCT (36.6°C versus 37.1°C) with clinical pregnancy rate as primary endpoint is planned in order to confirm the present findings.

Wider implications of the findings: Modern direct-heat bench-top incubators with individual incubation chambers allow extreme stable temperatures and fast recovery after lid openings, which might be the future for embryo culture. Strict temperature control during incubation is indicated to obtain excellent results.

Trial registration number: NA.

O-094 Key performance indicators (KPIs-Score) based on clinical and laboratorial parameters can establish benchmarks for internal quality control (IQC) in an embryo cryopreservation programme

A.L. Mauri[1,2], C.G. Petersen[1,2], L.D. Vagnini[2], A. Renzi[2], B. Petersen[2], M.C. Mattila[1], V.A. Comar[1], J. Ricci[1], F. Dieamant[1,2], J.B.A. Oliveira[1,2], R.L.R. Baruffi[1,2], J.G. Franco Jr.[1,2]

[1]Centre for Human Reproduction Prof. Franco Jr, Research, Ribeirao Preto, Brazil
[2]Paulista Center for Diagnosis- Research and Training, Research, Ribeirao Preto, Brazil

Study question: Could a KPIs-score with clinical and laboratorial parameters be used to establish benchmarks for internal quality control (IQC) in an embryo cryopreservation IVF/ICSI programme?

Summary answer: This KPIs-score was able to define benchmarks for clinical pregnancy(CP). IQC for cryopreservation programme could utilize this KPIs-score to detect problems in the clinical-laboratorial interface.

What is known already: KPIs have been employed for IQC in the cryopreservation laboratory by using indicators such as embryo cleavage rates within 24 hours, percentage of embryo survival, and number of top-quality embryos after freezing-thawing. Laboratory conditions (solutions for freezing and thawing, temperature, pH, humidity, air quality, culture media, equipment, etc.) could modify these variables and negatively impact the final results. However, in no time, clinical KPIs (C-KPIs) such as age, AMH and number of oocytes collected are added to laboratory KPIs (L-KPIs) for analysis, despite the fact that the final endpoint is the evaluation of clinical pregnancy (CP) rates after embryo thawing.

Study design, size, duration: In this prospective cohort study, 158 patients underwent cryopreservation IVF/ICSI cycles during 2015-2016. The total KPIs-score was obtained by the analysis of the following C-KPIs(fresh cycle data): age, AMH, and number of M-II oocytes, and L-KPIs(frozen-thawing cycle data): morphological quality of cryopreserved embryo Iot(MQCL) and embryo cleavage rate (ECR) after thawing. The total KPIs-score was correlated with

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presence or absence of CP. The relationship between the C-KPIs-score and L-KPIs-score was analysed to establish quality standards.

Participants/materials, setting, methods: A total of 158 patients (33.8 ± 3.2years) participated in this research. The total KPIs-score was determined by the analysis of 5 parameters: age, AMH, number of M-II oocytes, morphological quality of cryopreserved embryonic lot (MQCEL) and embryo cleavage rate (ECR) after thawing. The maximum total KPIs-score was 25 points (Table I).

Main results and the role of chance: The model of logistic regression (MLR) with respect to CP and the total KPIs-score (158 patients/53 CP) resulted in an odds ratio of 1.27 (95%CI: 1.12 to 1.43). Also, there was a significant difference ($P < 0.0001$) with respect to the total KPIs-score mean among the group of patients with CP (KPIs-score = 21.1 ± 2.8) and the group without CP (KPIs-score = 18.9 ± 3.3). Clinical pregnancy probabilities (CPP) could be obtained using the MLR (prediction key) with the total KPIs-score as a predictor variable. Therefore, KPIs-score = 25/CPP = 62% (95%CI: 46% to 75%), KPIs-score = 20/CPP=33% (95%CI: 26% to 41%), KPIs-score=10/CPP=4% (95% CI: 1.2% to 14%), etc. Moreover, the mean C-KPIs-score obtained in the pregnancy group was 13 ± 2, and the L-KPIs-score was 8.1 ± 2.0. Routinely, in all cases where the C-KPIs-score was ≥11 after the IVF/ICSI procedure but the L-KPIs-score (freeze-thawing) obtained was ≤6 (approximately one standard deviation less than the mean), a detailed revision of the laboratory procedure was performed to check quality standards.

Table I The KPIs-score system for the cryopreservation programme.

SCORE POINTS	AGE (years)	AMH (ng/ml)	OOCYTES-MII (n)	MQCEL EMBRYOS	ECR
1	≥40	< 1	≤3	ONLY LOW-QUALITY	EMBRYOS WITH ECR<100%
3	37–39	≥1-<2	4–6	1 TOP-QUALITY OR ≥1 INTERMEDIATE-QUALITY	1 EMBRYO WITH ECR = 100%
5	≤36	≥2	≥7	≥2 TOP-QUALITY	≥2 EMBRYOS ECR=100%

Limitations, reasons for caution: One of limitations of the KPIs-score would be the necessity of each clinic to set up their own KPIs-score and future benchmarks.

Wider implications of the findings: The total KPIs-score was able to establish benchmarks for clinical pregnancy in an embryo cryopreservation programme. Also, the KPIs-score can provide insights about the clinical-laboratory interface. A comparative analysis between the values of the C-KPIs-score and L-KPIs-score can detect laboratorial problems or pitfalls in the work of clinicians and embryologists.

Trial registration number: Not applicable. All patients provided written consent, and the local Research Ethics Committee approved the study.

O-095 A quick, easy and effective sperm wash procedure for HCV+ and HIV+ patients

J. De Creus, E. Van De Voorde, E. Van den Abbeel, K. Tilleman, P. De Sutter

University Hospital Ghent, Reproductive Medicine, Ghent, Belgium

Study question: To develop a quick and efficacious semen washing protocol to remove viral HCV and HIV particles from seminal plasma.

Summary answer: A protocol comprised of 1 density gradient and 2 semen washes was able to remove viral particles and resulted in higher sperm yield after processing.

What is known already: Despite the controversy whether antiretroviral medication has a negative effect on semen quality (Garrido et al. (2004), Kehl et al.(2011), ICSI is needed when sperm quality is low. Precautions need to be taken in treatment of serologically discordant couples in ART. Many centers use a semen wash procedure like density gradient, swim up and buffer washing to effectively remove viral particles from the semen sample and selecting motile spermatozoa. Although the risk for viral transmission from parents to children is marginally low through ART procedures, techniques and protocols are also established for the safety of laboratory staff.

Study design, size, duration: Optimisation of the semen wash protocol was performed by using semen samples of consenting patients artificially spiked with HCV+ blood plasma.Four semen wash procedures were compared: P1: standard in house HIV protocol; P2 described by Molina et al. (2013); P3: Savasi et al. (2013); P4: optimised short protocol. P4 was validated using 3 different ejaculates of 1 HCV RNA+ patient and 25 ejaculates of 17 HIV+ patients.

Participants/materials, setting, methods: In total 46 samples were used: artificially HCV spiked(n = 14), negative controls(n = 4), HCV+ patient samples(n = 3) and HIV+ patient samples(n = 25).P4 was implemented in the clinic for both HCV+ and HIV+ patients. A comparative descriptive retrospective analysis was conducted to verify the sperm yield after washing in HIV patients P1(n = 18 patients) versus P4(n = 7 patients). Quantification of viral load in supernatant and sperm pellet after every centrifugation step was measured using real-time PCR on Abbott m2000sp.

Main results and the role of chance: P1, the standard rigorous HIV protocol used in the lab, consisted of wash, gradient centrifugation, wash, swim up and a final washing step. It was insufficient for the removal of HCV particles in artificially spiked samples of which the viral load ranged from 8,3*10 IU/ml to 8,3*106 IU/ml. Processed samples showed 1,26 ± 0.28% of initial viral load indicating a huge reduction in viral load, but without complete removal. Additionally, P2 and P3, established protocols from literature, were unable to remove the viral particles in the spiked sperm samples: 3.58 ± 2.57% remained after processing. No HCV particles were detected in the artificially spiked HCV samples processed with P4. P4 consisted of 2-layer density gradient at 400 g 20', followed by 2 washes with buffer at 400 g 10'. Most of the viral load (97,15 ± 2,85%) was removed after the density gradient. Validation of the wash protocol was performed on a HCV patient having a viral load of 3.88*105 IU/ml. Three different ejaculates were processed, all samples were negative and the samples were released for clinical treatment. P4 was subsequently implemented for HIV+ patients (also RNA virus). Semen processing with P1 (n = 18 patients) and P4 (n = 7 patients) showed a statistically significant higher yield (Student's T-Test p = 0.04).

Limitations, reasons for caution: The viral load was measured by real-time PCR, with a quantifiable limit for HCV ≥30 IU/ml. Viral particles may still be present if concentrations are lower than the detection limit. The sample size of this study is rather low due to the specific patient cohort being studied.

Wider implications of the findings: Short and effective protocols result in fewer manipulation steps, with less possibilities for cross contamination and safer handling of the samples. A higher yield in semen after preparation can be very important for patients already compromised in semen quality like serologically positive patients.

Trial registration number: This research is conducted with the approval of the local ethics committee (2013/715).

INVITED SESSION

SESSION 26: ENDOMETRIAL STEM CELLS: HOPE AND EXPECTATION

Tuesday 4 July 2017 Plenary 1 08:30–09:30

O-096 The endometrium in women with PCOS

T. Piltonen

University of Oulu, Department of Obstetrics and Gynaecology- Oulu University Hospital- University of Oulu and Medical Research Center and PEDEGO Research Unit, Oulu, Finland

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Abstract text

The endometrium in women with PCOS

Several studies suggest that the endometrium in women with polycystic ovary syndrome (PCOS) is compromised. Indeed, the women have shown to present with several endometrial abnormalities possibly explaining some of the adverse endometrium-related clinical outcomes. PCOS and an increased miscarriage rate have been suggested to coincide, but the results remain conflicting as obesity and ART are also associating factors. On the other hand, recent studies have also shown an increased risk of pregnancy-induced hypertension, preeclampsia, and premature delivery in women with PCOS that may relate to altered decidualization/placentation in these women. In fact, histological and molecular studies have shown placental abnormalities including histological findings related to inflammation and poor placental development as well as altered steroid hormone signaling pathway activation in women with PCOS. Alarmingly, the findings have been reported to occur even in uncomplicated pregnancies. Beyond fertility issues, PCOS *per se* is associated with an increased risk for endometrial cancer, with obesity aggravating the risk. Previous studies have shown for example an altered steroid receptor and their co-activator expression, inflammatory profile and changes in targets related to glucose metabolism that might also relate to endometrial stress and increased cancer risk.

Despite several endometrial abnormalities in women with PCOS, up to date no common clinically relevant markers exist as a tool to screen endometrial abnormalities in these women. The most investigated markers have been related to steroid hormone action endometrial and receptivity/decidualization, glucose metabolism, apoptosis and inflammation/immune cell migration. However, as concluded, the clinical relevance of these findings still awaits future clarification and so far no common screening protocols/recommendations for women with PCOS are established. Some recent studies have indicated that as for interventions, weight reduction, exercise and perhaps metformin could be useful tools to improve endometrial health in women with PCOS. The improvement might stem from the improved endometrial environment where endometrial regeneration from stem cells results into healthier endometrium in the subsequent cycles. However, further studies are warranted to answer some of the clinically relevant questions. In order to further develop and design interventions, the future aim should be in testing optimal methods/protocols and cell types for endometrial sample collection, validating screening tools and identifying the most severe PCOS phenotypes relating altered endometrial health.

O-097 Stem cell treatment for endometrial pathologies

X. Santamaría Costa[1], S. Cabanillas[2], C. Arbona[3], I. Cervello[4], F. Raga[5], J. Ferro[6], J. Palmero[7], J. Remohí[8], A. Pellicer[9], C. Simon[10]

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Abstract text

The endometrium has a unique capacity to regenerate its functional layer during the menstrual cycle. Different factors, however, can cause destruction of the endometrium right through to its basal layer into the muscle layers of the myometrium. The damaged endometrium may not be repaired correctly which leads to the formation of Intrauterine Adhesions (IUA) and, therefore, lead to Asherman Syndrome (AS).

There is little high quality epidemiological data and the exact prevalence in terms of cases per 10,000 people is not reported in publications or patient associations. However, incidence and prevalence in Europe may be assessed after certain estmations.

Almost the entire majority of the causative factors on endometrial pathologies (iatrogenic and non-iatrogenic) have in common the triggering an inflammatory response. That is the reason why, some authors believe that the inflammatory pathway could play an important role in the pathogenesis of AS, resulting in the release into the intrauterine environment of factors which stimulate the formation of fibrotic tissue after endometrial trauma. On the other hand, it is determined that only 15–20% of curettages cause IUA adhesions suggesting that there may be some additional factors of susceptibility. Additionally, it is known that certain types of miscarriage or women with Müllerian duct malformation have a higher incidence of adhesions formation. All these facts, demonstrate that not only iatrogenic, but also genetic, anatomical and infection processes contribute to the ethiopahogenesis of this pathological entity.

Hysteroscopy is considered as the gold standard for diagnosis and treatment of AS. Although hysteroscopy is a technique very effective to solve mild cases of AS, the possibility of adhesion recurrence in moderate and severe cases is high according to published reports. Therefore, new strategies should be considered to treat these refractory cases.

Cells derived from bone marrow co-expressing CD133+ and vascular endothelial growth factor receptor 2 (VEGFR2) represent a subpopulation of cells known as endothelial progenitor cells (EPCs) with endothelial progenitor capacity that can be mobilized to the circulation and can improve neoangiogenesis of pre-existing endothelium. CD133+ Bone Marrow Derived Stem Cells (BMDSC) have been employed in clinical trials for regenerative medicine in non-haematological applications and have been shown to functionally contribute to neoangiogenesis during wound healing and limb ischemia, post-myocardial infarction, endothelialisation of vascular grafts, atherosclerosis, retinal and lymphoid organ neo-vascularization and tumour growth.

Some clinical and preclinical studies have shown that BMDSC CD133+ broadly act by regenerating the endometrium and thereby increasing fertility as a result of assisted reproductive treatment (ART)

In this sense, a pilot study CD133+ BMDSCs has been previously undertaken (Trial Registration Number: NCT02144987) that demonstrated that in the first 3 months, autologous cell therapy, using CD133+ BMDSCs in conjunction with hysteroscopy and hormonal replacement therapy (HRT), increased the volume and duration of menses as well as the thickness and angiogenesis processes of the endometrium while decreasing intrauterine adhesion scores and improved reproductive outcomes achieving the delivery of 5 healthy New Born after the treatment.

However, the precise molecular mechanism is still not well understood although pre-clinical and clinical studies in other pathologies can help to understand their mechanism of action. Therefore, further research and new lines of research have to be developed in order to increase the effectiveness of these novel therapy.

INVITED SESSION

SESSION 27: MAKING THE BEST OF PSYCHO-SOCIAL SUPPORT

Tuesday 4 July 2017 Plenary 2 08:30–09:30

O-098 Integrating Psychological Care into the Infertility Clinic Setting

A. Domar

Domar Center for Mind/Body Health, Waltham, U.S.A.

Abstract text

Integrating Psychological Care into the Infertility Clinic Setting

A Domar

Domar Center for Mind/Body Health, Boston IVF, Harvard Medical School, Boston MA, USA

Recent research documents once again the prevalence of negative psychological symptoms in both men and women experiencing infertility. The majority of women reported clinical levels of both anxiety and depression and the majority of men reported clinical levels of anxiety. Studies from across the world have documented the negative psychological impact of infertility.

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These psychological symptoms experienced by our patients have the potential to impact their medical care in three ways:

(1) Psychological distress is unpleasant to experience
(2) Distressed patients are more likely to drop out of treatment
(3) Anxious and depressed patients are more challenging to care for

Current recommendations include screening patients so as to identify those who are at highest risk of terminating treatment prior to achieving a pregnancy, providing written stress management materials, and streamlining the treatment protocols so as to decrease the burden of care. A mental health professional embedded into an infertility clinic setting can serve to address all three of these issues.

However, it is possible to do far more to serve the needs of patients and those who care for them. Based upon the results of a randomized controlled trial conducted in Spain, it is apparent that training physicians in empathic skills communication can have a significant impact on patient assessment of quality of care. It is not challenging for a psychologist to train all employees who have patient contact in empathic communication. Patients also benefit from structured behavioral and cognitive suggestions to decrease their stress level while cycling. Recent research showed that such interventions are associated with a decrease in treatment termination rates. Finally, the stress level of patients creates a challenging situation for the nurses who care for them. Burnout and turnover are commonly reported by infertility nurses. Mental health professionals can provide stress management training and support for nurses and others who care for patients.

This presentation will include a brief review of the research on the efficacy of psychological interventions to decrease the distress of patients, the impact on dropout rates, and methods to lower the stress levels of nurses and support staff. The main goal of the presentation however is to provide participants with concrete examples and suggestions of how to implement these changes into the infertility clinic setting.

O-099 How to help patients help themselves

D. Lancastle

University of South Wales, School of Psychology and Therapeutic Studies, Pontypridd, United Kingdom

Abstract text

How to help patients help themselves

Becoming a parent is a key personal life goal and people tend to assume that unprotected sexual intercourse will automatically lead to pregnancy as soon as they decide to conceive. For the 9% or so of couples that are infertile, however, such an assumption is challenged with monthly disappointment and the realisation that their futures will necessarily include treatment to help them conceive and/or the eventual acceptance that they will never become the parent of a child that is biologically their own. More than half of couples do seek treatment, but although treatment may enable couples to become parents this is not guaranteed and treatment itself is associated with physical, psychological, and social demands that can have a negative impact on quality of life and mental health.

The balance between the demands of life-experiences and the resources people have available to deal with those demands is a key principle of the Transactional Theory of Stress and Coping (Lazarus & Folkman, 1984). According to this approach, psychological stress is experienced when the demands of the situation exceed resources. In terms of demands, infertility and fertility treatment certainly heap a significant number of these onto the shoulders of infertile couples! There are cognitive demands such as decisions about treatment, the financial and physical demands of treatment itself, uncertainty about treatment success, worries about pregnancy, and distress from treatment failure, to name but a few. As it may not be possible, however, to alter specific treatment protocols to lessen demands and it is certainly not possible to guarantee treatment success, lessening the demands of treatment is not within the control of medical professionals. Bolstering the resources that patients have available to help them deal with those demands is therefore an appropriate way to try and redress the demands-resources balance and bolster patients' psychological wellbeing.

The ESHRE Guideline "Routine psychosocial care in infertility and medically assisted reproduction – A guide for fertility staff on the implementation of psychosocial care by healthcare professionals in infertility and medically assisted reproduction" differentiates specialised infertility counselling and psychotherapy for the minority of patients with clinically significant emotional problems or special needs, from 'routine' psychosocial care. This presentation speaks to the latter form of psychosocial support and provides information about theoretically driven self-help interventions that could provide the basis of a 'self-help tool kit' that medical professionals can guide patients towards as part of routine psychosocial care.

Effective coping is our first line of defence against stressful life experiences, and the self-help interventions presented can be broadly described as 'coping interventions' because they encourage individuals to employ coping strategies (e.g., positive reappraisal coping, distraction, relaxation, social support seeking) that have been shown to be appropriate and helpful during stressful life-experiences including fertility treatment. Such self-help interventions are a valuable adjunct to the high quality psychosocial support offered by medical professionals in face to face clinic sessions because patients can access these interventions in their own time to meet their ongoing needs when they are not in clinic. Bolstering coping resources in this way will help patients to negotiate their way through the various psychological demands of infertility and fertility treatment.

INVITED SESSION
SESSION 28: CONTROVERSIES IN CRYOPRESERVATION

Tuesday 4 July 2017 Room A 08:30–09:30

O-100 Oocyte cryopreservation: can it compete with cleavage stage and blastocyst cryopreservation?

D. Gook

The Women's Hospital, Reproductive Services /Melbourne IVF, Parkville- Victoria, Australia

Abstract text

For over 30 years embryo cryopreservation has played an important role in ART. Increasing rates of survival and the demonstration of implantation rates similar to those of equivalent fresh embryos, either at cleavage stages or after extended culture to the blastocyst stage, has influenced clinical IVF practice resulting in a major trend towards single embryo transfer in many parts of the world. More recently, the possibility of achieving improved perinatal outcomes, by transferring embryos in a menstrual cycle unaffected by ovarian stimulation, has led to a move towards the practice of cryopreserving all embryos generated in a stimulated cycle.

In contrast, oocyte cryopreservation has traditionally only been applied when embryo cryopreservation was not an option e.g. to provide a fertility preservation option in cases where there was no male partner, to circumvent legal restrictions that prohibited embryo storage and to defer insemination in cases where no sperm could be obtained in an ART cycle. This more limited application was based on a perception that outcomes from oocyte cryopreservation were likely to be inferior to those achievable with embryo cryopreservation.

Within the last decade, however, the outcomes from oocyte cryopreservation have improved significantly with the advent of reproducible vitrification techniques. Extensive application of this approach within large donor oocyte programs has demonstrated that high survival rates can be achieved and that the surviving oocytes can fertilise, develop and implant at rates indistinguishable from those of comparable fresh oocytes. This has led many to question whether oocyte cryopreservation can now match embryo cryopreservation in terms of efficiency and whether its use should now be considered for application in a wider context.

In this presentation, the evidence to support the contention that oocyte cryopreservation can compete with embryo cryopreservation in terms of overall efficiency will be analysed. In addition, if the outcomes from oocyte cryopreservation can be shown to approximate those from embryo cryopreservation, the advantages and disadvantages of extending the use of oocyte cryopreservation into other clinical contexts will be discussed.

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O-101 Can automation guarantee the standardization of cryopreservation?

L.F. Rienzi

Genera c/o Clinica Valle Giulia GENERA center for Reproductive medicine, GENERA center for Reproductive medicine, Roma, Italy

Abstract text

With the introduction of vitrification in ART a substantial improvement in oocyte and embryo survival rates is obtained and, in turn, an increased live birth rate is reported with the use of vitrified embryos as compared to slow-frozen ones. Successful cryopreservation programs are essential to maximize the efficacy of ovarian stimulation cycles in IVF treatments. Moreover, cryopreservation allows cycle segmentation, fertility preservation and the use of cryopreserved oocytes for egg-donation programs.

Vitrification approach permits the solidification of cells into a glass-like state without the formation of ice crystals. This procedure, that involves multiple steps, may vary substantially from lab to lab due to the different physical and chemical conditions. Moreover, when performed manually, differences between operators are common during the stringent times pick-and-place operations. There is thus a poor standardization between centres and across operators. All these aspects may affect the overall efficacy of the technique.

With the aim to standardize vitrification procedure automation is suggested. Automatic cell manipulation has several important potential advantages including:

– Minimization of operation-caused errors and discrepancies;
– Alleviation of operators from this task with better exploitation of human resources;
– Optimization of timing controls for each step, including the vitrification itself;
– Possibility to process multiples samples contemporaneously;
– Opportunity to normalize chemical and physical conditions (including temperature and pH).

Over from vitrification process, robotic platforms combining embryo culture, evaluation and manipulation are expected in the near future. Such integrated platforms are very likely to provide the standardization of IVF, aiming at guaranteeing high standard of quality for every IVF center.

INVITED SESSION

SESSION 29: ASRM EXCHANGE SESSION

Tuesday 4 July 2017 Room B 08:30–09:30

O-102 Intention to treat: A rational approach to the management of the older and low response IVF patient

O. Davis

New York- NY, U.S.A.,

Abstract text

IVF has a high probability of success in young patients with normal ovarian reserve, due to the generation of robust numbers of euploid embryos. Patients of advanced reproductive age confront the challenges of higher aneuploidy rates coupled with fewer oocytes/embryos. Currently, many IVF clinics are restricting their treatment regimens to those of potential merit for their best prognosis patients, i.e. day 5-only transfers and, increasingly, the routine application of PGS; the obvious theoretical benefit is eSET of PGS-normal blastocysts thus mitigating the risks of dizygotic twinning. Universal codification of this approach for all IVF patients poses significant pitfalls for those women with an intrinsically poorer prognosis, however. In this presentation, a flexible approach to treating each patient according to her individual needs on an intention-to-treat basis will be explored. Simply put, the focus should be shifted from maximizing the IVF clinic's per transfer success rates to optimizing every patient's chances for achieving a successful pregnancy when she enters into treatment. This discussion will emphasize the importance of a willingness to treat the well-informed poorer prognosis patient in an individualized manner, the selection of optimal stimulation protocols, the inevitable impact of embryo attrition imposed by prolonged culture to day 5 (for fresh transfer or PGS) and the potential for misdiagnosis following PGS which may lead to further embryonic "loss". Similar to onco-fertility patients presenting with a limited time horizon in which to maximize their fertility preservation, the older/low response patient also has a limited window of opportunity during which the loss of potentially healthy embryos in an effort to promulgate only the best prognosis embryo transfers may, in fact, undermine the individual patient's chances for success. The clinical emphasis should shift back to an intention-to-treat paradigm initiated at the point of entry of the patient into an IVF clinic, and specifically at the commencement of each stimulation cycle. A policy of systematic embryo de-selection following oocyte retrieval in an effort to maintain the best possible success rate at the point of embryo transfer is better suited to the best prognosis patients.

O-103 How to counsel the older man seeking hormone and fertility therapy

R. Sokol

Ventura- Ca, U.S.A.,

Abstract text

How To Counsel The Older Man Seeking Hormone And Fertility Therapy.

Rebecca Z. Sokol, M.D., M.P.H.
Professor Emerita
Medicine and Obstetrics and Gynecology
Keck School of Medicine
University of Southern California

Evidence supports the hypothesis that reproductive potential declines with age in men, and that as men age they have a small increased chance of passing on genetic defects to their children. A gradual decline in circulating testosterone levels is reported in aging men, though the levels tend to remain within the normal range for testosterone. Cross sectional and longitudinal studies report an estimated 0.4% year decrease in total testosterone levels and 1.3% decrease in free testosterone levels. The decline in testosterone is primarily a result of reduced production of testosterone by the testes associated with a concomitant increase in LH secretion. A gradual decline of spermatogenesis also occurs with age. Daily sperm production in the testes is negatively correlated with age. Semen parameters begin to decline by age 35 years, but rarely fall into the "infertile" range. Time to pregnancy significantly increases with the age of the male partner, fecundity may decrease with age of the male partner, and the rate of miscarriage is increased in partners of older men. Testosterone replacement therapy does not improve spermatogenesis and is contraindicated for men attempting to initiate a pregnancy because it acts as a contraceptive. There is an association between increased paternal age and increased genetic diseases in the offspring of older men (Paternal Age Effect Disorders). Sperm replications increase as men age, causing an increase in point mutations and resultant genetically inherited syndromes. The incidence of schizophrenia and autism also may be increased in the offspring of older men. The etiology for this increase is multifactorial. Although the absolute number of children born with a genetic or neurogenic disorder is small, and the decline in fertility potential with age is subtle, counseling the couple about the risks of older paternal age is recommended.

INVITED SESSION

SESSION 30: PARAMEDICAL INVITED SESSION - PROVIDING PATIENTS WITH HELPFUL ADVICE

Tuesday 4 July 2017 Room W+X 08:30–09:30

O-104 Supporting women who are pregnant after assisted conception

H. Vervenne

Leuven University Hospital, Leuven, Belgium

O-105 Describing embryos to patients?

V. Provoost

Bioethics Institute Ghent BIG Ghent University, Vakgroep Wijsbegeerte en Moraalwetenschap, Gent, Belgium

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Abstract text

Describing embryos to patients?

Assisted reproduction takes patients on a journey through an entirely new domain. One aspect of that journey consists of the decisions patients need to make about the embryos created during in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI) treatment. They will need to decide whether to cryopreserve the embryos, whether or not to use the embryos for treatment and what to do with the embryos that are no longer wanted or needed. In light of these decisions, professionals have a duty to inform the patient in a way that is adequate and comprehensible. In this presentation, we will explore ways to describe embryos to patients by first listening to how patients conceptualise and feel about their embryos. For this, we will use research insights about patients' beliefs and views.

Although patients also express awe and marvel about embryos 'coming back to life again' during the thawing process, research has shown that for most patients making decisions about embryos is difficult and stressful. Patients usually know little about the medical or technical procedures involved in treatments with cryopreserved embryos; a lack of knowledge that is compensated for by a high level of confidence in the medical team. However, when people are dealing with matters they do not fully grasp, they tend to fill the gaps in their knowledge with information from other, more familiar, domains of experience. For infertility patients this is often compensated by using metaphors, mainly from the kitchen. For instance, cryopreservation and storage of embryos in liquid nitrogen tanks at a temperature of -196° Celsius is often compared with freezing meat by the food industry or in the freezer at home. Such comparisons might seem harmless at first glance but they do entail some dangers. Projecting characteristics of food or food storage (such as the reduction in quality during storage time) onto embryo storage can result in a belief that stored embryos had a best before or expiry date. In line with this, the use of frozen embryos has been perceived as weird and unnatural, raising doubts about the effectiveness of using cryopreserved embryos as compared to fresh ones and about the effects of freezing, storage and thawing on the health of their future children.

The dangers of these misconceptions become more clear when we look at decisions that are inconsistent with the patients' wish for a child. One example is seen in patients who want to continue storage (often linked to feelings of anticipated regret) while not wanting a(nother) child. Another example shows exactly the opposite: women who want a(nother) child but decide to end storage of their embryos. The latter group of women sometimes returns to the clinic for a new cycle of IVF to create new embryos only months after their decision to discard their cryopreserved embryos. Apart from reducing their chances of becoming pregnant, these decisions also give rise to extra (and potentially unnecessary) financial and emotional investment in their treatment. Especially the latter group appeared to have misconceptions and negative beliefs about the embryo. These are the patients most in need for appropriate information. However, it is important to note that the women who had doubts or mistrusted the use of cryopreserved embryos did not talk to the medical staff about this, which suggests that these patients' worries go unnoticed by professionals.

Findings like these demonstrate the importance of listening to patients, taking into account their feelings, conceptualisations, social contexts and the emotional basis of their decision making. Only this way, we can find the most appropriate way to describe embryos to patients.

INVITED SESSION

SESSION 31: WHO SESSION - ADDRESSING THE UNMET NEED FOR INFERTILITY SERVICES

Tuesday 4 July 2017 Room C 08:30–09:30

O-106 Global prevalence of infertility

R. Kennedy

Warwick Medical School, Coventry, United Kingdom

O-107 Status of infertility services globally

M. Ali

WHO Headquarters, Geneva, Switzerland

O-108 Addressing barriers to access to infertility services, the European experience

P. De Sutter

Ghent University Hospital, Dept. Reproductive Medicine, Ghent, Belgium

O-109 WHO priorities in infertility

J. Kiarie, I. Toskin

WHO Headquarters, Geneva, Switzerland

SELECTED ORAL COMMUNICATIONS

SESSION 32: INSIDE THE EMBRYO

Tuesday 4 July 2017 Plenary 1 10:00–11:30

O-110 Factors affecting embryonic mosaicism

E. Fragouli[1], S. Alfarawati[1], M. Simpkins[1], G. Cutts[1], K. Spath[1], D. Babariya[1], N. Kubikova[1], L. Rubistello[2], S. Munne[2], D. Wells[1]

[1]Reprogenetics UK, Institute of Reproductive Sciences, Oxford, United Kingdom
[2]Reprogenetics LLC, Reprogenetics LLC, Livingston, U.S.A.

Study question: Do differences in IVF procedures (e.g. culture medium, biopsy practitioner, incubator types, etc) and patient characteristics influence the frequency of mitotic errors, leading to mosaicism?

Summary answer: The type of medium used during embryo culture affects mitotic malsegregation and blastocyst mosaicism rates. Additionally, certain patients generate an excess of mosaic blastocysts.

What is known already: Mosaicism - the presence of chromosomally distinct cell lines within the same embryo - is common during preimplantation development, caused by incorrect segregation of chromosomes during mitosis. Recent innovations (next generation sequencing, NGS) have, for the first time, allowed mosaicism to be accurately detected in trophectoderm (TE) samples taken from embryos for the purpose of preimplantation testing for aneuploidy (PGT-A). Mosaic embryos have been shown to be associated with reduced implantation rates and elevated miscarriage rates. Recent data has revealed that the frequency of mosaicism in TE biopsies differs between IVF clinics. The cause of this variation is currently unclear.

Study design, size, duration: The rate of mosaicism in TE biopsies was assessed for 27 different European and US clinics, by examining NGS results obtained during PGT-A. All clinics completed a detailed survey, capturing information about embryological practices (e.g. varieties of media used for oocyte collection and embryo culture, types of incubators, oxygen concentrations, timing of embryo checks, etc). Mosaicism rates were also considered with respect to the embryologist who performed the biopsy, as well as the specific patient.

Participants/materials, setting, methods: A total of 19,719 embryos were examined (average 730 per clinic). All these embryos were biopsied at the blastocyst stage. The biopsied TE samples were composed of ~5 cells and were subjected to comprehensive chromosome analysis using a next generation sequencing strategy. The NGS method underwent extensive validation and was demonstrated, in blinded experiments, to provide accurate detection of mosaic samples, even when only one cell out of five was aneuploid.

Main results and the role of chance: A total of 15% (2,880/19,719) of blastocysts had one or more mosaic chromosome abnormalities in their associated TE specimens. 45% of embryos (8,927/19,719) were characterized as euploid, while the remaining 40% (7,912/19,719) had aneuploidy affecting all of the cells in the TE sample, with or without additional mitotic errors. The mosaicism rate differed significantly between clinics ranging from 11% to 27%. Twenty of these clinics cultured their blastocysts in a continuous medium system, using three different brands of media. One of these was associated with significantly ($P < 0.05$) larger numbers of mosaic blastocysts, compared to the other two. A sequential blastocyst culture system was employed in the remaining 7 clinics, and three different media were used. Significantly more mosaic embryos ($P < 0.02$ and 0.0001) were seen for two of the three media used. A significant difference ($P < 0.0001$) in mosaicism rate was also observed between continuous and sequential culture

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systems, with the former associated with more mosaic embryos (17% vs.13%). There was no relationship between the frequency of mosaicism and different biopsy practitioners, neither other aspects of embryo culture. However, we did observe that patients with multiple unsuccessful IVF/PGT-A attempts had a tendency to generate more mosaic blastocysts than other patients.

Limitations, reasons for caution: Data analysis was based on NGS results obtained from a single TE biopsy specimen. This likely underestimates the true frequency of mosaicism as one TE sample cannot guarantee to contain representatives of all of the different cell lines within an embryo. Nonetheless, validation data indicates most mosaics are successfully detected.

Wider implications of the findings: This study provides evidence that different media formulations directly influence the frequency of mitotic errors and embryo mosaicism, with significant implications for IVF success. Additionally, certain patients have a predisposition to generating mosaic embryos. Our results suggest that maintenance of genetic competence should represent a new focus for media development.

Trial registration number: Not applicable.

O-111 The relationship between blastocyst morphology, euploidy, aneuploidy and mosaicism

M. Simpkins, S. Alfarawati, G. Cutts, K. Spath, N. Kubikova, D. Wells, E. Fragouli

Reprogenetics- UK, Institute of Reproductive Sciences, Oxford, United Kingdom

Study question: Does the presence of mosaic chromosome abnormalities affect the morphology of blastocysts? Do mosaic blastocysts behave more like chromosomally normal or fully aneuploid embryos?

Summary answer: Mosaic blastocysts tend to be morphologically more similar to fully aneuploid embryos, rather than completely euploid embryos.

What is known already: Mosaicism is the result of post-zygotic chromosomal malsegregation, most often occurring during the first three cleavage divisions, and leading to the presence of chromosomally distinct cell lines within the same embryo. With the recent increase in use of Next Generation Sequencing (NGS), a sensitive method capable of detecting mosaic abnormalities in trophectoderm biopsy specimens, issues have surfaced regarding the way in which mosaic embryos are dealt with clinically. Specifically, the implantation potential of mosaic blastocysts is unclear. Questions therefore have arisen about whether mosaic embryos should be treated in the same way as fully aneuploid ones, and excluded from transfer.

Study design, size, duration: The relationship between blastocyst ploidy and different morphological characteristics was assessed. These included expansion, and grade of inner cell mass and trophectoderm. The blastocysts investigated were grouped in the following categories: euploid, abnormal (non-mosaic), mosaic (euploid-aneuploid) and mosaic (aneuploid-aneuploid). The morphologies of euploid blastocysts and those carrying non-mosaic or mosaic abnormalities compatible with clinical pregnancy (i.e. certain aneuploidies involving chromosomes 13, 16, 18, 21, 22, X0, and XXY) were also compared.

Participants/materials, setting, methods: A total of 1696 embryos generated by 306 patients were examined. These patients were having IVF treatment combined with preimplantation genetic testing for aneuploidy (PGT-A) for various indications in 12 different clinics. The average female age was 38.4 years. All embryos were biopsied at the blastocyst stage of development. The trophectoderm (TE) samples removed were examined cytogenetically via a highly validated NGS strategy. The analysis of embryo ploidy and associated morphology took place retrospectively.

Main results and the role of chance: 25.5% of blastocysts (433/1696) were found to contain at least one non-mosaic aneuploidy, 25.4% (430/1696) were characterised as having one or more mosaic abnormalities, 27.0% (458/1696) contained a combination of mosaic and non-mosaic chromosomal abnormalities and the remaining 22.1% (375/1696) were euploid. Compared to the chromosomally normal embryos, blastocysts containing abnormal cells (uniformly aneuploid or mosaic) had significantly ($P \leq 0.001$) poorer blastocyst expansion scores. The same embryos were also associated with poorer inner cell mass (ICM) and TE grades ($P \leq 0.05$). The differences were insufficient to allow abnormal embryos to be clearly identified, but they do indicate that aneuploidy is already impacting embryo development at this early stage. When the three abnormal groups of embryos were compared to one another (abnormal non-mosaic, mosaic euploid-aneuploid, mosaic aneuploid-aneuploid), no significant differences in any aspects of morphology were observed. The fact that mosaic embryos closely resembled those that are uniformly aneuploid, suggests that they too are likely to be compromised. Finally, the morphology of euploid embryos was found to be indistinguishable from that of embryos having forms of aneuploidy (mosaic or non-mosaic) potentially compatible with a viable pregnancy. This indicates that those types of aneuploidy do little to impair preimplantation development.

Limitations, reasons for caution: Cytogenetic classification was based on TE samples removed from blastocysts during PGT-A analysis. As only a fraction of the cells from each embryo are tested, inevitably some mosaic embryos will be incorrectly classified fully euploid or aneuploid. However, this misclassification is expected to have little impact on the results.

Wider implications of the findings: In morphological terms, mosaic blastocysts closely resemble those that are uniformly aneuploid. In contrast, embryos affected by types of aneuploidy compatible with survival and formation of clinical pregnancies, had morphology equivalent to euploid blastocysts. These findings add to the debate over whether mosaic embryos should be considered for transfer.

Trial registration number: Not applicable.

O-112 Frequency and clinical relevance of mosaic segmental aneuploidy in blastocyst stage human embryos

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Study question: What are the factors influencing the occurrence of segmental aneuploidy in human embryos and what is the clinical significance of such errors?

Summary answer: Specific culture media formulations affect the frequency of segmental aneuploidy, the vast majority of which arises during the first few mitotic divisions after fertilisation.

What is known already: Segmental aneuploidy is characterised by the loss or duplication of chromosomal fragments. Such abnormalities occur at a considerable frequency during preimplantation embryo development, but their underlying cause and clinical significance are poorly understood. Further investigation is required in order to establish, and perhaps one day eliminate, the causes of segmental aneuploidy and to improve management of patients with affected embryos. Next generation sequencing (NGS) is used for preimplantation genetic screening (PGS) and provides highly accurate detection of segmental aneuploidy. Furthermore, NGS is capable of detecting mosaic aneuploidy (the presence of chromosomally distinct cell lines within the same embryo biopsy specimen).

Study design, size, duration: Trophectoderm biopsies from 9,037 blastocyst stage embryos underwent comprehensive chromosome screening via NGS. Patients were referred for PGS for various reasons including advanced maternal age (AMA), recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL). A questionnaire concerning IVF culture practices including culture media systems, brands, incubators, gas composition etc. was completed by all the IVF clinics that referred the patients.

Participants/materials, setting, methods: Trophectoderm biopsies were subjected to whole genome amplification followed by low-pass NGS using the Miseq platform (Illumina). Analysis of the chromosome complement was performed with BlueFuse software (Illumina). Prior to embryo analysis, mosaicism detection using this approach was validated by mixing cells from several aneuploid and euploid cell lines in specific ratios. These experiments demonstrated the accurate detection of different proportions of abnormal cells (including those with segmental aneuploidy) in small samples (~5 cells).

Main results and the role of chance: 20.0% of blastocysts were affected by segmental aneuploidy (1810/9037). The distribution of chromosomal breakpoints was not entirely random, with a number of hotspots revealed, some of

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which correspond to known fragile sites in human genome. Mosaic segmental errors were more common than segmental aneuploidies affecting all of the cells in the sample (1429 versus 507 instances; P < 0.0001), indicating that most of these abnormalities arise after fertilisation. Interestingly, patients with a history of recurrent implantation failure showed an elevated rate of non-mosaic segmental aneuploidy, suggesting that their gametes have an unusually high risk of suffering chromosome breakage during meiosis (16.8% RIF versus 4.8% AMA and 5.9% RM, respectively; P < 0.0001). There was no association between segmental abnormality and female age. Rates of blastocyst segmental aneuploidy were not identical for all clinics, ranging from 2.5%-25.2% of embryos affected (P < 0.0001). In an effort to determine the reason for this variation, culture methods were assessed in detail at ten of the clinics referring samples for PGS. Rates of meiotic segmental aneuploidies were identical across all clinics. However, levels of mosaic (i.e. post fertilisation) segmental abnormalities differed, tending to be higher in clinics utilising continuous culture media as compared to those using sequential (P = 0.019).

Limitations, reasons for caution: The degree of mosaicism may not be representative of the entire embryo in all cases, as it is determined from a small sample of trophectoderm (TE) tissue. Sometimes, mosaicism may be absent from TE sample, but present elsewhere in the embryo.

Wider implications of the findings: The study confirms that segmental aneuploidy is common in blastocysts and typically arises after fertilisation. The results indicate that culture environment modulates the risk of segmental abnormalities occurring, providing an opportunity to optimise embryological methods. Additionally, there is evidence that some patients are predisposed to segmental errors affecting their embryos.

Trial registration number: Not applicable.

O-113 A novel method to calculate the mitochondrial DNA/nuclear DNA ratio in human blastocysts as a biomarker for human embryo implantation potential

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Study question: What is the significance of the human blastocyst mitochondrial DNA/nuclear DNA ratio as an IVF biomarker tested by next-generation sequencing (NGS)?

Summary answer: Mitochondrial-DNA/nuclear-DNA ratio of human blastocyst, adjusted for size/level of chromosomal-aberrations, can be used as a biomarker of implantation potential of euploid embryos in patients >35years.

What is known already: Previous research evaluating levels of mitochondrial DNA and mitochondrial DNA/nuclear DNA ratio (mtDNA/nDNA ratio) of human blastocyst and its clinical significance has shown conflicting results. While some have enthusiastically announced this ratio as promising universal IVF biomarker, there were important arguments against it involving method of testing, study design and accuracy of ratio calculation.

Study design, size, duration: This was a retrospective study where we reanalyzed the PGS data from low pass whole genome sequencing of 1152 blastocysts and developed a mathematical method to accurately calculate mtDNA/nDNA ratio. The studied group also included reanalysis of genomic content and mtDNA/nDNA ratio calculation of 188 blastocyst transfers with pregnancy outcomes.

Participants/materials, setting, methods: Trophectoderm biopsies of D5/D6 blastocysts (n = 1152) from 387 patients were tested with NGS for PGS at Create Fertility Preimplantation Genetic lab. The calculation of mtDNA/nDNA ratio involved quality filtering of mtDNA and nDNA specific reads and included correction factors reflecting the size of nuclear DNA by gender, chromosomal aberration and the level of mosaicism if present.

Main results and the role of chance: Euploid embryos from patients over 35 years of age that implanted (n = 26, 72%) had significantly higher average mtDNA/nDNA ratio than embryos that didn't implant (n = 10, 28%) (0.001227 ± 0.000667 vs 0.0007915 ± 0.000356, p = 0.00172). This difference was not observed in the transferred euploid embryos from patients under 35 years (n = 119). Embryos that were euploid/mosaic in levels less than 40% (n = 32, 17%) had no difference in the levels of mtDNA/nDNA ratio regardless of patient's age and their implantation potential. When comprehensive ratio calculation was applied euploid (n = 574) blastocysts, had significantly lower levels of mtDNA/nDNA ratios (0.00108 ± 0.0007785) compared to aneuploid (n = 260, 0.0014813 ± 0.0018064) p = 0.0008 and mosaic (n = 269, 0.0012918 ± 0.0008516) embryos p = 0.0008. Patient age didn't have significant influence on the mtDNA/nDNA ratio regardless of ploidy.

Limitations, reasons for caution: This study was limited to the samples analyzed in our lab in 2016 and by the number of pregnancy outcomes available until December 2016.

Wider implications of the findings: Overall, our findings provide evidence that accurate calculation of mtDNA/nDNA ratio can be used to prioritize euploid embryos for transfer in patients that are over 35 years of age.

Trial registration number: N/A.

O-114 Quantitative and qualitative changes of mitochondria in human embryos

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Study question: What roles do mitochondria play in the development of human embryos?

Summary answer: The mitochondrial respiratory function of human embryos developed along with embryonic growth although the copy numbers of mitochondrial DNA (mtDNA) decreased transiently before blastulation.

What is known already: Although the rate of oxygen consumption (OCR) in mice and cattle changes during preimplantation embryogenesis, the number of mtDNA copy remains unchanged in mice but changes in cattle and pigs. However, dynamic aspects of mitochondrial functions and mtDNA copy numbers in human embryos during preimplantation development remains obscure.

Study design, size, duration: Sixteen oocytes and 100 embryos were used to assess mtDNA copy numbers and OCR. Three oocytes and 12 embryos were used to determine cytochrome c oxidase activity. All specimens were obtained between July 2004 and November 2014, and donated from couples after they had given informed consent.

Participants/materials, setting, methods: Mature oocytes and developed embryos to 2, 3-4, and 5–8 cell, morula and blastocyst stages after ICSI were used to assess their OCR in the presence or absence of mitotoxins. The mtDNA copy number was determined using the samples after analysis of OCR. The relationships between developmental stages and OCR, and developmental stages and mtDNA copy number were analyzed. Furthermore, cytochrome c oxidase activity was determined in oocytes, 4, 8, morula and blastocyst stages.

Main results and the role of chance: Mitochondrial OCR (mtOCR) was calculated by subtracting the value obtained in the presence of cyanide from those obtained without any mitochondrial toxins. No difference in mtOCR was found from oocyte to 8 cell stages. However, mtOCR increased rapidly at 9–14 cell and later stages (P < 0.01) compared with those until 8 cell stage. Furthermore, the mtOCR at blastocyst stage were significantly higher than those until 14 cell stage (P < 0.01). The number of mtDNA copy per specimen in embryos decreased transiently (P < 0.01) at 2 cell, 9–14 cell and morula stages compared with oocytes. The number of mtDNA copy significantly increased (P < 0.01) in expanded blastocyst stage compared with those in earlier stages. The numbers of mtDNA copy per cell in embryos decreased gradually (P < 0.01) from oocytes toward morula and blastocyst stages. Taken together, OCRs increased toward the morula stage ahead of an increase of mtDNA at the time of blastulation. The undifferentiated state of inner cell mass appears to be associated with a low OCR. On the other hand, the aerobic metabolism of mitochondria in trophectoderm cells increased.

Limitations, reasons for caution: All samples except for oocytes were used after vitrification and warming following the guidelines of the Japan Society of Obstetrics and Gynecology. To avoid contamination with mtDNA from spermatozoa attached to the zona pellucida, all embryos were obtained by using intra-cytoplasmic sperm injection.

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Wider implications of the findings: Modifying the energy sources required for mitochondrial functions would provide an environment with less stressful conditions for the culture of embryos. The present work showing changes in mitochondrial structure, function, and mtDNA copy numbers during preimplantation development would be useful in optimizing culture media for the development of human embryos.

Trial registration number: This study was approved by the IRB of IVF Namba Clinic and the Japan Society of Obstetrics and Gynecology (Registry numbers 135 and 138).

O-115 Single-Cell Analysis of Telomere Length Dynamics and DNA Damage Across Early Human Development Suggest Alternative Lengthening of Telomeres

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Study question: What happens to telomere length during human meiotic maturation and pre-implantation development? What is the impact of DNA damage and telomere attrition on human development?

Summary answer: DNA damage and telomere attrition limit oocyte maturation in vitro. Telomeres are short in oocytes, increase markedly, and develop increased heterogeneity during pre-implantation development.

What is known already: Telomere length reflects aging in many cell types. Sperm, which emerge from spermatogonia throughout the life of the man, have long telomeres. Oocytes from mouse and women maintain short telomeres. Telomerase, the enzyme that lengthens telomeres, is minimally active in mouse and human oocytes and pre-implantation embryos until blastocyst stage. Lacking appreciable telomerase activity, early mouse embryos elongate telomeres via Alternative Lengthening of Telomeres (ALT), which provides robust telomere elongation, but increased genomic instability. We do not know whether ALT occurs during early human development, and if so, when it takes place.

Study design, size, duration: 60 immature germinal vesicle (GV) and metaphase I (M1) human oocytes donated to research from women ages 18–45 were collected and *in vitro* matured (IVM) for up to 48 hours following the subjects' retrieval. Additionally, 28 cryopreserved human embryos donated to research from 7 couples (age 27–42 years old), at the New York University Langone Fertility Center.

Participants/materials, setting, methods: Immature oocytes were *in vitro* matured to metaphase II (M2). Frozen embryos between the 2 pronuclear (2PN) and day 3 (8-10-cell) stage were thawed and dissociated into single blastomere, intact blastocysts were processed whole. Telomere length was evaluated using Single Cell Amplification of Telomere Repeats PCR (SCATR-PCR), expressed as a telomere to reference gene ratio (T/R ratio). DNA damage was assessed by immunoflorescent staining. Statistical analysis was performed using one-way ANOVA or T-test where appropriate.

Main results and the role of chance: During oocyte maturation, immunostaining revealed that oocytes which arrested at the GV stage and failed to mature, contained robust and abundant DNA damage signaling on their chromosomes compared with successfully matured M2s (mean total florescent units = 35073.4 ± 10051.8 vs. 843.7 ± 74.9). Telomere length however did not differ significantly between arrested GV and M2 oocytes (mean T/R ratio = 0.074 ± 0.040 vs 0.105 ± 0.067). Telomere length increased significantly ($p < 0.05$) between M2 oocytes and both 2PN embryos (mean T/R ratio = 0.837 ± 0.546) and blastocysts (mean T/R ratio = 0.634 ± 0.260). The most significant elongation ($p < 0.0002$) occurred by day 2 (2–4 cell) (mean T/R ratio = 0.957 ± 767). Between day 2 and day 3 (mean T/R ratio = 0.385 ± 0.418) telomere length decreased. These data suggest early activation of a telomere lengthening mechanism, prior to zygotic genome activation. Additionally, Intra-embryo telomere length increased until its peak day 3 (Coefficient of Variation = 108.51%) and was at its lowest at the blastocyst stage (Coefficient of Variation = 40.97%) when telomerase becomes active. Together these data suggest that an Alternative Lengthening of Telomeres (ALT) mechanism may be responsible for both increases in telomere length and increased heterogeneity among blastomeres within early embryos.

Limitations, reasons for caution: The limited sample size for some of the earliest embryonic stages in addition to the freezing method for the embryos may have affected quality, and an inability to infer developmental ability as the embryos were not cultured to blastocyst.

Wider implications of the findings: Our study is the first to perform a molecular characterization of telomere dynamics and the role of DNA damage in human oocytes and embryos at the single cell level and provides the first evidence that ALT is part of normal embryonic development in humans.

Trial registration number: N/A

INVITED SESSION
SESSION 33: LIVE SURGERY SESSION
Tuesday 4 July 2017 Plenary 2 10:00–11:30

SELECTED ORAL COMMUNICATIONS
SESSION 34: DRUGS AND PROTOCOLS IN ART
Tuesday 4 July 2017 Room A 10:00–11:30

O-116 Submaximal doses of ghrelin do not inhibit gonadotrophin levels but stimulate prolactin secretion in postmenopausal women

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Study question: Does ghrelin suppress gonadotrophin levels in estrogen-deprived postmenopausal women? Does ghrelin stimulate PRL secretion in postmenopausal women and what is the role of estrogen?

Summary answer: In estrogen-deprived postmenopausal women, ghrelin administration affects neither FSH nor LH levels but stimulates PRL secretion, that is amplified by exogenous estrogen administration

What is known already: Previous studies in normally menstruating women regarding the role of ghrelin in gonadotrophin secretion have shown either no effect or a suppressive action. It has been suggested that the pattern of ghrelin administration (multiple vs single dosage) may have an impact and that there may be a facilitating action of estrogen on ghrelin-induced gonadotrophin suppression. However, the effect of ghrelin on gonadotrophin secretion in estrogen-deprived postmenopausal women has not been previously investigated. It is well known that exogenous ghrelin stimulates the secretion of PRL, but its effect in postmenopausal women has not been studied as yet

Study design, size, duration: Prospective intervention study in 10 volunteer postmenopausal women 52–74 years old (within 2–29 years past menopause) and a BMI of 22.3–34.6 kg/m^2. All women were studied during two 15-day periods of oral estrogen treatment (A and B) a month apart. The study was conducted between September 2015 and May 2016

Participants/materials, setting, methods: Four experiments (Exp) were performed on days 1 (Exp1A, Exp1B) and 15 (Exp15A, Exp15B) of the two study periods (09.30 h). The women received two ghrelin injections iv (0.15 μg/kg at time 0 min and 0.30 μg/kg at 90 min) in Exp1A and Exp15A and

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normal saline (2 ml) in Exp1B and Exp15B. Blood samples were taken at -15, 0, 30, 60, 90, 120, 150 and 180 min. Statistical analysis by Student's t-test and one-way ANOVA

Main results and the role of chance: Hormonal levels are presented as mean±SEM. Before the study onset, serum FSH, LH and estradiol levels were 52.2 ± 6.1 IU/l, 31.6 ± 3.8 IU/l and 27.4 ± 2.6 pg/ml, respectively. After estrogen treatment, serum estradiol values increased significantly on day 15 to levels similar to those in the late follicular phase of the normal menstrual cycle (Period A: 146.9 ± 22.6 pg/ml; Period B: 147.5 ± 29.3 pg/ml, P < 0.001). Total plasma ghrelin levels increased significantly after the two injections of ghrelin in both Exp 1 A (from 630 ± 26 pg/ml at 0 min to 5111 ± 135 pg/ml at 30 min and 5190 ± 158 pg/ml at 120 min, P < 0.001) and Exp 15 A (from 699 ± 55 pg/ml at 0 min to 5053 ± 512 pg/ml at 30 min and 5804 ± 274 pg/ml at 120 min, P < 0.001). Following the two injections of ghrelin (Exp 1 A and Exp 15 A), serum FSH and LH levels remained stable, while GH and PRL levels increased significantly with peak values occurring at 30 and 120 min (P < 0.001). In Exp 15 A, serum PRL increment in response to ghrelin (area under the curve) (1st injection: 140.3 ± 42.7 ng/ml/90 min; 2nd injection: 189.4 ± 45.2 ng/ml/90 min) was significantly greater than in Exp 1 A (16.6 ± 36.4 ng/ml/90 min and 41.5 ± 35.9 ng/ml/90 min respectively, P < 0.05)

Limitations, reasons for caution: LH pulsatility was not investigated in this study. Nevertheless, in normally menstruating women an inhibitory effect of ghrelin on FSH and LH levels has been shown previously in daily blood samples

Wider implications of the findings: The present results do not have direct application to practice. However, they are the basis for further research regarding the role of ghrelin in the physiology of the hypothalamo-pituitary axis

Trial registration number: N/A.

O-117 A second dose of kisspeptin safely optimizes oocyte maturation in women undergoing in IVF treatment: a phase 2 randomized controlled trial

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Study question: Determine whether increasing the duration of LH-exposure by administering a second dose of kisspeptin could further optimize oocyte maturation.

Summary answer: Administering a second dose of kisspeptin safely improves oocyte yield in women at high risk of developing OHSS undergoing IVF treatment.

What is known already: In vitro fertilization is an effective therapy for infertility, but can result in the potentially life-threatening complication ovarian hyperstimulation syndrome (OHSS).

We have previously reported that a single dose of kisspeptin results in an LH-surge of ~12-14hrs duration, which safely triggers oocyte maturation in women at high risk of OHSS.

Study design, size, duration: Phase-2 single-blinded randomized placebo-controlled trial of 62 women at high risk of OHSS were recruited between October 2013 and August 2014.

Participants/materials, setting, methods:
Setting: Hammersmith Hospital, London, UK

Protocol: Following a recombinant FSH/GnRH-antagonist IVF protocol, all patients (n = 62) received a subcutaneous injection of kisspeptin-54 (9.6 nmol/kg) 36hrs prior to oocyte retrieval.

Patients were then randomized 1:1 to receive either a second dose of kisspeptin (D; Double, n = 31), or saline (S; Single, n = 31) 10hrs thereafter.

Primary Outcome: Proportion of patients achieving an oocyte yield (percentage of mature oocytes retrieved from follicles ≥14 mm) ≥60%.

Secondary Outcomes: Reproductive hormone levels, Implantation rate, OHSS occurrence.

Main results and the role of chance: A second dose of kisspeptin induced further LH-secretion at 4hrs and 10hrs after injection compared to saline (P < 0.0001). A higher proportion of patients achieved an oocyte yield ≥60% following a second dose of kisspeptin (S:45.2%, D:71.0%; absolute difference +25.8%, CI 2.1–49.5%, P = 0.042). Patients receiving two doses of kisspeptin had a variable LH-response following the second kisspeptin dose, which appeared to be determined by the LH-response following the first kisspeptin injection. Patients who had a lower LH-rise following the first dose of kisspeptin had a more substantial 'rescue' LH-response following the second dose of kisspeptin. The variable LH-response to the second dose of kisspeptin meant that the proportion of patients achieving an oocyte yield ≥60% was improved, but without also increasing the frequency of ovarian over-response and OHSS.

Limitations, reasons for caution: Further studies are warranted to directly compare kisspeptin to more established triggers of oocyte maturation.

Wider implications of the findings: We observe that a second dose of kisspeptin administered 10hrs following the first is a safe option to improve the proportion of patients achieving an oocyte yield of at least 60%, but without also increasing the risk of ovarian over-response and OHSS.

Trial registration number: Clinical Trials Registration Number: NCT01667406

O-118 Randomized clinical trial to compare the pregnancy rates of vaginally applied progesterone 400 mg pessary and progesterone 8% gel after in-vitro fertilization

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[9]*Merck KGaA since October 2015, Global Medical Affairs- Fertility, Darmstadt, Germany*

Study question: Is twice daily progesterone 400 mg vaginal pessary non-inferior to daily progesterone 8% gel after 38 days of luteal phase support (LPS) concerning pregnancy rate?

Summary answer: Progesterone 400 mg vaginal pessary is non-inferior to progesterone 8% (90 mg) gel in clinical pregnancy rate after 38 days of luteal phase support.

What is known already: A pharmacokinetic (PK)/pharmacodynamics (PD) trial compared various dosing regimens of progesterone vaginal pessaries to progesterone 8% (90 mg) gel once daily and placebo in 125 healthy female subjects (EudraCT # 2012-001726-95). With respect to endometrial transformation rates, non-inferiority to progesterone gel was demonstrated for twice daily progesterone 200 mg and 400 mg vaginal pessaries. The PK profile after 10 days of multiple dosing with progesterone vaginal pessary 400 mg bid was most stable and steadily reached physiological plasma progesterone concentrations. The frequency for vaginal spotting and bleeding was lowest after 400 mg progesterone pessary.

Study design, size, duration: This was a multi-center, multi-national, open, randomized, two-parallel groups, non-inferiority trial. Patients were stratified according to center and age groups (≤35, >35 years). 812 patients were screened (randomized: 769). Patients were randomized 1:1 to progesterone vaginal pessary or progesterone gel treatment self-administered twice or once daily, respectively, for a period of up to 70 (±3) days, starting in the evening of the oocyte retrieval day.

Participants/materials, setting, methods: Women undergoing fresh embryo transfer after in vitro fertilization or intracytoplasmic sperm injection as part of their regular ART therapy were included. Clinical pregnancy defined as gestational sac with fetal heart movement was assessed by transvaginal

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ultrasonography on Day 38 and Day 70. Primary efficacy endpoint was clinical pregnancy on Day 38. Secondary outcomes included β-hCG levels (Day 18, Day 38) and implantation rate (N implanted embryos/N embryos transferred) on Day 38 and Day 70.

Main results and the role of chance: For the primary end-point, non-inferiority of progesterone pessary versus progesterone gel was met in the full analysis set (FAS) but could not be formally shown for the per protocol set (PPS) based on the pre-defined -9% non-inferiority margin (actual value: -9.5%). Pregnancy rates on Day 38 were 38.3% for progesterone pessaries and 39.9% for progesterone gel (FAS). These rates were higher than anticipated in sample size calculations (assumed a reference rate: 30%) leading to an increased variability, thus to wider confidence intervals than expected and explaining the marginal violation of the non-inferiority criterion for the PPS. Pregnancy rates on Day 70 were 34.5% (progesterone pessary) and 37.6% (progesterone gel). In the progesterone pessary group, the pregnancy rate was higher in younger patients (≤35 years: 41.4%; >35 years: 33.8%) and transfer of two or three embryos led to a higher rate (42.1%) compared to single embryo transfer (31.1%). Pregnancy rates on Day 38 were 21.1%, 38.9%, 37.9%, 38.3%, and 42.4% in Belgium, Bulgaria, Czech Republic, Hungary, and Serbia, respectively. Implantation rates for progesterone pessaries compared to progesterone gel on Day 38 were 24.6% and 26.5% and on Day 70, 22.5% and 24.5% respectively. Both treatments were safe and well tolerated.

Limitations, reasons for caution: A logistic regression analysis was applied to investigate potential 'country' effects. The test for any interaction between country and treatment (p = 0.13) indicated a tendency for country specific differences between treatments. However, since this test is not statistically significant, treatment comparisons over all countries are considered valid and reliable.

Wider implications of the findings: Progesterone pessary, shown to be non-inferior to progesterone gel in regards to clinical pregnancy rates in this study, can be regarded as a reasonable alternative to current treatment options for LPS in ART cycles. Additional available options allow an increasing role for patient centered decision making in LPS.

Trial registration number: EudraCT number 2013-001105-81

O-119 Combination tocolytics on the inhibition of OT-induced contractions of human pregnant myometrium in vitro

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Study question: Can OBE002, a novel FP receptor antagonist, which inhibits $PGF_{2\alpha}$- and OT-induced myometrial contractions enhance inhibitory effects of existing tocolytics on OT-induced myometrial contractions *in-vitro*?

Summary answer: The combination of OBE002 with existing tocolytics, atosiban and nifedipine, enhanced their inhibitory effect on OT-induced contractility.

What is known already: Preterm labour (PTL) is a major cause of neonatal morbidity and mortality. Currently, management of PTL aims to reduce uterine contractions. Since most tocolytics have been developed specifically for the inhibition of uterine contractions, tocolytics are not uterospecific and thus have dose-dependent multi-organ side effects. It is suggested that combined use of tocolytics with drugs interfering with different signaling pathways lead to additive inhibitory effects allowing lower doses with reduced side effects and increased efficacy.

Study design, size, duration: The inhibitory effects of OBE002 in combination with either atosiban or nifedipine, in OT-induced myometrial contractions, were investigated in human pregnant myometrial strips For each combination tocolytics experiment, six non-labouring human myometrial biopsies were used from six different patients undergoing elective caesarean section at term. All experiments were performed within 24 hours from tissue collection to ensure myometrial viability and optimal contractility performance.

Participants/materials, setting, methods: Myometrial strips from the same patient were mounted on a DMT Myograph 800 MS. Regular baseline contractions were recorded for 20 mins prior to treatment with OBE002 at 60 and 600 nM alone or with atosiban or nifedipine (6 nM) and effects on spontaneous contractility was measured in the next 10 min. The effect of the tocolytics upon OT stimulation was measured with increasing OT concentrations (1, 10, and 100 nM) at 10 min intervals.

Main results and the role of chance: Atosiban reduced OT-induced myometrial contractility at 6 nM and this effect was enhanced in a dose-dependent manner when combined with OBE002 ($p < 0.01$ vs Ato 6 nM, ANOVA). Concurrent administration of OBE002 and atosiban, at maximum concentration of 600 nM, reduced the contractility to basal level. Nifedipine and OBE002 alone inhibited the OT-induced contractions, and their inhibitory effects were increased further when the two drugs were used together. The combination of OBE002 and nifedipine was more effective than OBE002 alone ($p < 0.01$ OBE002 60 nM vs Nif+OBE002 60 nM, ANOVA).

Limitations, reasons for caution: Synergistic effects of OBE002 and atosiban/nifedipine were observed but further dose-response treatments could strengthen our findings. This study was carried out using term, non-labouring samples, but preterm or labouring samples will be useful to be examined in the future.

Wider implications of the findings: Targeting the FP receptors with OBE002 in combination with the existing tocolytics may therefore be a more effective strategy for preventing or delaying preterm delivery.

Trial registration number: N/A.

O-120 Melatonin in assisted reproductive technology: the MIART trial - A pilot double-blind randomised placebo-controlled clinical trial

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Study question: Does oral melatonin, when given during ovarian stimulation, improve clinical pregnancy rate after IVF?

Summary answer: When given at the doses tested, melatonin does not improve clinical pregnancy rate after ART.

What is known already: Melatonin is a potent oxygen scavenger and for this reason, it is thought to protect the oocyte and embryo from oxidative damage during the ART process. Previous uncontrolled and non-randomised studies have indicated that melatonin may improve ART outcomes, including the total number and quality of oocytes and embryos as well as pregnancy rate. Based on this, melatonin is currently used by many infertility specialists as an adjunct to ovarian stimulation with the intention of improving IVF success rates.

Study design, size, duration: A pilot double-blind, dose-finding, randomised placebo-controlled clinical trial of 160 women recruited between September 2014 and September 2016.

Participants/materials, setting, methods: Women undergoing their first cycle of IVF or ICSI were randomised to receive one of four different regimes of trial medication (placebo, n = 40; melatonin 2 mg, n = 41; melatonin 4 mg, n = 39; melatonin 8 mg, n = 40) twice per day from Day 2 of their cycle until the night before oocyte retrieval. Primary outcome was clinical pregnancy rate (presence of a live intrauterine fetus at 7-week ultrasound). Secondary outcome measures included oocyte number and maturity and embryo number and quality.

Main results and the role of chance: Compared with placebo, the geometric mean concentrations for melatonin in the highest dose group (8 mg bd) on the day of oocyte retrieval were 2-fold ($P < 0.03$) and 8-fold ($P < 0.001$) higher in serum and follicular fluid respectively. Despite these significant changes in serum and follicular fluid concentrations, there was no statistically significant difference in clinical pregnancy rates between the four groups. There were no

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statistically significant differences between the groups in total oocyte number, number of MII oocytes or number of fertilised oocytes or in the number or quality of embryos between the groups. There was also no difference in the number of utilised embryos. When comparing placebo with any dose of melatonin, there were no statistically significant differences in the median number of embryos (3.5 vs 3.0, p = 0.85) or good quality embryos (1.5 vs 2.0, p = 0.76). Compared with placebo, when taking any dose of melatonin, there was also no significant difference in cancelled cycle rate (19.3% vs 5.6%, OR 4.07, 95% CI 0.91–18.22, p = 0.07) or clinical pregnancy rate (22.8% vs 16.7%, OR 1.48 95% CI 0.56–3.94, p = 0.43). Miscarriage rate did not differ between groups.

Limitations, reasons for caution: The sample size of this pilot study may be insufficient to detect small differences in clinical pregnancy rate. Because we recruited women with an expected good ovarian response, we cannot conclude that melatonin has no effect on success rates in women with poor ovarian reserve or poor oocyte quality.

Wider implications of the findings: The lack of effect at any dose seen in our study suggests that the use of melatonin with the intention of improving ART success rates should be discontinued until high quality evidence demonstrates its efficacy in other patient populations.

Trial registration number: ACTRN12613001317785.

O-121 Testosterone treatment in women with poor ovarian repsonse: fertility and live birth rates

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Study question: The main question was to evaluate the efficacy of testosterone treatment before controlled ovarian stimulation (COS) on clinical pregnancy and live birth rate in poor responders undergoing IVF

Summary answer: Testosterone undecanoate treatment may increase pregnancy and live birth rate women with diminished ovarian response undergoing IVF.

What is known already: Many different technologies are used to improve pregnancy and live birth rates in poor responders, but none of them showed any statistically significant increase in these parameters, except androgen treatment. Testosterone treatment showed possible increase in pregnancy rate in women with poor ovarian response.

Study design, size, duration: 149 women with poor ovarian response, defined according to ESHRE consensus/the Bologna criteria of low ovarian response were included. Patients were randomly divided into 2 groups: Testosterone undecanoate (TU) treatment group (98 women) or control group (51 women). For TU group Testosterone undecanoate oral form 40 mg was given daily for at least 40 days (median 54 days) preceding COS for IVF.

Participants/materials, setting, methods: Primary outcome measures were clinical pregnancy (PR) and live birth (LR) rates in different age groups. Statistical research was made using a software package statistics (StatSoft Inc. U.S., version 12). Quantitative data is presented as median and quartile range. When comparing the quantitative data of two independent groups Mann Whitney U-test and Fisher exact two-tailed test were used. Values were considered statistically significant if p less than 0.05

Main results and the role of chance: There were no differences in patients' characteristics between the two groups. There was no significant difference in starting FSH dose or total dose of gonadotropins administered between the groups. There was a significant increase in number of oocytes retrieved 2 [1; 3] vs 1 [1;2] in TU group (p = 0.04). Clinical pregnancy rate (per cycle) was significantly higher in TU group (24.5%) than in control group (7.8%), p = 0.015. Live birth rate was higher in TU group than in control group 17.3% vs 5.9%, respectively, though the difference was not statistically significant (p = 0.07).

The treatment and control groups were divided into 2 subgroups each according to age: 1 subgroup less than 40 years old and 2 subgroup – 40 years and older. There was a significant increase in clinical pregnancy and live birth rate in TU 1 subgroup (less than 40 years old) compared to the control 1 subgroup: 38% vs 8.7% (p < 0.001) and 29.3% vs 6.5% (p < 0.01), respectively. There was no difference neither in clinical pregnancy nor in live birth rate between TU and control 2 subgroups.

Limitations, reasons for caution: no placebo control

Wider implications of the findings: This is the first study using oral testosterone undecanoate as a possible substance for treatment women with poor ovarian response. The effect observed in women less than 40 years is much higher, women 40 years and older, they may need more prolonged pretreatment with TU for better success rate.

Trial registration number: not available

SELECTED ORAL COMMUNICATIONS
SESSION 35: OOCYTES, DONORS AND OUTCOMES

Tuesday 4 July 2017 Room B 10:00–11:30

O-122 Quantifying the parameters affecting the success of egg donor program: A predictive model and optimization tool

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Study question: Which donor/ recipient parameters are relevant to predict blastocyst formation, implantation and live birth rate in an egg donor program with fresh and vitrified oocytes?

Summary answer: The predictive model included six factors: donor and recipient age, donor's estradiol (E2) at trigger, oocyte retrieval number, oocyte source and morphological embryo category.

What is known already: Currently there are predictive models that analyze clinical and embryo parameters to predict clinical outcomes after IVF, e.g. risk of multiple pregnancies. However, there are no reports of models to determine the optimal number of oocytes needed for successful egg donation with fresh and vitrified oocytes, which is definite by blastocyst formation, implantation and live birth rate. Moreover, survival rates in vitrified oocytes aren´t 100%. Additionally, vitrified oocytes yield less blastocyst formation than cycles using fresh donor oocytes. Thus, the number of vitrified oocytes needed in an oocyte donation cycle is likely higher than a fresh donor cycle.

Study design, size, duration: We performed a retrospective observational study in ovum donation program. The study group is composed by all patients who attended Spain and Portugal IVI Clinics to carry out assisted reproduction treatment with egg donation The data analysis was performed between 2013 and 2015. The study includes 20.551 cycles of oocyte donation. A total number of 263.124 oocytes were analyzed, an amount of 135.253 were fresh and 127.871 were vitrified.

Participants/materials, setting, methods: Econometric modelling was applied to build a model capable of providing an estimate for the appropriate number of oocytes needed in a recipient cycle. The dependent variables were discrete (e.g. vitrified/ fresh) and continuous (e.g. estradiol levels); on continuous variables optimal levels are also calculated. When possible, we used multinomial logit models and otherwise we used generalized lineal models representing a binomial process with a logit link function. Variable significance was analyzed through a t-test.

Main results and the role of chance: With the data analysis, 6 important parameters were determined for development of the model; donor and recipient age, donor's estradiol (E2) at trigger, oocyte retrieval donor number, oocyte source (fresh and vitrified) and morphological embryo category.

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According to our data, implantation rate and live birth do not show difference related with oocyte origin when we transferred the same quality blastocyst, but blastocyst formation was less in vitrified oocytes compared to fresh. Using the model, we may calculate the additional number of vitrified oocytes cycles needed to obtain the same number of blastocyst formation. That number varies depending on the clinical characteristics, donors and patients cycle parameters considered by the model.

The impact of oocyte vitrification is illustrated by the following example: while keeping other parameters constant, 8 fresh versus 10 vitrified oocytes are required to obtain the same number of blastocyst. Moreover, when we transfer 1 blastocyst classified with A quality, probability of implantation was 60% and probability of life birth was 48% at age of 40 years old, both independent of oocyte origin. This model allows a user to enter data to calculate the probability of blastocyst formation, implantation and live birth based on number of oocytes.

Limitations, reasons for caution: Not all factors that impact success of IVF were included. In addition, the correlations identified may vary in different populations or countries.

Wider implications of the findings: A predictive model capable of estimating the probability of implantation and live birth would be extremely helpful to improve outcomes and assist clinicians deciding how many oocytes are needed in oocyte donation programs, according to the odds to get a blastocyst and live birth for each mature oocyte.

Trial registration number: 1607-SCL-062-MC

O-123 Perinatal outcomes following gestational surrogacy versus autologous IVF: analysis of 87,815 singleton live births

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Study question: Does gestational surrogacy affect perinatal outcomes of preterm birth (PTB) and low birth weight (LBW) compared to autologous IVF treatment?

Summary answer: The present study did not demonstrate significant differences in the risks of PTB and LBW between gestational surrogacy and autologous IVF.

What is known already: Gestational surrogacy is when a woman carries and gives birth to a child through IVF for another individual or couple who are the intended parent/s. Since the first birth following gestational surrogacy in 1985, there has been an increasing trend in IVF-surrogacy. However, little information is available regarding obstetric or infant outcomes following surrogacy. There is a higher risk of pregnancy complications following ART compared to spontaneously conceived pregnancies and recent literature has shown oocyte donation pregnancies to be associated with higher risk of adverse obstetric and perinatal outcomes. It is a matter of interest if surrogacy influences perinatal outcomes.

Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of assisted reproduction treatment (ART) in the UK. The HFEA has collected data prospectively on all ART performed in the UK since 1991. Data from 1996 to 2011 involving a total of 87,815 singleton live births (244 following IVF-surrogacy cycles and 87,571 following stimulated fresh autologous IVF ± ICSI cycles) were analysed.

Participants/materials, setting, methods: Data from all women who underwent IVF-surrogacy or fresh autologous IVF cycles during the study period were analysed to compare perinatal outcomes (PTB, early PTB, LBW, very LBW) following singleton live births. Occurrence of live birth at <37 weeks gestation is defined as PTB and at <32 weeks gestation as early PTB. Birth weight <2500 grams is defined as LBW and <1500 grams as very LBW. Logistic regression analysis was performed adjusting for potential confounders.

Main results and the role of chance: The unadjusted odds were OR 0.89, 95% CI 0.56 to 1.41 for PTB, OR 0.23, 95% CI 0.03 to 1.67 for early PTB, OR 0.87, 95% CI 0.55 to 1.38 for LBW and OR 0.45, 95% CI 0.11 to 1.81 very LBW. After adjusting for potential confounders such as female age category, year of treatment, number of previous IVF cycles, previous live births (yes or no), cause of infertility (male factor, tubal disease, ovulatory disorder, endometriosis, unexplained, cervical factors), number of embryos transferred and initial singleton or multiple pregnancies that lead to singleton live births, there was no significant increase in the risk of adverse perinatal outcomes following surrogacy: PTB (adjusted odds ratio (a OR) 0.90, 95% CI 0.56 to 1.42), early PTB (a OR 0.23, 95% CI 0.03 to 1.68), LBW (a OR 0.90, 95% CI 0.57 to 1.43) and very LBW (a OR 0. 0.46, 95% CI 0.11 to 1.87).

Limitations, reasons for caution: Although the analysis was adjusted for a number of important confounders, the dataset had no information on confounders such as body mass index and medical history of women during pregnancy to allow adjustment. Given the relatively smaller number of events with IVF-surrogacy cycles, the results need validation by further studies.

Wider implications of the findings: The demonstration that gestational surrogacy is not associated with a significantly higher risk of PTB and LBW compared to autologous IVF provides assurance towards its current expanding application.

Trial registration number: Not applicable.

O-124 Shared motherhood: high delivery rates in a large series of egg donation treatments for lesbian couples using partner-donated eggs

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Study question: What are the patient characteristics and overall clinical outcome of a large series of lesbian couples undergoing ROPA (Reception of Oocytes from PArtner) treatment?

Summary answer: For lesbian couples wishing to share biological motherhood ROPA is a highly successful and safe treatment with reassuring obstetrical and perinatal outcome.

What is known already: After its first presentation in the reproductive literature in 2010 (Marina et al, 2010) and following an extensive ethical debate (Dondorp et al, 2010; De Wert et al, 2014) ROPA treatment has become increasingly accepted among ART practitioners and patients. It is currently an established treatment in several European countries (Spain, UK and Belgium, for example) for lesbian couples wishing to share their biological motherhood. Despite this, there is only limited data - a case-series of merely 14 couples - on the overall efficiency of ROPA treatment.

Study design, size, duration: All consecutive lesbian couples (n = 120) having ROPA treatment in a single private HFEA-regulated centre between 2011 and 2016 were included in this retrospective analysis. All fresh and frozen-thawed embryo transfers were followed-up until they achieved live birth (in addition to 19 currently ongoing pregnancies) or until the patients stopped further treatment. In 36% of unsuccessful and 70% of successful patients unused surplus embryos remain in storage.

Participants/materials, setting, methods: 120 lesbian couples underwent 133 treatment cycles (120 first, 10 second and 3 third attempts). There were no significant differences between donors and recipients in age (32.5 ± 4.1 vs 33.4 ± 4.9, p = 0.12) or ovarian reserve markers AMH and AFC; however, recipients were more parous than donors (0.8% vs 15%, p < 0.0001). Most (75%) donors were ≤35 years of age (range: 20–42 years). Recipients received an artificial endometrial preparation with oral oestrogens and vaginal progesterone synchronised with their donor.

Main results and the role of chance: Donors were mostly (82%) stimulated with a GnRH antagonist protocol and triggered with a GnRH agonist yielding an average 11.9 ± 7.1 eggs per cycle (range: 1–34). No moderate/severe OHSS cases were recorded among the oocyte donors. The fertilisation rates per retrieved eggs using donor sperm through IVF (79%) or ICSI (21%) were 66 ± 20% resulting in 7.6 ± 5 fertilised eggs per cycle. Embryos were cultured to blastocyst and cleavage stages in 68% and 31% of cycles, respectively. One treatment cycle (0.8%) was cancelled due to arrested embryo development. In 13 (10%) non-synchronised cycles all embryos were electively frozen. One-hundred-nineteen fresh and 44 frozen-thawed embryo transfers were performed with the replacement of an average 1.4 ± 0.5 embryos mostly (74%) at the blastocyst-stage. The single embryo transfer rate was 60% (98/163). For

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the 120 recipient patients the cumulative live birth / ongoing pregnancy rate recorded was at 60%, resulting in 53 live births and 19 ongoing pregnancies (for those conceived in the latter half of 2016). Twinning rate was at 12% (9/72). For singletons premature delivery rate (between 32–36 weeks) and low birth weight (<2.500 g.) rate was 12 % and 13%, respectively. The oldest oocyte donor achieving a live birth was 40 years of age.

Limitations, reasons for caution: No live birth or perinatal data is as yet available for pregnancies conceived in the second half of 2016. Although this series is substantial, it was not large enough to perform a multivariate analysis to determine potential prognostic factors influencing treatment outcome.

Wider implications of the findings: Shared motherhood is the preferred form of ART treatment amongst many lesbian couples. Increasingly the autonomy of ROPA patients is being respected even though complex IVF treatment is performed for (mostly) non-medical indications. To this end, OHSS-free ovarian stimulation with single-blastocyst-transfer provides a uniquely safe and highly efficient treatment modality.

Trial registration number: n/a.

O-125 Oocyte donation in women cured from cancer provide similar live birth rates compared to women without previous history of cancer

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Study question: What are the reproductive outcomes of women cured from cancer undergoing oocyte donation (OD)?

Summary answer: Live birth rate in women treated and cured of cancer following OD is 29.8%.

What is known already: About 5.4% of women worldwide will have cancer by the age of 50. Improvements in oncologic treatments have increased both their long-term survival and quality life; unfortunately, treatments such as chemotherapy (CT) and radiotherapy (RT) can impair fertility by uterine damage and/or diminishing the ovarian reserve in reproductive age women. At present, majority of cancer survivors who wish to become parents will undergo ART, in particular OD. Due to the limited number of the studies available, the current literature provides inconsistent results about reproductive outcomes for these women.

Study design, size, duration: Retrospective cohort study including 172 women cured from cancer, which underwent OD treatment between 2006 and 2015. We describe the reproductive outcomes of their first OD treatment performed at our clinic.

Participants/materials, setting, methods: The study included 172 patients, treated and cured from cancer by one or more of 4 oncological treatments (CT, RT, surgery, bone marrow transplant). All patients started ART after 5 years of follow-up from cancer and with authorization and declaration of cancer-free from their oncologists. All patients were analyzed for common demographic parameters and for reproductive outcomes.

Main results and the role of chance: The average age at OD cycle was 37.2 ± 6.2, all patients were of BMI between 18–25. The majority (166; 96.5%) were Caucasians; 46 were treated for Hodgkin lymphoma (3 also having thyroid, breast cancer or leukemia), 40 lymphoblastic leukemia, 21 borderline ovary cancer, 20 breast cancer, 15 no Hodgkin lymphoma, 7 colon-rectal cancer, 5 bone cancer (1 with ovarian borderline cancer), 4 thyroid cancer, 4 urinary system cancer, 10 others. The majority of patients (64.5%) were given a mixed therapy: 73.3% QT, 51.2% RT, surgery 41.9%, bone marrow transplant 39%. Time between being disease-free and ART was 10.7 years on average. Iatrogenic menopause affected 104 (60.5%) women. In 147 (85.4%) cycles, oocytes were fertilized with partner sperm. ET was performed on day 2-3 in 160 cases (93%). Single ET was performed in 30 (17.4%) cycles, 2 embryos were transferred in 141 (82%) and 3 embryos were transferred once (0.6%). Overall, clinical pregnancy rate as 40.1%, ongoing pregnancy rate 31.4%, and live birth rate 29.8%. When comparing patients who received a bone marrow transplant or not, the differences in reproductive outcomes were not statistically significant (clinical pregnancy: 43.6% vs. 39.1%, ongoing pregnancy 33.3% vs. 30.8%, and live birth 30.1% vs. 28.4%; p>0.05).

Limitations, reasons for caution: The main limitation of this study is its retrospective design, and the fact that patients were recruited along a period of 10 years. During this time, both oncological and ART treatments have undergo significant changes, possibly affecting the generalizability of the presented results.

Wider implications of the findings: According to our results, OD treatments in patients cured of cancer provide satisfactory results, with a live birth rate that is comparable to that reported for women that did not suffer from cancer. Physicians should discuss this option with patients cured of cancer in the context of reproductive counseling.

Trial registration number: NA.

O-126 Impact of the number of retrieved oocytes on cumulative live birthrates after repeated cycles of assisted reproductive technology – A Danish national cohort study

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Study question: Is the number of aspirated oocytes in the first assisted reproductive technology (ART) cycle associated with the cumulative live birthrates (CLBR) in subsequent cycles?

Summary answer: An increasing number of aspirated oocytes was associated with higher CLBR, and lower risk of discontinuing treatment. Initial treatment-response predicts outcome in subsequent cycles.

What is known already: Previous reports have shown a positive association between the number of retrieved oocytes and live birthrate per fresh treatment cycle. This has also been shown for the CLBR in one complete ART-cycle. One complete cycle is defined as one fresh ART-treatment including possible subsequent frozen-thawed transfers (FER). However, it has not been studied whether the number of oocytes in the initial cycle predicts live birthrates in subsequent fresh/complete cycles. Based on HFEA-data, women with 13 oocytes had increased CLBR compared with women with 5 oocytes, but only 40% of the women had more than one fresh cycle.

Study design, size, duration: The Danish National IVF-registry includes all ART treatments in public and private clinics since 1994. Treatment-cycles were cross-linked with the Medical Birth Registry, identifying treatment-related births and spontaneous conception (SC) births. This national cohort study includes all women starting ART treatments with homologous eggs between 2002 and 2011, N = 30,486. Subjects were followed for up to three fresh ART-cycles including subsequent FER-cycles (= three complete cycles), until the first live-birth, or until December 2011.

Participants/materials, setting, methods: The CLBR within 1–3 complete ART-cycles were calculated as the proportion of women with a livebirth, out of all women initiating ART-treatment, including drop-outs (no livebirth or continued treatment within follow-up). The number of retrieved oocytes in the first treatment-cycle was categorized in four groups: 0–3, 4–9, 10–15 and >15. In women with complete follow-up, multivariate logistic regression analysis assessed impact of retrieved oocytes on CLBR, adjusting results for female age and cause of infertility.

Main results and the role of chance: The mean female age at first treatment was 33.1(SD±4.7) years. After one, two and three complete cycles, the observed CLBR after ART-conception was 26.0% [95%CI 25.5–26.5], 40.7% [40.2–41.3], and 48.1% [47.5–48.6], respectively. The CLBR after SC was 4.7% [4.4–4.9], 7.2% [7.0–7.5] and 8.9% [8.6–9.3] after one, two and three complete

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cycles. The drop-out rate was 19.9% within three cycles, whereas 15.8% had additional treatments. The CLBR within three cycles, stratified by initial treatment-response were: 0–3 oocytes: 30.5% [29.0–31.9], 4–9 oocytes: 47.0% [46.0–48.0], 10–15 oocytes: 55.8% [54.5–57.2], >15 oocytes: 58.0% [55.8–60.1]. Compared with women with 4–9 oocytes in the first cycle, the adjusted odds ratios (AOR) for livebirth within three complete cycles were 0.5 [0.5–0.6] with 0–3 oocytes, 1.3 [1.2–1.4] with 10–15 oocytes and 1.5 [1.3–1.7] with >15 oocytes. Compared with women with 4–9 oocytes in the first cycle, AOR of livebirth in the 2nd and 3rd cycle were 0.8 [0.8–0.9] with 0–3 oocytes, 1.2 [1.1–1.3] with 10–15 oocytes and 1.4 [1.2–1.6] with >15 oocytes. The risk of discontinuing treatment decreased with increasing number of oocytes. Compared to women with 4–9 oocytes, the AOR for dropping out was 1.7 [1.6–1.9] with 0–3 oocytes, and 0.8 [0.7–0.9] with 10–15 or >15 oocytes.

Limitations, reasons for caution: Although mandatory, there may be treatment-cycles not registered in the IVF-registry.

Wider implications of the findings: The results emphasizes that the ovarian response to stimulation is an important prognostic factor, irrespective of age, for infertile couples entering ART-programs where the success is often based on repetitive cycles and combinations of both fresh and frozen thawed embryo replacements.

Trial registration number: The study was approved by the Danish Data Protection Agency (J.nr. 2012-41-1330).

O-127 Subcutaneous progesterone for endometrial preparation in substituted cycles for oocyte donation recipients: a randomized controlled trial

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[2]*Instituto Bernabeu, Molecular Biology, Alicante, Spain*

Study question: Is there any difference in clinical outcomes between using aqueous formulation of subcutaneous progesterone (SP) or micronized vaginal progesterone (VP) in substituted cycle for fresh embryo transfer in oocyte recipients?

Summary answer: No differences were found between groups in terms of ongoing pregnancy rate at 12 weeks of gestation

What is known already: Luteal phase support has been shown to improve pregnancy rates in women undergoing IVF. Two phase III randomized controlled trials and the subsequent meta-analysis found no differences in clinical outcomes using SP or vaginal progesterone for luteal support in IVF patients after fresh embryo transfers. Another study showed the efficacy of SP for inducing the transformation of the endometrium in substituted cycles. However, to date, clinical outcomes using SP for endometrial preparation have not been studied

Study design, size, duration: Single centre, blinded for the investigator, controlled, prospective, randomized, pilot trial. Over 18 months, 120 oocyte recipients were randomized on the day of egg retrieval of the donor based on a computer-generated randomization list to receive 25 mg daily SP or 200 mg tid daily VP.

Participants/materials, setting, methods: Patients included fulfilled medical and legal criteria for oocyte donation and followed the standard preparation protocol using transdermal estradiol. Randomization took place the day of the oocyte retrieval from the donor and in all the cases the embryo transfer was performed at the blastocyst stage. For the statisical analysis, t-test or anova test were used for continuos variables and Fisher test for categorical variables

Main results and the role of chance: Both groups were homogeneous in terms of age (40.2 years SP; 40.0 years VP p = 0.784), BMI (23.9 Kg/m^2 SP; 22.8 Kg/m^2 VP p = 0.074) and duration of infertility (3.8 years SP; 3.1 years VP p = 0.127).

Ongoing pregnancy rate at 12 weeks of gestation was 33.3% in the SP group and 50.0% in the VP group this difference was no significant (p = 0.086). Positive pregnancy test was similar between the groups, 66.07% SP and 64.81 VP (p=1.000). However, we found significant differences in clinical pregnancy rates (36.84% SP; 59.26% VP p = 0.022), implantation rates (32.46% SP; 53.71 VP p = 0.017) and biochemical pregnancy per embryo transfer (26.32% SP; 5.56% VP p = 0.004).

No differences were found in progesterone levels neither the day of the pregnancy test (6.66 ng/ml SP; 9.62 ng/ml VP p = 0.111), at 6 weeks of gestation (21.90 ng/ml SP; 12.98 ng/ml VP p = 0.138) or at 12 weeks of pregnancy (30.85 ng/ml SP; 25.47 ng/ml VP p = 0.387). However, lower levels of progesterone were found in the SP group at the moment of the embryo transfer (5.09 ng/ml SP; 9.59 ng/ml VP p = 0.004).

Limitations, reasons for caution: This is a pilot study and the sample size was not calculated

Wider implications of the findings: Despite ongoing pregnancy rate was not different in the group treated with subcutaneous progesterone biochemical miscarriage was higher so additional studies are needed to demonstrate its utility in substituted cycles.

Trial registration number: EudraCT: 2014-004784-20; ClinicalTrials: NCT02363127

SELECTED ORAL COMMUNICATIONS
SESSION 36: AN UPDATE ON SPERM FUNCTION
Tuesday 4 July 2017 Room W+X 10:00–11:30

O-128 Label-free phosphoproteomics reveals a novel calcium signaling pathway activated via kappa-opioid receptor in human spermatozoa

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Study question: Do the signaling pathways underlying G-protein coupled receptors in human spermatozoa differ from those found in somatic cells?

Summary answer: Phosphoproteomic approaches describe a different calcium signaling pathway underlying kappa-opioid receptor (KOR) which lasts in the inhibition of the acrosome reaction in human spermatozoa.

What is known already: The G-protein coupled receptors (GPCR) perceive different molecules from the extracellular environment, activate a broad variety of signaling pathways to finally last in cellular responses and they are the best therapeutic targets for the development of drugs. Some years ago the presence of different GPCRs in human sperm cells was described suggesting that they could participate in the modulation of the sperm fertilizing capacity. Taking into account that human spermatozoa are unable to transcribe and translate proteins, the study of signaling pathways underlying GPCR would be essential to identify different targets which seem to be unique in these cells.

Study design, size, duration: We studied KOR as GPCR model in order to analyze the signaling pathways that differ from the somatic cells. To understand the beginning of the pathway, we stimulated the receptor with U50488, a specific KOR agonist, and phosphorylated proteins were analyzed by LC-MS/MS. Acrosome Reaction was measured by flow cytometry. We used 60 human normozoospermic seminal samples from the Cruces University Hospital, to carry out both phosphoproteomic and functional studies.

Participants/materials, setting, methods: The samples were isolated and capacitated by *swim up*. The control samples were untreated and the rest were stimulated with U50488 for 1 and 60 minutes. To study the beginning of the signaling pathway, the 1 minute treatment samples were divided in 4 biological replicas, processed and analyzed by Q Exactive Mass spectrometer and MaxQuant Software v1.3.0.5. After the 60 minute treatment, the acrosome reaction was conducted by Flow cytometry using the anti-CD46 antibody.

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Main results and the role of chance: The phosphoproteomic study shows a decrease in the phosphorylation of four protein targets related to the calcium signaling pathway, after 1 minute of U50488 treatment. Among these potential proteins, CABYR is a Calcium-binding tyrosine phosphorylation-regulated protein, SPANX is a sperm protein associated with the nucleus on X chromosome, PGRMC2 is the Membrane-associated progesterone receptor component 2 and the FAM71B protein, is directly related to the CatSper sperm specific calcium channel. At the same time, CABYR and SPANX, are two testis specific proteins whose expression is limited to the testis and spermatozoa. These results highlights the fact that the U50488 stimulates a novel calcium signalling pathway which differs from those found in somatic cells supporting the idea that GPCR present unique features in their molecular mechanisms. Moreover, this finding could describe the first steps of the signalling pathways activated via the kappa-opioid receptor and which last in an inhibition of the acrosome reaction at one hour, as the functional studies confirm. This outcome is consistent with the idea that the GPCR, participate in the regulation of the acquistion of the fertile capacity in human sperm via cell specific molecular mechanism.

Limitations, reasons for caution: We need further studies to analyze more in deep the molecular mechanisms by which these 4 proteins participate in the inhibition of the acrosome reaction in human spermatozoa.

Wider implications of the findings: A better understanding of the molecular mechanisms that are involved in the physiology of the spermatozoid and differ from the somatic cells could be very helpful to grasp the aetiology of many cases of infertility and to develop new therapeutic targets and strategies.

Trial registration number: CEISH/61/2011.

O-129 Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of sperm DNA fragmentation: systematic review and meta-analysis

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Study question: To assess outcomes of intracytoplasmic sperm injection (ICSI) using testicular compared with ejaculated sperm in men with high sperm DNA fragmentation.

Summary answer: The existing evidence supports the use of testicular sperm in preference over ejaculated sperm for ICSI in men with high sperm DNA fragmentation (SDF).

What is known already: Recent studies have suggested potential benefits of using testicular versus ejaculated sperm for ICSI among couples with repeated ICSI failure. Oxidative-induced SDF during epididymis transit has been postulated to lower ejaculate sperm quality that may negatively impact ICSI outcomes. However, the evidence is not unequivocal, and some studies have found no benefit of testicular versus ejaculated sperm among men with cryptozoospermia. Therefore, we sought to assess the available evidence concerning outcomes of ICSI using testicular versus ejaculated sperm among infertile men with a strong rationale for using testicular sperm, namely, those with confirmed post-testicular sperm DNA fragmentation.

Study design, size, duration: We conducted a systematic search using PubMed, Scielo, and Google Scholar to identify all relevant studies published until December 2016. The search combined terms related to "sperm DNA fragmentation", "sperm DNA damage", "sperm chromatin integrity OR damage", "testicular sperm", "ejaculate", "intracytoplasmic sperm injection", with the filters "human" in any language. For the advanced search, article types selected were: clinical study, comparative study, journal article, meta-analysis, observational study, randomized controlled trial, review, and systematic review.

Participants/materials, setting, methods: Participants were couples undergoing ICSI with the use of ejaculated sperm (Eja-ICSI) or testicular sperm (Testi-ICSI) whose male partners had normo-/oligozoospermia and high SDF. Studies that included men with unexamined SDF were excluded. Subgroup analysis included the comparison among SDF testing methods. The levels of SDF in ejaculated and testicular sperm and live birth rates (LBR) were the primary outcomes. The secondary outcomes were clinical pregnancy rates (CPR), miscarriage rates, and fertilization rates.

Main results and the role of chance: Our electronic search retrieved 112 articles. After screening titles and abstracts, 11 articles were deemed eligible for full-text evaluation. Among these, we excluded four articles with reasons and included seven studies, involving 507 cycles and 3,840 injected oocytes, for qualitative and quantitative analysis. SDF rates were lower in testicular than in ejaculated sperm (Mean difference [MD] -24.58% [95% CI -32.53%,-16.64%, $P < 0.001$). The live birth rates per embryo transfer were higher when testicular sperm were used for ICSI compared with ejaculated sperm (Odds ratio [OR] 2.35 [95% CI 1.40–3.94; $P < 0.001$). Fertilization rates were not different between the two sperm sources, but the Testi-ICSI group had a trend to lower fertilization rates (OR 0.82 [95% CI 0.58–1.16]. Pooled results showed that CPRs were higher when testicular rather than ejaculated sperm was used for ICSI (OR 2.27 [95% CI 1.48–3.49; $P < 0.001$) whereas miscarriage rates were reduced with the former (OR 0.34 [95% CI 0.15–0.77; $P = 0.01$). Overall, heterogeneity was low but for SDF levels between testicular and ejaculated sperm. Subgroup analysis based on SDF method reduced heterogeneity estimates. Sensitivity analyses did not affect the overall effect size of pooled estimates.

Limitations, reasons for caution: Not all risk factors such as participant age, use of medication, and smoking, which might have affected SDF rates and ICSI outcomes were consistently reported. All included studies were observational. Another limitation refers to the quality of included studies, which also varied.

Wider implications of the findings: Pooled results from evaluated studies suggest that testicular sperm is preferred over ejaculated sperm for ICSI among men with high levels of SDF in the semen. The full clinical implications of using Testi-ICSI for men with high SDF deserves further investigation using randomized controlled trials.

Trial registration number: NA.

O-130 Topographic mapping of sperm chromatin fragmentation within the male reproductive tract and associated reproductive outcomes

A. Melnick, N. Pereira, S. Chow, A. Parrella, S. Cheung, Z. Rosenwaks, G. Palermo

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Study question: To assess sperm chromatin fragmentation (SCF) of sperm isolated from various levels of the male reproductive tract and how it affects reproductive outcomes.

Summary answer: There is an increase in SCF from the testes to the ejaculate, suggesting progressively increasing oxidative stressors through the male reproductive tract.

What is known already: The integrity of DNA in sperm has important fertility-related implications. During the later stages of spermiogenesis, breakage of a sizable amount of single- or double-stranded DNA occurs to allow tight chromatin compaction. Much of this DNA breakage is repaired, although reactive oxygen species (ROS) within the male reproductive tract can cause additional damage. While seminal anti-bodies can protect against damage by ROS in the ejaculate, DNA may still remain considerably fragmented. The topography mapping of SCF is particularly important in men with high SCF in ejaculates, where retrieving spermatozoa from the epididymis or testis may bypass such a chromatinic insult.

Study design, size, duration: Men with high SCF in their ejaculates underwent urologic evaluation and bilateral surgical sampling from vas deferens, epididymis, and testis. SCF of the ejaculated and surgically retrieved sperm samples was assessed by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Clinical outcomes for men undergoing ICSI treatment was recorded and stratified by the sperm source.

Participants/materials, setting, methods: Ejaculated samples were processed in standard fashion for SCF assessment with TUNEL. Surgical samples were minced and prepared for TUNEL, after which they were cryopreserved; these samples were thawed at a later date for ICSI treatment. SCF was measured by TUNEL on specimens isolated from all surgical sites. A commercially available

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kit was used for SCF assessment and at least 500 spermatozoa were counted per site under fluorescent microscopy with an adopted threshold of 15%.

Main results and the role of chance: Of the original 86 patients, 73 were treated by ART with an average SCF of 30.8 ± 18.4% (range 7.7–96.0). In 10 men aspiration of the vas deferens resulted in 16.4 ± 8% SCF (range 5.8–30.0) while in 44 men epididymal sampling yielded 15.8 ± 6.8% SCF (range 5.3–34.8) and in 83 the SCF on testicular spermatozoa was 11.3 ± 5.2% (range 2.0–27.0). The SCF progressively decreased as TUNEL was performed proximally from the ejaculate toward the vas deferens ($P = 0.05$), the epididymis ($P = 0.01$), and testis ($P = 0.01$). ART outcome with ICSI utilizing these surgical sources yielded a clinical pregnancy of 29.8%, while with the ejaculated counterpart only 16%. Based on these preliminary findings, a subgroup of patients (n = 19) with SCF of 40.1 ± 18% bypassed the prerequisite cycle with ejaculated spermatozoa. By opting to directly undergo TESE with ICSI, a clinical pregnancy rate of 36.0% per cycle was achieved that translated to 62% per couple treated.

Limitations, reasons for caution: Surgical sampling of the vas deferens, epididymis, and testis in men with high SCF in their ejaculates should only be performed after extensive and individualized counseling. Moreover, such an approach is preliminary and requires further evidence, given that some men may not achieve a pregnancy even with surgically retrieved sperm.

Wider implications of the findings: The topographic mapping of SCF evidenced that oxidative stressors progressively compromise DNA integrity toward the ejaculate. Thus, men with high SCF in their ejaculates who are unable to achieve a pregnancy may benefit from undergoing surgical retrieval of sperm for diagnostic and therapeutic purposes.

Trial registration number: Not applicable.

O-131 ORP: a reliable and reproducible method of evaluating oxidative stress - a multicenter study

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[1]*The Cleveland Clinic Foundation, American Center for Reproductive Medicine-Department of Urology- Cleveland Clinic, Cleveland- Ohio, U.S.A.*
[2]*Hamad General Hospital, Urology Department, Doha, Qatar*

Study question: To investigate the reproducibility and reliability of ORP measurement as an indicator for semen quality across different fertility centers.

Summary answer: ORP is a reproducible and reliable method to differentiate fertile from infertile semen samples.

What is known already: Seminal oxidative stress (OS) is well reported to affect male fertility status. The discrepancy in the measurement of OS has hindered its clinical use as a quality indicator for semen. Few studies measured reactive oxygen species alone while others measured only reductants leading to lack of standardization of results. Oxidation reduction potential (ORP) is a better representative for OS as it provides an overall measure of the activity of both oxidants and reductants. Very recently, ORP assessment by MiOXSYS has been introduced as a measure of OS with high specificity in differentiating fertile from infertile semen samples.

Study design, size, duration: This is a multicenter retrospective study comparing data from semen analysis and ORP measurement between two andrology laboratories in two different demographic areas (USA and Qatar) over a period of 1 year (2015 – 2016).

Participants/materials, setting, methods: The same protocol was followed by both laboratories. Semen analysis was performed according to WHO, Fifth Edition. ORP was measured using MiOXSYS. Each data set contains infertile patients' group and normal fertile donors group. Data was analyzed separately from each laboratory. The data from both laboratories was then combined and analyzed. To compare between two groups a Student's t-test was used. Receiver operator characteristic (ROC) analyses were used to determine cutoff values for sORP.

Main results and the role of chance: The first data set from USA contains 194 patients and 51 fertile donors, while the second data set from Qatar contains 400 patients and 50 fertile donors. In both data sets and in combined data, infertile group had significantly lower sperm concentration, total and progressive motility and normal morphology as well as higher ORP level when compared to fertile men ($P < 0.05$).

When comparing data from both centers, the infertile group showed significant difference between both data sets regarding progressive motility and morphology ($P < 0.001$). Also, the percentage of patients with abnormal semen volume, sperm count, total and progressive motility were significantly different between both data sets ($P < 0.05$). sORP level showed no significant difference between both data sets ($P < 0.08$)

ROC analysis indicated that sORP cut-off value of 1.42 mV/10^6/mL in USA group and in Doha group can accurately differentiate fertile from infertile semen groups. When combining both data together the cut-off value for sORP was again found to be 1.42 mV/10^6/mL.

Limitations, reasons for caution: The retrospective design of the study. However all data was available and both centers followed the same protocol.

Wider implications of the findings: Although other semen parameters showed significant differences between the two centers, sORP remained consistent in both data sets individually or in combined data. This proves its reproducibility and reliability. sORP is a measure of semen quality which adds more weight to semen testing in identifying fertile from infertile semen samples.

Trial registration number: N/A.

O-132 Four-dimensional analysis of sperm flagellar waveform as an extension for CASA

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[2]*University of Washington, Physiology and Biophysics, Seattle, U.S.A.*

Study question: Analysis of sperm movement in 4D in order to gather information about trajectories, chiralities, rolling and beating pattern for understanding the journey to the oocyte.

Summary answer: 4D analysis of sperm movement revealed a wide variety of swimming behaviors used as an answer to environmental circumstances on the oocytal track.

What is known already: Most past studies were only able to draw on 2D methods like CASA (computer assisted sperm analysis) to investigate swimming behavior and flagellar waveform of sperm. In general, they used projections onto the XY plane provided by conventional microscopic imaging in combination with stop-motion imaging. Previous results show that sperm with a linear trajectory rotate around their long axis at a time span correlated to beat frequency. A lack of rotating leads to a circular trajectory. Furthermore, flagellar excursions in the XY plane were described.

Study design, size, duration: Murine, bovine and human sperm were examined in 4D with reference to trajectories, chiralities, rolling behavior, beating pattern and flagellar waveform. A comparison was made between free swimming, adherent and circling sperm as well as a comparison between digital holographic microscopy (DHM) and CASA.

Participants/materials, setting, methods: Murine sperm from NMRI mice (Charles River Labs), bovine ejaculated sperm from a Holstein Friesian bull (HB-No 678525, Rinder-Union West eG) and human sperm from 5 healthy donors (collected under approved ethical protocols) were allowed to swim into physiological buffer at 37 °C. They were used for both, CASA and high speed digital holographic microscopy to record flagellar waveforms and sperm swimming paths in 4 dimensions.

Main results and the role of chance: DHM allows 4D tracking of the head of free-swimming sperm. With distinguishable left and right surfaces of the sperm head, we were able to monitor rolling of sperm around their long axis with correlating changes between left-face- and right-face-downmost configurations. DHM also allows 4D tracking of the flagellum. We were able to identify flagellar excursions into the Z plane as large as the excursions into the XY plane that are travelling down periodically the flagellum as sinusoid waves during each beat cycle. These waves correlate with rolling and beat frequency. The chirality of rolling is always alternating between clockwise and counterclockwise for a roll-counter-roll cycle. Without rolling, sperm obtain a circular trajectory with a planar movement revealed by DHM. Up to now, the current method to analyze sperm is CASA. We performed a comparative measurement between CASA and DHM to demonstrate the benefit of high speed holographic imaging as an extension to the 2D measurements.

Limitations, reasons for caution: Experiments were performed in vitro which means that not all in vivo questions could be answered.

Wider implications of the findings: Our findings will change the concept of sperm movement fundamentally: They indicate a chiral memory in form of a hypothetical elastic linkage within the flagellar machinery which stores torque of

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a roll for the following counter-roll. Our CASA measurements demonstrate a powerful extension for CASA with clinical relevance.

Trial registration number: none.

O-133 Oxidation reduction potential and sperm DNA fragmentation levels in sperm morphologic anomalies

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[2]Cleveland Clinic Foundation, Urology, Cleveland, U.S.A.

Study question: Is there a significant correlation between sperm morphology and structural anomalies and the measures of oxidative stress (OS) and sperm DNA fragmentation (SDF) in seminal plasma?

Summary answer: The degree of abnormality in sperm morphology and anatomic structure has a significant positive correlation with seminal OS and SDF measures.

What is known already: Controversy surrounds the significance of abnormal sperm morphology evaluated with routine semen analysis in predicting male fertility potential. Advanced sperm function assays are ancillary tests utilized to improve the diagnostic accuracy of semen parameters during male fertility evaluation. OS, the state of imbalance between oxidants and reductants, has been recognized to have detrimental effects on spermatogenesis through aggravating sperm apoptosis, lipid peroxidation and DNA fragmentation. Oxidation reduction potential (ORP) is a novel method that assesses the balance between oxidants and reductants, thereby reliably measuring OS in biologic samples.

Study design, size, duration: This is a cross sectional study of 1162 patients presenting to the male infertility unit of a tertiary medical center over a period of 12 months. After the collection of demographic and clinical data, patients were asked to provide a semen sample for analysis after > = 2 days of sexual abstinence.

Participants/materials, setting, methods: Semen samples were analyzed according to the 5th Edition WHO manual. Morphology was evaluated by a single experienced technician based on sperm stained using the Diff-Quik protocol. The percentage of abnormality in sperm head, neck or tail was recorded in each semen sample. SDF was tested using the sperm chromatin dispersion assay, while seminal ORP levels were assessed using the MiOXSYS system. Pearson's correlation was used to assess the relationship between study variables.

Main results and the role of chance: Patients mean age ± standard error of mean was 35.9 ± 0.2 years. Infertility was primary in 69.6% and secondary in 30.4% of patients. After a mean abstinence time of 3.7 ± 0.04 days, semen analysis results revealed a sperm concentration of 32.7 ± 0.78 million/ml, total motility of 50.1 ± 0.57% and normal morphology of 5.7 ± 0.22%. Sperm with abnormal head, neck and tail were observed in 53.8 ± 0.35%, 23.7 ± 0.25% and 17.1 ± 0.24% of patients, respectively. An inverse relationship existed between the percentage of normal sperm forms and measures of ORP and SDF (Table 1). A significant positive linear correlation existed only between the % of abnormal sperm heads and levels of ORP (r 0.34, CI 0.24 – 0.38, p < 0.001) and SDF (r 0.73, CI 0.49 – 0.96, p < 0.001).

Table 1 ORP and SDF levels in different %s of normal sperm forms.

	% normal sperm forms					
	0% (n = 56)	**1%** (n = 140)	**2%** (n = 181)	**3%** (n = 187)	**≥ 4%** (n = 598)	**p value**
ORP (mv/10^6/ml)	13.12 ± 1.8	12.04 ± 1.2	7.3 ± 0.7	4.9 ± 0.5	1.9 ± 0.2	<0.001
SDF (%)	56 ± 8.2	38.5 ± 3.7	31.5 ± 2.7	26.5 ± 2.16	22.6 ± 1.04	<0.001

Limitations, reasons for caution: Results were obtained from semen samples of patients presenting with primary or secondary infertility and hence were not compared with a control group or with men of proven fertility

Wider implications of the findings: Studying the correlation between sperm morphology indices and advanced sperm function tests is important as this would help in resting the controversy surrounding its clinical implication on fertility potential. It may also provide insights for developing novel sperm selection techniques that can be utilized during assisted reproduction.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 37: CLINICAL ASPECTS OF ENDOMETRIOSIS

Tuesday 4 July 2017 Room C 10:00–11:30

O-134 What factors affect mental health in women with endometriosis? Towards the development of a comprehensive explanatory model

F. Facchin[1], G. Barbara[2], E. Saita[3], E. Somigliana[4], D. Dridi[4], D. Alberico[4], L. Buggio[4], P. Vercellini[4]

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[2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Obstetrics and Gynecology and Service for Sexual and Domestic Violence SVSeD, Milan, Italy
[3]Catholic University of Milan, Department of Psychology, Milan, Italy
[4]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Obstetrics and Gynecology, Milan, Italy

Study question: What specific factors affect the mental health of women with endometriosis?

Summary answer: The psychological health of women with endometriosis can be affected by age, intimate relationship status, time from diagnosis, pelvic pain, and self-concept.

What is known already: Although it is well known that endometriosis as a chronic disease has a negative impact on mental health and quality of life, recent studies have shown that women's experience of endometriosis is characterized by remarkable variability in psychological outcomes. Such variability is partly explained by the presence of pelvic pain, which is associated with impaired mental health and whose severity can be influenced by personality. However, endometriosis is a multidimensional disease that involves a complex interaction of multiple variables (including individual characteristics such as self-concept) and further research is necessary to identify what specific factors may affect women's mental health.

Study design, size, duration: A total of 187 consecutive endometriosis patients were included in this cross-sectional study conducted between 2015 and 2017 at an Italian academic department of obstetrics and gynaecology located in Northern Italy.

Participants/materials, setting, methods: Participants were 187 endometriosis patients (mean age: 36.8 ± 7.1 years). Demographic and endometriosis-related information (i.e. surgical and hormonal treatment, pelvic pain severity [dysmenorrhea, dyspareunia, chronic pelvic pain, dyschezia], time from diagnosis) was collected. Self-concept was assessed using three validated measures evaluating self-esteem (Rosemberg Self-Esteem Scale), emotional self-efficacy (Emotional Self-Efficacy Scale), and body image (Body Esteem Scale). Mental health was assessed using the Hospital Anxiety and Depression Scale (HADS) and the Ruminative Response Scale (RRS).

Main results and the role of chance: A multivariable approach (i.e. hierarchical multiple regression) was used to examine the psychological impact of three sets of putative predictors identified on the basis of the extant literature: *Set 1* – demographic factors (age and intimate relationship status); *Set 2* – endometriosis-related factors (hormonal treatment; surgical interventions; current infertility; time from diagnosis; global pelvic pain severity); *Set 3* – global self-concept (a single factor, derived from principal component analysis [KMO test = .64, Bartlett's test of sphericity = 91.65, *ps* < .001], summarizing the information of the three self-concept scales to avoid multicollinearity problems). Significance tests were performed at *P* < .05. A shorter time from

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diagnosis was associated with greater anxiety (HADS-A: β = -.256; p = .001) and rumination (RRS: β = -.189; p = .014). Pelvic pain severity affected all psychological variables (*ps* < .01). Younger women displayed higher levels of depression (HADS-D: β = -.207; p = .003). Being in a stable relationship (coded 1 ['yes'] or 0 ['no']) was associated with decreased rumination (RRS: β = -.231; p < .001). Greater self-concept led to better psychological outcomes in all dependent variables (*ps* < .001).

Limitations, reasons for caution: A comprehensive model explaining how endometriosis can negatively affect mental health should test the impact of cultural differences, gender believes, and the quality of couple relationships. These variables were not included in our model and our sample was entirely composed of Caucasian women. These should be acknowledged as important limitations.

Wider implications of the findings: Although most endometriosis research focused on the role of pelvic pain, other factors should be considered to explain the psychological impact of the disease. Newly diagnosed younger women, especially with low self-esteem, may present higher levels of distress, which indicates the importance of timely psychological intervention aimed at empowering patients.

Trial registration number: Not applicable.

O-135 Endometriosis and women's sexual functioning: a systematic review of the available evidence

G. Barbara[1], F. Facchin[2], L. Buggio[1], M.P. Frattaruolo[1], N. Berlanda[1], P. Vercellini[3]

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Study question: What is the impact of endometriosis and its pharmacological and surgical treatments on female sexual functioning (FSF)?

Summary answer: Endometriosis has a negative impact on multiple domains of sexual functioning. Medical interventions can lead to medium-/long-term improvement, but not necessarily to a definitive resolution.

What is known already: Endometriosis is associated with an increased risk of dyspareunia, witch often coexists with other forms of sexual dysfunctions, such as hypoactive sexual desire, low lubrication and arousal, and orgasm disorders. This is often due to women's fear and anticipation of pain, which represents one of the most powerful inhibitor of the sexual response cycle. As endometriosis affects about 5–10% of women of reproductive age, a large proportion of young women in their most sexually active period of life could present sexual dysfunctions caused by the disease, which may interfere with physical and psychological quality of life and with conception.

Study design, size, duration: We conducted a systematic review of studies on the association between endometriosis and FSF. According to the PRISMA guidelines, we evaluated articles published between 2000 and 2016 on the correlation between: 1) the mere fact of having endometriosis and the presence of related female sexual dysfunctions; 2) the association between surgical and/or pharmacological treatments for endometriosis and related effects on FSF.

Participants/materials, setting, methods: Observational and retrospective studies on the impact of endometriosis and its treatments on FSF based on adequate description of participants, setting, medical intervention, and sexual outcomes were included. In order to investigate all aspects of FSF, we included only studies in which sexual outcomes were evaluated with a comprehensive sexual questionnaire, focusing not only on dyspareunia, but also on all the other aspects of FSF. Exclusion criteria were: qualitative research, case-report, commentaries or review articles

Main results and the role of chance: 31 studies were included in this systematic review and divided in 3 categories: no-intervention studies (n = 9), investigating the association between endometriosis and FSF; surgical intervention studies (n = 17), examining postoperative sexual outcomes of surgery for endometriosis; pharmacological intervention studies (n = 5), evaluating the effects of pharmacological endometriosis treatments on FSF. The clinical scenario described by the "no-intervention studies" was that around 70% of women with endometriosis suffer from sexual dysfunction (pain at intercourse, low satisfaction, lack of desire, low arousal, orgasm difficulties), with a negative impact on women's psychological well-being and intimate relationships. The "surgical intervention studies" showed that surgery for endometriosis may lead to improved sexual functioning, although the extent and the length of these positive effects were often poorly defined. Most articles had an exclusive focus on deep infiltrating endometriosis and radical laparoscopic surgery. Although several studies considered a post-operative pharmacological adjuvant therapy, surgery and hormonal treatment were directly compared in one study only. The paucity of "pharmacological intervention studies" might seem surprising. However, almost all of this kind of studies focused exclusively on deep dyspareunia, without considering the global female sexual functioning. Overall, pharmacological treatment leads to a medium-long term amelioration of FSF.

Limitations, reasons for caution: The heterogeneity of population, instruments, and sexual outcomes considered in the different studies did not allow to pool data and accurately estimate the sexual effects of the different intervention programs for amelioration of FSF in women suffering from endometriosis.

Wider implications of the findings: Since FSF is a multidimensional phenomenon deriving from the interaction of multiple physical, psychosocial, and emotional factors, we think that the best treatment program for women affected by endometriosis related sexual dysfunctions can be provided by multidisciplinary teams composed of gynaecologists, sexologists, and psychotherapists.

Trial registration number: not applicable.

O-136 Endometriosis - How male partners experience sexuality

S. Haemmerli[1], A. Kohl Schwartz[2], D. Fink[3], P. Imesch[4], M. Wölfler[5], S. Von Orelli[6], B. Leeners[1]

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Study question: How do male partners experience sexuality in partnership with women suffering from endometriosis?

Summary answer: Partners of endometriosis patients are less satisfied with their sexual relationship and their sexual problems interfere with relationship happiness.

What is known already: Sexuality is strongly associated with wellbeing, partnership satisfaction and the long-term development of couples' relationship. Endometriosis-associated pain, dyspareunia and fatigue influence couples' sexuality, but little is known about the male perspective in affected couples.

Study design, size, duration: A multi-center case-control study was performed between 2010 and 2015 in Switzerland, Germany and Austria. 236 partners of endometriosis patients and 236 partners of age-matched control women without endometriosis with a similar ethnic background.

Participants/materials, setting, methods: Participants were asked to answer a modified form of the Brief Index on Sexual Functioning (BISF) and the Global Sexual Functioning (GSF) questionnaire. The questionnaire was analyzed with SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Main results and the role of chance: Partners of endometriosis patients were less satisfied (p = 0.002) with their sexual relationship and their sexual problems interfered stronger with relationship happiness (p = 0.001) than in partners of control women. The wish for sexual activity (p = 0.387) and sexual desire (p = 0.919) was similar in both groups. Frequencies of sexual intercourse (p < 0.001) and other sexual activities were significantly higher in the control group.

Limitations, reasons for caution: Women asked their partners to participate in this study which might reflect a more satisfied study sample compared to the general population.

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Wider implications of the findings: Symptoms of endometriosis are related to a reduced satisfaction with sexual relationships and the associated relationship happiness in male partners. Therefore, counselling should include potential effects of endometriosis on sexual activity and partners should be invited to participate in aiming to find solutions for a fulfilling sexual and partner relationship.

Trial registration number: NCT 02511626.

O-137 Determinants of patients' experiences with patient-centeredness in endometriosis care

A. Schreurs[1], W. Nelen[2], W. Kuchenbecker[3], P. Van de Ven[4], L. Van der Houwen[5], E. Dancet[6], S. Apers[6], C. Lamballk[5], V. Mijatovic[5]

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[5]*VU University Medical center, gynecology, Amsterdam, The Netherlands*
[6]*University Hospitals Leuven, gynecology, Leuven, Belgium*

Study question: Which demographic and medical characteristics of woman are associated with their assessment of patient-centeredness in endometriosis care?

Summary answer: A lower educational level, dyspareunia and membership of a patient-organization were found to be independently associated with patient-centeredness as experienced by women in endometriosis care.

What is known already: Patient-centered care (PCC) is one of the six dimensions of quality of care. Indeed, focusing on improving patients' experiences with patient-centered care is necessary to improve quality of care. The patient-centeredness of endometriosis care can be measured with the validated and reliable Endocare Questionnaire (ECQ), generating patient-centeredness scores (PCS). Those PCS can subsequently facilitate the development of care improvement projects. A previous study found that a lower educational level was correlated with a higher overall PCS in patients from solely two tertiary clinics.

Study design, size, duration: A cross-sectional study was conducted in surgically diagnosed patients with endometriosis. A total of 401 patients were eligible and received the ECQ in 2015 and 2016 and, if needed, two reminders were sent.

Participants/materials, setting, methods: All patients were selected from a Dutch tertiary and a Dutch secondary clinic. All 401 patients underwent endometriosis surgery between 2013 and 2014. Univariate and multivariate regression analyses with a forward elimination procedure were conducted to identify variables associated with PCC.

Main results and the role of chance: The overall response rate was 56.9%. In total, data from 209 patients was eligible for analysis. Univariate analyses showed 'educational level', 'membership of a patient organization', 'child wish in the future', 'subfertility', 'dysmenorrhea', 'dyspareunia', 'chronic pelvic pain' and 'the degree of which disease is suppressed' as potential determinants of PCC (all $p < 0.2$). 'The degree of which disease is suppressed' was excluded from multivariate analysis due to the low response rate ($n = 90$).

After adjustment for clinic, multivariate analysis showed a lower educational level ($B=0.551$, $p = 0.027$), presence of dyspareunia ($B=0.790$, $p = 0.001$) and a being member of a patient organization ($B=1,670$, $p < 0.001$) to be independently associated with higher overall PCS ($R^2=0.21$).

Limitations, reasons for caution: Since endometriosis is a chronic disease and care is very dynamic, one cross-sectional study will not comprehend the possible change in patients' experiences to its fullest. A larger sample size would be valuable for identifying determinants of the overall PCS.

Wider implications of the findings: The afore-mentioned determinants of PCC are of value for studies benchmarking clinics for their patient-centeredness. In addition, they help clinicians to determine how to tailor their care to their individual patients.

Trial registration number: not applicable.

O-138 Reproductive outcome in severe endometriosis in different age groups with abnormal intra-follicular markers: A prospective observational study in IVF cycles

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Study question: Does severe endometriosis in younger age with abnormal cytokines affect reproductive outcome in women undergoing in- vitro fertilization (IVF)?

Summary answer: Younger women with severe endometriosis had similar reproductive outcome as tubal factor infertility irrespective of lower oocyte/embryo yield and abnormal intra-follicular cytokines/angiogenic factors.

What is known already: Endometriosis, a chronic inflammatory state, can impair reproductive outcome for reasons incompletely known. Women with endometriosis have high levels of macrophages, pro-inflammatory cytokines and angiogenic factors in peritoneal fluid when compared to fertile controls. It is also reported that altered intra-follicular cytokines and angiogenic factors (interleukin-8, interleukin-12, adrenomedullin) may adversely affect the oocyte and embryo quality. A recent systematic review and meta-analysis suggested that severe endometriosis is associated with low IVF success. Various studies have suggested that this is due to both diminished number and quality of oocytes.

Study design, size, duration: This prospective observational study included 652 infertile women who underwent IVF at a tertiary infertility centre during the period January 2012 to December 2015. Subjects were divided into two groups- study group/group A ($n = 294$) consisted of women with severe endometriosis (ASRM stage III/IV) and control group/group B ($n = 358$) had women with tubal factor as sole cause of infertility. All subjects belonged to age group of 25–40 years.

Participants/materials, setting, methods: Follicular fluid samples were collected during oocyte retrieval. ELISA was used to measure cytokines and angiogenic factors. The groups were sub-stratified based on age (group A:A1 (<35), A2(≥35) and group B:B1(<35), B2(≥35)). Number of Metaphase II oocytes, grade I/II embryos, pregnancy rate and miscarriage rate per number of pregnancies were compared. Significance of differences were evaluated by Chi-square test and multivariate analysis was applied for follicular fluid markers and its relation to oocyte and embryo quality.

Main results and the role of chance: Endometriosis group had a reduced pregnancy rate in comparison with tubal group (27.21% vs 34.08%, $p < 0.06$), though not statistically significant. Majority of the women in endometriosis group were <35 years (68%) compared to 57.3% in tubal group. Pregnancy and miscarriage rates were comparable between A1 and B1 [(31.84% vs 36.58%, NS) and (18.75% vs 16%, NS) respectively]. In contrast, when A2 was compared to B2, pregnancy rate was significantly lower in A2 (17.2% vs 30.7%, $P < 0.02$) but miscarriage rate was similar. A significant elevation of cytokines (IL-1β, TNF-α, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ) and angiogenic molecules (vascular endothelial growth factor, adrenomedullin, angiogenin) was found in endometriosis group compared to tubal group ($P < 0.001$). Number of Metaphase II oocytes and grade I/II embryos were significantly lower in group A1 versus B1 (6.63 ± 3.28 vs 7.66 ± 3.68; $p < 0.003$ and 3.38 ± 1.8 vs 4.02 ± 1.54; $p < 0.001$). Similar results were observed in group A2 versus B2 [Metaphase II oocytes (4.63 ± 2.33 vs 5.69 ± 1.39; $p < 0.001$) and grade I/II embryos (2.6 ± 1.28 vs 3.55 ± 0.98; $p < 0.001$)]. IL-8, IL-12 and adrenomedullin were identified as most important markers in follicular fluid that negatively affects oocyte and embryo quality ($p < 0.001$).

Limitations, reasons for caution: The present study does not necessarily impart evidence towards a direct correlation between elevated intra-follicular biomarkers and pregnancy outcome since more than one embryo is usually transferred per IVF cycle. A relatively smaller sample size of women with endometriosis ≥35 years perhaps reduces the potentiality of this observation.

Wider implications of the findings: In our study, women > 35 years with severe endometriosis have poorer IVF outcome compared to younger women.

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Since the diagnosis of endometriosis is often late, earliest opportunity should be sought to reduce its adverse effect on reproductive outcome.This can be avoided by early resortment to ART and fertility preservation.

Trial registration number: Not applicable.

O-139 Endometriosis-related infertility: severe pelvic pain is associated with more severe disease

T. Thubert[1], P. Santulli[1], M.C. Lamau[1], L. Marcellin[1], M. Bourdon[1], C. Maignien[1], V. Gayet[1], K. Pocate-Cheriet[2], C. CHAPRON[1]

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Study question: What is the significance of severe preoperative painful symptoms for patients presenting with endometriosis-related infertility?

Summary answer: In case of endometriosis-related infertility, severe pelvic pain is significantly associated with more severe endometriosis with deeply infiltrating lesions.

What is known already: Endometriosis is associated with infertility. In the setting of infertility the management of endometriosis is actually still debated. The extent of the disease plays an important role in the decision process between surgery or ART. The impact of painful symptoms in case of endometriosis related infertility is debated without reaching a consensus.

Study design, size, duration: This was an observational, cross-sectional study using data prospectively collected in all non-pregnant patients aged between 18 and 42 years, who were surgically explored for benign gynaecological conditions at our institution between January 2004 and December 2016. For each patient, a standardized questionnaire was completed during a face-to-face interview conducted by the surgeon during the month preceding surgery. Complete surgical excision of was performed for each patient.

Participants/materials, setting, methods: Surgery was performed in 1374 histologically proven endometriosis affected women. 505 women had endometriosis-related infertility. Of them, 167/505 patients had severe pain. Pain was considered as severe when visual analogue scale (VAS) was ≥ 7. Prospective preoperative assessment of type and severity of pain symptoms (VAS) was compared with preoperative and operative findings. Correlations were sought with univariate analysis to determine characteristics of infertile severe painful patients compared to non painful patients

Main results and the role of chance: Among infertile women suffering from endometriosis, patients who had severe pain had significantly more history of scholar absenteeism (60.2% vs. 15.6%, $p < 0.001$), more previous prescription of contraceptive pills because of severe primary dysmenorrhea (26.3% vs. 11.9%, $p = 0.004$) and more previous history of surgery for endometriosis (47.0% vs. 14.4%, $p < 0.001$) than infertile women without severe pelvic pain. Phenotype of endometriosis was significantly different between both group with higher rAFS total score in the painful group (39.2 ± 33.7 vs. 17.9 ± 24.5, $p < 0.001$). According to the surgical phenotype of endometriosis, infertile painful women had more frequently deeply infiltrating lesion than non painful women (57.9% vs 17.3%, $p < 0.001$). However, there was no more endometrioma in the painful group (16.6% vs 22.2%, $p < 0.001$). Deeply infiltrating lesion were more often localized in the intestine (64.8% vs 2.4%, $p < 0.001$) or in the vagina (48.5% vs 27.6%, $p < 0.001$) in the painful patients.

Limitations, reasons for caution: We cannot exclude that infertile women with a diminished ovarian reserve, as assessed during their infertility work-up, were referred less frequently to surgery and might therefore be underrepresented. In addition we cannot exclude that our group of women present other causes of infertility.

Wider implications of the findings: In case of severe pain, infertility among endometriosis women was significantly associated with deeply infiltrating lesions. In this situation, the practitioner should perform an appropriate preoperative imaging work-up in order to plan the correct strategy of patient management (surgery or assisted medical reproduction).

Trial registration number: none

INVITED SESSION

SESSION 38: EUROPEAN AND GLOBAL ART MONITORING SESSION

Tuesday 4 July 2017 Room A 11:45–12:45

O-140 Assisted Reproductive Technology (ART) in Europe 2014. Preliminary results generated from European registers by ESHRE

C. Calhaz - Jorge

Faculdade de Medicina de Lisboa, Human Reproduction Unit, Hosp. de Santa Maria, Lisbon, Portugal

O-141 ICMART World Report 2013

G.D. Adamson[1], J. De Mouzon[2], S. Dyer[3], G. Chambers[4], O. Ishihara[5], M. Banker[6], R. Mansour[7], S. Vanderpoel[8,9], F. Zegers-Hochschild[10]

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Abstract text

Study question: In 2013, what was: global utilization of ART as measured by number and type of cycles; effectiveness as measured by clinical pregnancy, miscarriage and live birth rates; safety as measured by multiple births and other complications, and; the status of extensive additions and revisions to the ICMART-WHO 2009 glossary?

Summary answer: Globally, ART utilization continues to increase but with wide variation in utilization, effectiveness and safety. Participation in data collection is gradually improving through regional as well as national registries. The International Glossary on Infertility and Fertility Care, a partnership effort led by ICMART, provides significantly enhanced standardization of concepts, processes, procedures, and clinical events for data collection and research essential for precise and harmonized global communication.

What is known already: ICMART began ART global data collection in 1991. Utilization, effectiveness and safety have continuously improved with more cycles, higher pregnancy rates and lower rates of multiple births. This last outcome has been coupled with a proportional increase in the number of frozen embryo transfer (FET) cycles; however, wide variations exist globally. Over 6.5 million ART babies have been born. ICMART has helped develop African registries in association with ANARA. Data collection and quality remain challenging. The 2009 glossary has been widely utilized.

Study design, size, duration: Countries and regions annually collect ART data, some prospectively and others retrospectively. ICMART retrospectively requested these data from all known global sources for 2013, analyzed them and presents preliminary results. The 2009 Glossary revision involved a 3-year interactive consultative and consensus process with subject experts, then reassessment over one year together with international and regional societies and patient representatives.

Participants/materials, setting, methods: The European IVF Monitoring Consortium (EIM), SART, Latin American Network of Assisted Reproduction (REDLARA), Australian/ New Zealand Registry, ANARA and other countries,

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totaling approximately 60, contributed national data through standard ICMART forms. Data were reviewed, corrected, analyzed and summarized by ICMART working with the University of Uppsala, Sweden using standard statistical tests. ICMART led the glossary revision with experts and its society partners, ASRM, ESHRE, IFFS, March of Dimes, AFS, GIERAF, ASPIRE, MEFS, REDLARA, and FIGO.

Main results and the role of chance: Data collection and analysis are ongoing. Preliminary results are presented at ESHRE. The number of ART cycles continues to increase, but utilization is still very influenced by affordable access to ART which is related to insurance or public funding. Regional differences persist in the age of the population treated, number of embryos transferred, rate of multiple births, and other factors.

Additionally, the revised 2009 glossary, now named The International Glossary, has been expanded to 283 terms with definitions covering: Clinical terminologies, Outcome terminologies, Epidemiology and public health, Laboratory including andrology, and Embryology. Some 2009 definitions have been modified or expanded.

The role of chance is minimal.

Limitations, reasons for caution: Global ART results are limited to reporting countries and clinics representing approximately 2/3 of global cycles. Many countries have limited data validation and ICMART can perform only minimal verification of submitted data. The International Glossary definitions are based on a detailed and pre-determined consensus process that was sometimes challenging across many disciplines and different stakeholders.

Wider implications of the findings: It is accepted that measurement of human activities improves quality and outcomes. ICMART World Reports standardize data, track trends, enable comparisons, stimulate questions and improve ART quality in clinics, countries, regions and globally. Additionally, better understanding of ART increases societal acceptance and eventually creates broader support for ART research and clinical access. The International Glossary on Infertility and Fertility Care represents a unique global partnership outcome and provides a terminology platform that will harmonize clinical data reporting, research and communication.

O-142 The International Glossary on Infertility and Fertility Care: Led by ICMART in Partnership with ASRM, ESHRE, IFFS, March of Dimes, AFS, GIERAF, ASPIRE, MEFS, REDLARA, FIGO

L.F. Zegers-Hochschild[1], D. Adamson[2], S. Dyer[3], C. Racowsky[4], J. de Mouzon[5], R. Sokol[6], L. Rienzi[7], A. Sunde[8], L. Schmidt[9], I.D. Cooke[10], J.L. Simpson[11], S. Van der Poel[12]

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[12]WHO:HRP / staff at time of study implementation, HRP, Geneva, Switzerland

Abstract text

Study question: To generate a consensus and evidence-driven set of terms and definitions to be used globally in order to ensure consistency when reporting on infertility issues and fertility care interventions, as well as to harmonize communication among the medical and scientific communities, policy-makers, and lay public including individuals and couples experiencing fertility problems.

Summary answer: A set of 283 consensus-based and evidence-driven terminologies used in infertility and fertility care has been generated through an inclusive consensus-based process with multiple stakeholders.

What is known already: In 2006 ICMART published a first glossary of 53 terms and definitions. In 2009 ICMART together with WHO published a revised version expanded to 87 terms, which defined infertility as a disease of the reproductive system, and increased standardization of fertility treatment terminology. Since 2009, limitations were identified in several areas and enhancements were suggested for the glossary, especially concerning male factor, demography, epidemiology and public health issues.

Study design, size, duration: Twenty-five professionals, from all parts of the world, representing their expertise in a variety of sub-specialties, were organized into five working groups: clinical definitions; outcome measurements; embryology laboratory; clinical and laboratory andrology; and epidemiology and public health. Assessment for revisions, as well as expansion on topics not covered by the previous glossary, were undertaken. A larger group of independent experts and representatives from collaborating organizations further discussed and assisted in refining all terms and definitions.

Participants, setting, methods: Members of the working groups and glossary coordinators interacted through electronic mail and face-to-face in international/regional conferences. Two formal meetings were held in Geneva, Switzerland, with a final consensus meeting including independent experts, as well as observers and representatives of international/regional scientific and patient organizations.

Main results and the role of chance: A consensus-based and evidence-driven set of 283 terminologies used in infertility and fertility care was generated to harmonize communication among health professionals, scientists, and the lay public, patients and policy makers. Definitions such as "fertility care" and "fertility awareness" together with many other terminologies used in epidemiology and public health as well as in Embryology and andrology have been introduced in the glossary for the first time. Furthermore, the definition of "infertility" was expanded in order to cover a wider spectrum of conditions affecting the capacity of individuals and couples to reproduce. The core of the definition of Infertility remains as a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse; however, it also acknowledge that the failure to become pregnant is not always the result of a disease, and therefore introduces the concept of an "impairment of a person's capacity to reproduce" which can lead to a disability as an impairment of function.

Limitations, reasons for caution: All stakeholders agreed to the vast majority of terminologies included in this glossary. In cases where disagreements were not resolved, the final decision was reached after a vote, defined before the meeting as consensus if passed with 75%. Over the following months an external expert group, which included representatives from non-governmental organizations, reviewed and provided final feedback on the glossary.

Wider implications of the findings: Some terminologies have different definitions, depending on the area of medicine, e.g. demographic or clinical as well as geographic differences. These differences were taken into account and this glossary represents a multinational effort to harmonize terminologies that should be used worldwide.

INVITED SESSION

SESSION 39: DNA REPAIR IN OOCYTES AS A KEY DETERMINANT OF REPRODUCTIVE FAILURE

Tuesday 4 July 2017 Room B 11:45–12:45

O-143 Role of DNA Repair and BRCA Gene Function in Oocyte Aging and Reproduction

K.H. Oktay

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Abstract text

Emanating from our first observations of reduced ovarian reserve in women with BRCA mutations, we have previously shown that oocyte DNA DSB repair declines with age, resulting in increased accumulation of DNA DSB and increased oocyte apoptosis. Furthermore, ovarian aging was accelerated in BRCA1 mutant mice and the overexpression of BRCA1 in mouse oocytes rendered these oocytes more sensitive to genotoxic stress. Based on these revelations, we hypothesized that declining DNA DSB repair may be a fundamental mechanism in oocyte aging. Multiple recent prospective studies have now confirmed that the serum AMH levels are lower in BRCA mutation carriers compared to controls and those carriers may experience early menopause. Large GWAS studies also identified BRCA and DNA repair genes in general, among the most significant determinants of age at natural menopause. While the impact of BRCA mutations on fertility is unclear in younger women, these differences may have impact on fecundity in later reproductive years. Further, BRCA gene and DNA repair pathways may also be involved in the maintenance of meiotic spindle and hence any deterioration may have adverse consequences for oocyte quality. Because of the elimination of women with most severe BRCA dysfunction from the reproductive pool via risk reducing salpingo-oophorectomy or cancer-treatment-induced ovarian failure, the women who are available for most studies likely represent the tip of the iceberg. This also explains why some small and retrospective studies may not easily identify BRCA-mutation-related ovarian compromise. Given the strong concordance of laboratory and clinical studies and confirmation across numerous trials, BRCA-related DNA Repair and its dysfunction appears to be a critical component of oocyte aging.

O-144 Meiosis-quality assurances and their failures

R. Jessberger

Technische Universität Dresden, Medical Faculty Carl Gustav Carus, Dresden, Germany

Abstract text

Meiosis – Quality Assurances and Their Failures

The faithful transmission of genetic information between generations is essential for human health. Safeguarding mechanisms act at various stages of gem cell development, including in female and male meiosis. Yet, the quality assurance processes that ought to ensure proper meiotic chromosome structure and dynamics are not error-free. In meiotic prophase I the completion of repair of programmed DNA double-strand breaks and the proper synapsis of homologous chromosomes are monitored. Spermatocytes that fail in these processes are efficiently eliminated through an X/Y chromosome-based mechanism, which is lacking in oocytes. The dictyate stage at the end of prophase I in which oocyte remain arrested for a very long period poses a particular challenge to chromosomal integrity. The well-known increase in aneuploidy with increasing age of oocytes poses the most severe problem to faithful inheritance. In recent years, the gradual loss of cohesin, the protein complex responsible for sister chromatid cohesion, was found in a number of mouse models to prominently contribute to loss of chiasmata in metaphase I and to dissociation of sister chromatids in metaphase II. The role of cohesin and its decay, as well as the potential impact of cohesin regulatory factors during oocyte ageing will be discussed.

INVITED SESSION

SESSION 40: PROGESTERONE SUPPORT - EXAMINING THE EVIDENCE

Tuesday 4 July 2017 Room W+X 11:45–12:45

O-145 Progesterone support in ART

P. Humaidan

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Abstract text

The luteal phase of all stimulated IVF/ICSI cycles is abnormal. The main reason for the luteal phase defect (LPD) is the multi-follicular development achieved during ovarian stimulation, leading to supra-physiological levels of steroids (progesterone and estradiol) secreted by a high number of corpora lutea during the early luteal phase, which directly inhibit the release of LH from the pituitary via feedback actions at the hypothalamic-pituary axis level. This reduction in circulating endogenous LH has a detrimental effect on the early-mid luteal phase, as LH plays a crucial role for the steroidogenic activity of the corpus luteum in terms of progesterone production. Thus, luteal phase support with progesterone remains mandatory in fresh transfer cycles after ovarian stimulation for IVF/ICSI treatment. Moreover, with the introduction of new embryo culture systems and in particular vitrification of supernumerary embryos, the live birth rate after frozen-thaw embryo transfer is now similar to, and in many cases superior to that of fresh embryo transfer. This has created a paradigm shift in stimulation policies, in which GnRHa is used for ovulation trigger, followed by segmentation and subsequent transfer in either an HRT frozen-thaw cycle or a natural cycle. For scheduling purposes many centers favor the HRT cycle. Although, poorly defined, until recently a "standard" luteal phase progesterone support was considered sufficient for all patients undergoing fresh as well as frozen-thaw embryo transfer; however, recent scientific evidence questions this policy. During the last decade personalization - or individualization became the "mantra" of ovarian stimulation, and the concept subsequently moved on to the choice of ovulation trigger. Indeed, near future suggests personalization of the luteal phase support as well. This will demand monitoring of the mid-luteal phase in terms of serum progesterone, as the mid-luteal progesterone level seems to play a pivotal role for reproductive success in ART.

O-146 Progesterone in early pregnancy

Y. Cheong

University of Southampton Academic Unit of Human Health and Development, Faculty of Medicine Consultant in Obstetrics and Gynaecology Subspecialist in Reproductive Medicine and Surgery, Southampton, United Kingdom

Abstract text

Much scientific progress has been made since progesterone was first isolated in 1930 s from the corpus luteum. We now know that progesterone is a 21-carbon atoms sex steroid, as opposed to androgens (19-cardon atoms) and estrogens (18-carbon atoms). Progestogen is a class of steroid hormones, which binds to, and activates the progesterone receptor and includes the natural hormone progesterone and synthetic forms (progestin). Progesterone and progestin are structural but not functional analogues. Progesterone is produced from cholesterol and acts as precursors to all other endogenous steroids including androgen, oestrogen, glucocorticoid, mineralocorticoid and neurosteroid. Hence, whilst progesterone is central to many aspects of reproduction through nuclear and extra-nuclear receptor mechanisms, it is also crucial in many non-reproductive processes on bones, central nervous system and metabolism.

In early pregnancy, progesterone is produced by the corpus luteum until the placenta takes over at around the 7th week of gestation. A defective luteal phase, where there is insufficient progesterone exposure to maintain a normal secretary endometrium to facilitate normal embryo implantation and growth has long been proposed as a possible cause of subfertility and miscarriage. However, until now, there is yet no consensus on the diagnosis of this condition. Clinical treatment strategies with progesterone supplementation to improve pregnancy outcomes face the conundrum of having to evaluate the treatment of a disease that cannot be accurately diagnosed. Results from studies on the supplement of progesterone to improve pregnancy outcomes remain conflicting. It is, however, clear that progesterone use during early pregnancy is non beneficial in women with unexplained recurrent miscarriage.

In assisted conception with ovarian stimulation, it is recognized that luteal phase support is required due to the presence of a dysfunctional luteal phase although the mechanisms behind this is still not completely clear. Studies have highlighted the presence of non-synchronized delay in endometrium development in approximately 25% of women undergoing assisted reproduction (IVF). Re-synchronisation of the implantation window, by way of personalized progesterone therapy, segmentation of luteal phase and/or realigning the circadian clock may be potential strategies to improve the outcomes of early pregnancy

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SELECTED ORAL COMMUNICATIONS

SESSION 41: PARAMEDICAL SESSION - PLANNING A FAMILY

Tuesday 4 July 2017 Room C 11:45–12:45

O-147 Contraceptive counseling is a golden opportunity to talk about fertility and reproductive health

Y. Skogsdal[1], H. Fadl[2], J. Karlsson[3], Y. Cao[4], T. Tydén[5]

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[2]*Orebro University Hospital, Department of Obstetrics and Gynaecology, Orebro, Sweden*
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[4]*4Clinical Epidemiology and Biostatistics, School of Medical Sciences, Örebro University*

Unit of Biostatistics, Institute of Environmental Medicine, Karolinska Institutet
[5]*Uppsala University, The Medical Faculty, Uppsala, Sweden*

Study question: Can information about fertility and the use of a Reproductive Life Plan in contraceptive counseling increase women's knowledge about fertility and reproductive Health?

Summary answer: Information about fertility and factors affecting reproductive health was appreciated of the majority of women and increased their knowledge of fertility and reproductive Health.

What is known already: Traditionally counseling about contraceptive methods has emphasis on the efficacy and safety. Other aspects of reproductive health such as reproductive capacity are not routinely discussed and consequently women lack an important piece of information for their own understanding of fertility and reproductive health. The Center for Disease Control and prevention (CDC) recommends a tool called Reproductive Life Plan (RLP). It is a plan for having children or not and how to achieve these goals. Healthcare professionals should encourage their patients to consider an RLP, but few studies have evaluated the effectiveness of using the RLP in clinical settings.

Study design, size, duration: A randomized controlled trial was conducted between 2015 and 2016. Women (n = 6354), who attended 86 midwives at 28 clinics for contraceptive counseling, were assessed for eligibility. Main outcome measures were knowledge of fertility and reproductive Health.

Participants/materials, setting, methods: Participants (n = 1946) were randomly assigned to control group (CG, n = 970) or intervention group (IG, n = 976). Before the counseling they answered a baseline questionnaire. Both groups received standard counseling, and the IG also received the RLP-based intervention including questions on the RLP, oral information about fertility and reproductive health and a specially designed brochure about fertility. Two months later a questionnaire was mailed for follow-up.

Main results and the role of chance: The two groups were comparable regarding background factors. Women in the IG increased their knowledge significantly ($p < 0.05$) in all questions about fertility; age and fertility, chances of getting pregnant at time of ovulation, fecundity of an ovum and chances of having a child with help of IVF. The IG also increased their knowledge of factors impacting reproductive health such as stop using tobacco, to refrain from alcohol, to be of normal weight and to start with folic acid before a pregnancy.

Women in IG who did not plan any more children, used more long acting reversible contraception than women in CG, 56.5% vs. 44.6%. We found no difference between IG and CG at what age women wished to have their first (28 years) and last (34 years) child.

At baseline, 42 % planned to do lifestyle changes before a pregnancy. The corresponding figure after the intervention was 78 %.

We evaluated the experience of the intervention: 75 % had read the brochure, 60 % were very or fairly positive to the RLP discussion, 76 % felt that RLP discussion should be a routine during visits to the midwife or other health professional.

Limitations, reasons for caution: The follow-up was only two months. We have no information on whether the increased knowledge will turn into a behavioral change before a future pregnancy. The study included only Swedish-speaking women who were between 20 to 40 years old.

Wider implications of the findings: Most women appreciated the intervention and we therefore suggest that the RLP-concept with increased information about fertility can be used in contraceptive counseling. The screening question; Do you wish to have (more) children in the future turned out to be relevant in contraceptive counseling.

Trial registration number: ISRCTN 32759.

O-148 Hope for the best …. But prepare for the worst

S. Bailey[1,2], C. Bailey[3], Y. Cheong[4], E. Kitson-Reynolds[1], N.S. Macklon[5,6]

[1]*University of Southampton, Health Sciences, Southampton, United Kingdom*
[2]*University Hospital Southampton NHS Foundation Trust, Research and Development, Southampton, United Kingdom*
[3]*University of Nottingham, Health Sciences, Nottingham, United Kingdom*
[4]*University of Southampton, Human Development and Health- Faculty of Medicine, Southampton, United Kingdom*
[5]*University of Southampton, Obstetrics and Gynaecology, Southampton, United Kingdom*
[6]*University of Copenhagen, Obstetrics and Gynaecology, Copenhagen, Denmark*

Study question: How do women experience the waiting period (the first twelve weeks) of a new pregnancy following recurrent miscarriage?

Summary answer: Women prepare for the worst, fully expecting another miscarriage to occur. Extreme levels of anxiety are accompanied by feelings of guilt, loneliness and frustration.

What is known already: Recurrent miscarriage is an extremely distressing condition and can be both physically and emotionally traumatising. Studies investigating miscarriage and its effects on emotional morbidity indicate that increased levels of anxiety are often experienced by women with a history of reproductive loss during subsequent pregnancies and that the experience of recurrent miscarriage frequently results in a period of 'marked stress reaction' when the woman becomes pregnant again, whilst they wait for confirmation by ultrasound scan that their pregnancy is ongoing and viable. Some women will elect not to conceive at all rather than face this period of troubling uncertainty.

Study design, size, duration: A qualitative process evaluation employed semi-structured interviews to investigate women's subjective experiences of study methods and explored in-depth women's initial experience of pregnancy following repeated miscarriages. This was nested within a two-centre RCT feasibility study of a novel self-help intervention aimed at improving psychological well-being during the waiting period of a new pregnancy following recurrent miscarriage. Recruitment (n = 75 feasibility RCT, n = 14 qualitative component) took place over a two-year period.

Participants/materials, setting, methods: Participants were recruited from the Recurrent Miscarriage Clinics in two major hospitals in the United Kingdom. Participants were randomised and either received the intervention and weekly questionnaires to assess their psychological well-being up until twelve weeks of their new pregnancy, or simply completed the same weekly questionnaires. Study participants accepted an invitation to participate in semi-structured interviews. Qualitative data was analysed using a thematic network approach and data saturation was achieved after 14 interviews.

Main results and the role of chance: This abstract presents the findings from the qualitative component of the study. Thematic network analysis identified 6 main organising themes:

'Preparing for the worst'

Women reported that they 'prepare for the worst,' fully expecting a further miscarriage to occur. There is a reluctance to emotionally invest in their new pregnancy, taking one day at a time and avoiding looking forward to a successful pregnancy outcome.

Uncertainty and emotional turmoil

Women continually ruminate between the potential outcomes of their new pregnancy. Levels of anxiety and worry are extreme, affecting all aspects of their lives.

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Checking for symptoms of ongoing pregnancy

This was often excessive and included compulsive checking for vaginal bleeding and monitoring of pregnancy symptoms. Fluctuating symptoms caused increased distress.

Social isolation and loneliness

Social isolation was seen as a method of controlling the potential threat of social interaction with other pregnant women.

Guilt

Participants reported a 'constant guilt'. They were to blame for their miscarriages and they were letting their partner and family down at their 'failure' to have a successful pregnancy.

Professional care

Women expressed the importance of supportive care and understanding health professionals. This made a positive difference to the negative emotions they were experiencing.

Limitations, reasons for caution: While every effort was made to support the recruitment of a diverse sample to the feasibility study, the UK setting may limit extrapolation to other national and cultural contexts. The purposive sampling strategy enabled an inclusive approach to data collection from the ethnicities represented in the main study sample.

Wider implications of the findings: This study investigated an issue which causes distress and anxiety to women affected by recurrent miscarriage. It adds valuable information to the body of evidence regarding the understanding of the initial experience of pregnancy following recurrent miscarriage and highlights the unmet needs of this vulnerable patient population.

Trial registration number: This study is registered with the ISCTRN registry. The registration number is: ISRCTN43571276.

O-149 Have we left family out of family planning?

B. Grace[1], J. Shawe[2], J. Stephenson[3]

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[2]School of Health Sciences, Faculty of Health & Medical Sciences, University of Surrey- Guildford- Surrey- GU2 7XH, United Kingdom

[3]UCL Institute for Women's Health, Sexual & Reproductive Health, University College London- Medical School Building- 74 Huntley Street- London WC1E 6 AU, United Kingdom

Study question: What are the current experiences of women and men of reproductive age and healthcare professionals regarding fertility and family planning education in the UK?

Summary answer: There is bias in reproductive health education towards preventing pregnancies. New terminology is needed to encourage more balanced messages encompassing planning to have a family.

What is known already: Although the term family planning implies planning to have a family, it is typically understood to mean birth control, use of contraception or prevention of pregnancy. According to Oxford dictionaries, family planning can be defined as 'the practice of controlling the number of children one has and the intervals between their births, particularly by means of contraception or voluntary sterilisation"[1]. There is currently no widely accepted equivalent terminology for planning to have children. In our study, we introduced the concept of 'family-building" as an alternative terminology for construction or formation of a family and investigated participants' experiences.

Study design, size, duration: We carried out 34 qualitative in-depth interviews with 13 women, 13 men and 8 healthcare professionals. Interviewees were purposively sampled to include men and women from the reproductive age range (18–45 years), ethnicity and education background. Interviews were conducted between October 2016 and January 2017.

Participants/materials, setting, methods: The study was a qualitative component of a wider mixed methods study. Participants were sampled from a UK cross-sectional survey on Fertility Awareness with 1080 participants who agreed to a follow-up interview. Survey participants were recruited nationwide via online newspaper and social media adverts. Healthcare professionals included doctors and nurses. Healthcare professionals were recruited from RCN, RCGP, RCP, doctors.org.uk. Data was transcribed and analysed via thematic analysis. Favourable ethical opinion was given by UCL.

Main results and the role of chance: Participants stated that the term'family planning' meant the avoidance of pregnancy but could not state similar terminology for planning a family. We introduced the concept of family-building to elicit views.

We found recurring themes towards postponement family-building for various socioeconomic reasons. However respondents reported little or no school education on preparation for family-building later in life. Topics such as age-related fertility decline and assisted reproductive therapy were not covered. There was no other source of formal education on family-building in the continuum until men and women were ready to start families, often with inaccurate expectations and fertility issues. Many would like family-building education in work-settings.

Interviewees were also unsure of how to access reliable information. Access e.g. GP appointment was reported as one of the main barriers to seeking information from healthcare professionals. Men reported that they were unlikely to visit walk-in family-planning clinics; and for women who find it easier, focus is on the use of contraception. Information on family-building was seldom provided.

Healthcare professionals were also more likely to view family-planning in the context of pregnancy-prevention. They highlighted knowledge gaps amongst patients and themselves but were uncertain about where the responsibility lies for improving education.

Limitations, reasons for caution: Although we gathered rich data, interviewees were self-selected and results principally reflect views of those who we willing to participate. Due to the online recruitment method, there is a bias towards more educated respondents with online access.

Wider implications of the findings: Emphasis on pregnancy-prevention means that relatively little attention's being drawn to issues associated with postponement of childbirth and impact of infertility. There remains an important need for advocacy to improve access to contraception as part of family-planning education, but should be balanced by messages about fertility, healthy pregnancy and family-building.

Trial registration number: Not applicable.

O-150 The organisation of patient recruitment in directly competing IVF studies to optimise recruitment rate and patient satisfaction

S. Wellstead[1], J. Forbes[1], Y.C. Cheong[2,3], N.S. Macklon[2,3]

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[2]University of Southampton, Unit of human development and health- faculty of medicine, Southampton, United Kingdom

[3]University Hospital Southampton NHS foundation Trust, Complete fertility centre- Department of Obstetrics and Gynaecology, Southampton, United Kingdom

Study question: How should recruitment to intervention studies be managed to optimise numbers and reduce conflict when competing trials have similar eligibility criteria?

Summary answer: Presenting patients with the study most appropriate for them when multiple complex IVF studies are being carried out in one unit, reduces patient disengagement.

What is known already: Research active fertility units often have more than one intervention study ongoing. The challenge is to ensure that all trials are recruited to successfully and within the target period. Failure to do so risks being unable to answer the research question, impacts on the reputation of the unit and has financial consequences for the researchers and sponsors. Previous studies report that placing the choice with patients can be overwhelming, and result in disengagement from research. The optimal strategy for addressing this issue remains unclear.

Study design, size, duration: This retrospective study over a three month period analysed the engagement of patients with the research team and recruitment rates, when more than three complex studies was discussed concurrently during the initial consultation with patients.

Participants/materials, setting, methods: Women referred to the research nurse by their clinician, were approached to discuss the studies in which they were eligible to participate. As the number of studies increased, a drop in recruitment was observed, we considered alternative approaches to disclosing all studies.

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Main results and the role of chance: Having competing complex IVF studies had a negative impact on patient engagement with the research team. We examined strategies for managing competing studies when introducing them to patients, including 'disclosure of all trials' to all eligible patients, or randomly dividing the population and presenting them with only one study. As a team, we reflected on the ethical implications of withholding a study from a patient and the positive and negative impacts this could have on both the patient and the trial. This posed the dilemma of who is responsible for deciding which studies were offered to which patients. We therefore introduced a flow diagram for the research team. This clarified which studies patients were eligible for, and then the expected period of time until their treatment would commence. Patients were then approached and presented with the most appropriate study for them and their circumstances thus avoiding overwhelming them and hence patient disengagement. Following the implementation of this decision making model, engagement (defined by continued ongoing dialogue) with the research team improved greatly to 87.5% over the subsequent two months, compared to 69% before.

Limitations, reasons for caution: This was a retrospective observational study and was not powered for patient engagement before and after the decision making model was implemented.

Wider implications of the findings: The management of multiple studies within a limited population has a potentially significant impact on recruitment. Caution should be taken when interpreting the data but the concepts reported may be of assistance when multiple trials are competing for recruitment from one patient population.

Trial registration number: Not applicable

INVITED SESSION

SESSION 42: MHR SYMPOSIUM - TRANSGENERATIONAL PROGRAMMING VIA THE PATERNAL GENOME

Tuesday 4 July 2017 Plenary 2 14:00–15:00

O-151 High-dose folic acid supplementation alters the human sperm methylome and is influenced by the MTHFR C677T polymorphism

J. Trasler

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Abstract text

Male and female germ cells show distinct differences in the timing of epigenetic patterning, including that of DNA methylation. Such sex-specific differences suggest that males and females will be susceptible to the induction of epigenetic defects in their germ cells at different times in their lives, with the possibility of passing such defects on to their offspring. In the male, the major period of DNA methylation acquisition occurs before birth in male germ cells of the fetal testis. Postnatally, the patterns must be maintained in the male germ line stem cells and a small amount of additional methylation occurs as germ cells develop from spermatogonia to spermatocytes. Folate in the diet is an important source of methyl groups for DNA methylation. Folate deficiency, either due to diet or folate pathway enzyme defects, and folate supplementation are important clinical situations in which methyl donors required for DNA methylation are either lower or higher than normal. High dose folic acid supplementation (4.0–5.0 mg/day) is prescribed to women at high risk for neural tube defects and pregnancy complications, such as preeclampsia, and in the treatment of men with infertility. The potential adverse effects of high dose folic acid on the sperm epigenome have not been examined. We hypothesize that folate status will perturb DNA methylation in male germ cells that are exposed at the key prenatal and postnatal times (susceptibility windows) when DNA methylation patterning occurs. In mouse and human models, we are using array and next generation sequencing based assays to identify DNA methylation alterations in sperm associated with altered folate status. In the mouse we have administered folic acid supplemented diets to model prenatal and postnatal exposures and have found evidence of intergenerational adverse reproductive effects and sperm DNA methylation defects. In men with no history of infertility, low doses of folic acid (0.4 mg/day) given for 3 months did not result in alterations in sperm DNA methylation. In contrast, in men with idiopathic infertility receiving supplements of 5.0 mg/day of folic acid for 6 months, DNA methylation at imprinted loci remained unchanged; however, there was unexpected loss of methylation across the sperm epigenome, effects that were exacerbated in men homozygous for a common polymorphism in the folate pathway enzyme methylenetetrahydrofolate reductase (MTHFR). In summary, both the mouse and human data indicate that clinically relevant high dose folic acid supplements can impact the sperm epigenome. In addition, the mouse studies suggest that DNA methylation defects in male germ cells associated with folic acid supplementation may be heritable and have adverse effects on future generations. (Supported by CIHR).

O-152 Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress

T. Bale[1], J. Chan[2]

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[2]*University of Pennsylvania, Biomedical Sciences, Philadelphia, U.S.A.*

Abstract text

Neurodevelopmental disorders including autism and schizophrenia have been highly associated with parental factors, including lifetime stress experience. We have developed a mouse model of paternal stress in which adult male mice exposed to chronic stress prior to breeding produce offspring with hypothalamic-pituitary-adrenal (HPA) stress axis dysregulation. Paternal sperm was examined for changes in miRNA content where 9 specific miRNAs were identified as significantly increased in the sperm from previously stressed fathers. To test the relevance and potential mRNA targets of these miRNAs following fertilization, we synthesized and injected the 9 miRNAs into single cell zygotes and found that the resulting offspring phenotype completely recapitulated that found from paternal stress sires. In addition, we have now completed single cell amplification from injected zygotes using Fluidigm technology and determined the maternal stored mRNAs that were targets of these sperm miRNAs. Gene set enrichment analysis of the offspring hypothalamus demonstrated broad changes in transcription, largely increasing gene repression in the brains from stressed dads. We hypothesize that the epididymal epithelial cells in the caput of the epididymis that secrete miRNA-containing exosomes are involved in the this sperm reprogramming. Our current studies are studying these cells in vitro and in vivo, to determine how stress interacts with the exosome processing machinery and changes in the miRNA content. We are targeting these cells in an attempt to rescue this paternal transmission and offspring phenotype. Overall, these results demonstrate that paternal experience across the lifespan can induce long-term germ cell epigenetic reprogramming and impact offspring hypothalamic regulation, and may therefore offer novel insight into factors influencing neuropsychiatric disease risk. Identification of the specific miRNAs in germ cells that are altered long-term following stress experience may point to unique biomarkers that could identify at-risk populations.

INVITED SESSION

SESSION 43: ENDOMETRIOSIS MANAGEMENT - LESS OF THE PAST, MORE OF THE FUTURE

Tuesday 4 July 2017 Room A 14:00–15:00

O-153 A vision of the future direction of surgery for endometriosis

T.C. Li

Prince of Wales Hospital, Department of Obstetrics & Gynaecology - Chinese University of HK, Shatin, Hong Kong

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Abstract text

Endometriosis is a common gynaecological condition resulting in reproductive failure or pelvic pain. Surgery has played an important role in the management of the condition. In endometrioma, the key question is how to preserve ovarian function if surgery is indeed indicated. The various strategies to be discussed will include a flexible approach to cystectomy or cystotomy and ablation, as well as reduction of mechanical and vascular injury. Specifically, the preliminary experience of the use of an haemostatic agent, floseal, based on an ongoing RCT will be highlighted. In adenomyosis and adenomyoma, there is a limited role of surgery but recent development in hysteroscopic approach to the management of cystic adenomyoma and its advantages will be outlined. In Stage I/II endometriosis, the choice between ablation and excision will be discussed with emphasis on how to select suitable cases for the two techniques. In stage III/IV disease and deep infiltrating endometriosis (DIE), the ongoing debate of treatment by accredited surgeons in specialized center and its alternatives will be revisited. In the future, surgery will continue to play an important role; there will be some refinement of surgical techniques but important advances in treatment including prevention of postoperative recurrence will follow breakthrough in understanding the genomics & cell biology of different sub-types of endometriosis and the development of specific medical or adjuvant "target" therapies.

O-154 Future perspectives on the medical management of endometriosis

E. Somigliana

Fondazione Ospedale Maggiore Policlinico U.O. Centro Sterilita, Obstetrics-Gynecology and Neonatology, Milano, Italy

Abstract text

During the last decades, endometriosis management have been inspired by the observation of the natural history of the disease. Endometriosis is exceedingly rare before menarche, it transiently improves during pregnancy and it resolves after menopause. Accordingly, progestins, estroprogestins and GnRH analogues have all been demonstrated to markedly improve pain and prevent recurrences. However, once these agents are discontinued, symptoms tend to resume and protection against recurrences vanishes.

Surgical excision of lesions is sometimes demanding and risky but significantly improves symptoms. However, it is poorly effective on adhesions and, again, does not protect from recurrences. A crucial point here is that surgery can remove the lesions but cannot remove the causes of the disease, leaving women exposed to recurrences.

On these bases, researchers have struggled to find out the holy gray for the definite cure of the disease. These efforts have been up to now disappointing. Interfering with the molecular pathway of endometriotic lesions was systematically shown to have concomitant repercussions also on ovulatory mechanisms or on the eutopic endometrium, thus limiting their clinical interest. Overall, we failed to identify agents that are superior to the already available classical hormonal treatments.

Research must continue. No-one knows the long term future of the treatments of endometriosis. However, despite recognizing this difficulty, pessimism is not justified. To date, we cannot cure women with endometriosis but we can effectively treat them. We should re-think the treatment of of the disease in a more pragmatic and woman-centered manner. The future (the present) is a personalized and more conscious management. Physicians and patients should be aware of the relevant beneficial effects of currently available therapies and concomitantly also accept and deal with their limitations. Progestins, estroprogestins, GnRH analogues, surgery and IVF represent an outstanding armamentarium provided that they are used within a personalized medicine that puts women, their symptoms, their fears and their whishes in the Center. Physicians are called to establish a in-depth relationship with the patients, based on honest and detailed information in order to tailor the best treatment for every woman. The future is improving the satisfaction of the women with the available instruments rather than seeking for the holy gray. And we can do it.

INVITED SESSION

SESSION 44: PARAMEDICAL INVITED SESSION 3: DEBATE – 'SHOULD WE PERFORM PGS ON ALL PATIENTS?'

Tuesday 4 July 2017 Room B 14:00–15:00

O-155 Pro

L.F. Rienzi

Genera c/o Clinica Valle Giulia GENERA center for Reproductive medicine, GENERA center for Reproductive medicine, Roma, Italy

Abstract text

Pre-implantation genetic testing for aneuploidy (PGS or PGT-A) is an early form of prenatal diagnosis. The embryos obtained during an *in vitro* fertilization cycle (IVF) are tested with the goal to identify those that are affected by chromosomal aneuploidies. As for pre-implantation genetic testing for monogenic disease (PGD or PGT-M), this strategy has the principal objective to avoid the instauration of a pregnancy with an affected embryo. To date there are not yet a clear consensus about the population of patients that would benefit from PGT-A approach. The main indications suggested in the literature are: advanced maternal age, history of recurrent miscarriages and/or repeated IVF failures. However, other indications have been proposed over time including: previous genetically abnormal pregnancy, poor embryo quality, previous radiotherapy and single embryo transfer policy. Chromosomal aneuploidies are indeed the main reason for implantation failure and pregnancy loss, and their prevalence in the embryos is strongly related to female age. In theory, all patients undergoing an IVF cycles have a potential risk to produce aneuploid embryos and could thus benefit from this test. The debate about the usefulness of PGT-A has started after the clinical failure of its first version based on cleavage stage biopsy and 9 chromosome-FISH analysis. Today, PGT-A is performed by comprehensive chromosome screening (CCS) techniques on trophectoderm (TE) biopsies. To understand the potentiality of the technology as performed now, 3 aspects have to be considered: invasivity of the biopsy, accuracy of the test and clinical efficiency in terms of sustained pregnancy rate per transfer and miscarriage rate. Unlike blastomere biopsy, TE biopsy is a safe and extensively validated approach with low biological and technical margin of error. The prevalence of mosaic diploid/aneuploid embryos is estimated to be low at the blastocyst stage, between 0 and 16 %. All CCS technologies adapted to, or designed to conduct PGT-A are highly concordant. The false positive error rate is estimated to be around 0.5% while a clinically recognizable error rate per transferred blastocyst of 0.2 %. Moreover, RCTs, observational and prospective studies were able to show that in both young and advanced maternal age populations, PGD-T resulted in a higher delivery rate per embryo transferred. To this regard, in particular, it was shown that single euploid blastocyst transfer equals double untested embryo transfer in terms of live birth rate, but with significantly better obstetrical outcomes. These data collectively represent a considerable body of evidence in favour of PGT-A clinical efficiency. An analysis of the cost-effectiveness of PGT-A is however still missing. Ideally, it should also take into account pre-natal test cost and obstetrical and neonatal costs. In conclusion, there is a sufficient body of evidence to support the clinical application of CCS-based PGT-A on TE biopsies. The main limiting factor is the need for a high-standard laboratory to conduct blastocyst culture, biopsy, vitrification, and genetic test and, in turn, the cost of the procedure.

O-156 Con

S. Repping, S. Mastenbroek

Center for Reproductive Medicine, Center for Reproductive Medicine, Amsterdam, The Netherlands

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Abstract text

The aim of preimplantation genetic screening (PGS) is to select out embryos that are aneuploid. The reason to do so has generally been to increase the chance of live birth in couples undergoing IVF/ICSI. However, data that supports the notion that PGS increases live birth rates is lacking. In fact, PGS using day 3 biopsy and FISH results in a significant reduction in live birth rates. For novel technologies using day 5 biopsy and array based or NGS analysis, well designed trials are largely lacking. Simple reasoning demonstrates that no selection method will ever increase cumulative live birth rates per started cycle and the scientific consensus is that it does not. At best, PGS could perhaps reduce time to pregnancy or reduce the number of miscarriages but at what cost? And how should we inform patients about these (un)certainties and how are they informed?

INVITED SESSION

SESSION 45: PATIENT SESSION: PUSHING THINGS FORWARD: ACCELERATING CHANGE - PATIENT ASSOCIATION ROLE IN MAR

Tuesday 4 July 2017 Room W+X 14:00–15:00

O-157 25 million people matter. Patients' network as a driving force in Europe

S. Rautakallio-Hokkanen

Lapsettomien yhdistys Simpukka ry, Infertility Association, Tampere, Finland

Abstract text

Infertility is a very personal and intimate issue. There is a need to talk to someone who shares your thoughts and knows what your going through. This is where patient associations have their role. There are cultural and legislational differences in Europe, but the main idea of patient associations everywhere is still the same: to support, to understand, to listen and to reach out a helping hand. National associations are very important when there is a need to discuss infertility related issues with authorities, lawmakers and health care professionals in their own countries.

But when it comes to patients' rights on European level, it's very hard to influence or make things move forward from national level. Fertility Europe is an umbrella association for European infertility associations. It was founded in 2009 with 9 members and has since then reached most of the European countries. With 23 member associations from 21 countries Fertility Europe has a significant role in joining over 25 million people facing infertility in Europe. What can an umbrella association do for its members? What has Fertility already done to promote the rights of infertile patients? What does it mean that #InfertilityMattersEU?

O-158 Questions? Questions! How can patients contribute to improvement of fertility care?

J. Knijnenburg

Groesbeek, The Netherlands,

Abstract text

The basis of science is a curiosity, that leads to questions for which scientists seek and find answers with some hard work. Guidelines summarize the best answers on these scientific questions in order to implement a good standard of care. That's all in the world of the scientist and professional caregivers. And it's all about groups and not about individuals.

Patients come to you for answers on their personal questions. But how do they know that they are in the right place, handled with the right care? And how do you know that you give them the care that suits them in their personal lives?

Patient organization Freya has developed several tools in order to try and bring caregivers and patients together in their mutual quest for the best fertility care. We use different questionnaires to measure the quality of care from a patients' perspective, to compare the care actually given to the guidelines and try to make fertility care more transparent. Furthermore, we encourage patients to ask more questions in the consulting room so they get the individual answers they seek.

O-159 Reproductive health education of adolescents as a tool for early prevention of infertility

I. Popova

Zachatie Association Member of Fertility Europe, Sofia, Bulgaria

Abstract text

Zachatie Association is the patient organization of people with reproductive health issues in Bulgaria. Established in 2004, the NGO is entirely volunteer structure and some of its main goals are to promote public debate on infertility in Bulgaria; to assist the implementation of national strategy for reproductive health; to initiate and support changes in legislation, related to reproductive health and infertility treatment; to provide information and support on prevention and treatment of infertility-inducing diseases.

Zachatie Association supports the vision of the International Federation of Gynecology and Obstetrics (FIGO), that „*prevention reduces or sometimes avoids the harm of infertility for some of the population at risk, even if some of those affected later have access to treatment. Prevention generally involves not only access to healthcare but also to health education.*"

In our understanding, the education about the human reproductive health should start at early age – namely at puberty – in order to have a maximum preventive effect. The main issues we focus on are as follows:

(1) Education about functioning of the human reproductive system;
(2) Education about sex and birth control;
(3) Education about healthy lifestyle and possible risk factors for the reproductive health.

Zachatie Association considers the government-provided mandatory education in high schools as not adequate and sufficient in that matter. Therefore, as a socially responsible organization, the Association has decided to launch its own educational and awareness campaign among the teenagers of the country, in favour of public health.

The awareness campaign, called "**Stay in the Loop: Your Reproductive Health**" is aimed mostly at school boys and girls aged 13–17. The campaign was launched in 2011 and is still going on.

This presentation will describe into details the main goals, aspects, tools and outcomes of the reproductive health awareness campaign for teenagers in Bulgaria, as well as our conclusions and vision for potential future development of the project on European scale. Our hope is that this campaign will serve as an example for the government and that reproductive health education will be considered part of the mandatory high school education in the future. It is our strong belief, that reproductive health education will lead to prevention of some forms of infertility in the future and to economic benefits for the society in the long run.

O-160 MAR in Croatia: Retrospective and Prospective. The role of patients' organisation in positive change of MAR practices

I. Lodder Rozic

Zagreb, Croatia,

Abstract text

On the July, 17 2009 Croatian government pass one of the most restrictive law on the assisted reproduction and infertility treatments. Among other, it limited number of fertilized oocytes to three (3) within a cycle, banned cryopreservation of embryos and MAR was made available for married couples only. As consequence of such political act, patients went through numerous unnecessary

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ART treatments, number of high-risk multiple pregnancies increased and more couples opted for cross-border treatments.

Although NGO Roda (Stark) dealt with the issues of (in)fertility since 2003, this law shifted focus from patient information and support to advocacy for the best medical practices, becoming virtually the only voice of reason. This presentation will give an overview of actions and campaigns for change and public awareness.

After almost three years of struggle and with a governmental change, on July 13, 2012 new MAR law came into force. Even so, current legislative still tried to conciliate opposite personal and political views on the expense of medical standards and minority groups. So, five years later, where are we?

SELECTED ORAL COMMUNICATIONS
SESSION 46: FRONTIERS OF EMBRYOLOGY
Tuesday 4 July 2017 Plenary 1 15:15–16:30

O-161 Establishment of appropriate methods for human oocyte-activation by PLCZ1 cRNA (PLCZ) injection

T. Yamaguchi[1], A. Tanaka[1], M. Nagayoshi[1], T. Ichiyama[1], M. Ohno[1], K. Kuroda[2], M. Ito[3]

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[2]Juntendo University School of Medicine, Department of Obstetrics and Gynecology, Tokyo, Japan
[3]Hamamatsu University School of Medicine, Department of Infectious Diseases, Hamamatsu, Japan

Study question: Is PLCZ injection into human oocyte a better method for oocyte-activation compared to other methods like Electrical stimulation or Ionomycin?

Summary answer: We found the optimal concentrations of PLCZ to activate oocytes physiologically, with a similar pattern of Ca^{2+} oscillations to that of in vitro fertilization oocytes.

What is known already: At fertilization, mammalian oocytes show repetitive transient Ca^{2+} transients each of which is due to Ca^{2+} release from the endoplasmic reticulum through Inositol 1,4,5-trisphosphate(IP_3) receptors. During fertilization, the so called sperm factor, is released into the oocyte and induces series of Ca^{2+} spikes that are required for oocyte activation. They are called Ca^{2+} oscillations. IP_3-producing enzyme phospholipase C zeta (PLCZ1) is a strong candidate to be the sperm factor.

Study design, size, duration: We performed this retrospective study to investigate the usefulness of PLCZ injection into human oocyte. 94 fresh M-II oocytes from 34 patients were used to find optimal concentration to activate human oocytes and 67 oocytes from 29 patients were used to compare with the other oocyte activation methods from January 2013 to December 2015.

Participants/materials, setting, methods: Optimal RNA concentration for oocyte activation was examined by injection with various concentrations of PLCZ into human oocytes from IVF patients who had consented to this experiments. After injection, the rate of PN formation was checked and the intracellular Ca^{2+} concentration of injected oocytes was monitored by Fluo8H AM fluorescent Ca^{2+} indicator.

Main results and the role of chance: Optimal concentration of PLCZ1 to activate human oocytes was 100 ng/μl in the examination using 94 fresh M-II oocytes (Table 1).

The pattern of Ca^{2+} oscillations by PLCZ injection was similar to the pattern of Ca^{2+} oscillations seen in the in vitro fertilized oocytes (Table 2).

	Oocytes (n)	Day 1	Day3 (>= 7 cell)
1PN+ First polar body number (%)	Cleaved embryos number (%)		
Ionomycin	15	66.7 (10/15)	40.0 (4/10)
PLCZ1	35	65.7 (23/35)	60.1 (14/23)
Electrical stimulation	17	52.9 (9/17)	44.4 (4/9)

In comparison with the other oocyte activation methods, cleavage rate was the highest in the oocytes activated by PLCZ. However it showed no significant differences in pregnancy rates.

Limitations, reasons for caution: Birth of healthy offspring and reproduction of healthy second generation from mouse oocytes activated by PLCZ was reported in 2008. However healthy human births have not been reported after using this method. The safety of PLCZ injection to human health should be further examined for future clinical applications.

Wider implications of the findings: First it will rescue the unfertilized oocytes after ICSI due to insufficient oocyte activation, which accounts for approximately 40% of all unfertilized oocytes. Secondly, this new oocyte activating method which is more physiologically oocyte activation will be beneficial for improving clinical outcome of ROSI.

Trial registration number: UMIN Clinical Trials Registry: UMIN000020860.

O-162 Using artificial intelligence to improve blastocyst morphology evaluation

J.C. Rocha[1], F.D. Matos[2], A.C. Basso[3], C.F.L. Hickman[4], S. Lavery[4], M.F.G. Nogueira[5]

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[2]Institut de Biologie de l'ecole normale Superieure de Paris, Biologie, Paris, France
[3]In Vitro Brasil SA, IVF, Sao Paulo, Brazil
[4]The Fertility Partnership, Boston Place Clinic, London, United Kingdom
[5]Sao Paulo State University UNESP, Laboratory of Embryonic Micromanipulation, Sao Paulo, Brazil

Study question: Can time-lapse images of embryos be assessed by an Artificial Intelligence (AI) based on a genetic algorithm associated with an artificial neural network (ANN)?

Summary answer: AI can be used to reduce inter-operator variation and to acquire additional parameters not detectable by manual optical observation of blastocysts.

What is known already: Morphological grading of blastocysts can lead to information predictive of the implantation potential of embryos. This information can then be used to determine number of embryos to be transferred, to advise the patient on their chances of pregnancy as well as to assess the quality assurance of a laboratory. The issue is that morphological grading by humans leads to wide inter- and intra-operator variation, a problem that may be

	Concentration of PLCZ (ng/μl)	0.01 (n = 13)	0.05 (n = 14)	0.1 (n = 4)	1 (n = 6)	5 (n = 25)	10 (n = 11)	100 (n = 18)	1000 (n = 3)
Day 1	Second polar body (%)	23.1	14.3	0	0	16.0	9.1	66.7	66.7
1PN (%)	23.1	14.3	0	0	8.0	9.1	66.7	66.7	

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resolved using advances in AI that use mathematical variables derived from blastocyst images to automatically classify images with the objective of increasing the predictive value of pregnancy.

Study design, size, duration: 482 early to expanded blastocyst images were assessed by three embryologists and processed for standardisation. 70% of images were used for training the system, 15% were used for validation and 15% for blind testing. Inter (blind classification of 48 images by 3 embryologists) and Intra-operator (thrice evaluation of the same 48 images by each embryologist) variation was compared between the embryologists versus AI.

Participants/materials, setting, methods: Of the 36 numerical variables extracted from the standardised images and analysed for collinearity, 24 remained which formed the input of the ANN architecture associated with the genetic algorithm to produce a predictable output of blastocyst grading. This AI was assessed using ROC, confusion matrix and Kappa Index against three experienced embryologists.

Main results and the role of chance: The confusion matrix (AI as the output class and the embryologists as the target class) demonstrated that serious errors (differing in more than two quality grades) occurred in 6% (4/72) of images, and the AI had a 76% (55/72) accuracy on test data. ROC curves demonstrated an excellent performance of the AI. The AI demonstrated an improved inter-operator variation (embryologist Kappa 0.358 was lower than the AI Kappa 0.757) and improved intra-operator variation (Intra embryologist 0.28, 0.41, and 0.47 Kappa scores leading to a low overall Kappa with 50–54% agreement among embryologists, versus Intra AI 1.0, 1.0, 1.0 Kappa leading to an overall perfect Kappa score and 87.5% of agreement.

Limitations, reasons for caution: This technology is in its early stages for implementation in human IVF time-lapse practice. Collaborative work between UNESP and Boston Place Clinic is ongoing to establish the evidence-base supporting the already observed benefits of these technologies when used together. The work was supported by FAPESP funding processes # 12/50533-2, 13/05083-1, 06/06491–2 and 12/20110–2.

Wider implications of the findings: By increasing objectivity and repeatability in embryo assessment, we can improve our efficacy of diagnosing embryo viability. Clinics can use this information to customise treatment strategies and patients can be better informed of their chances of pregnancy.

Trial registration number: not applicable.

O-163 The effect of gonadotropin releasing hormone (GnRH) agonist and antagonists stimulation protocols on the viability and metabolism of human oocytes and early embryos

C. Leary[1], R. Sturmey[2]

[1]*East Riding Fertility Services, The Hull IVF Unit, Hull, United Kingdom*
[2]*The Hull York Medical School, Centre for Cardiovascular and Metabolic Research, Hull, United Kingdom*

Study question: Does ovarian stimulation protocol influence oocyte viability and subsequent in-vitro embryo development and metabolic regulation?

Summary answer: GnRH antagonist protocol was associated with higher blastocyst rates, yet these blastocysts had a distinct metabolic phenotype and were less likely to implant.

What is known already: Ovarian stimulation influences IVF success. GnRH agonist and antagonists protocols are both used. Comparative studies have focused primarily on clinical pregnancy rates and ovarian response, yet the impact on on oocyte and embryo viability has received little attention.

Attempts to investigate the effects of exogenous gonadotrophins on embryo development in rodent models have indicated delayed development to the blastocysts. Whilst this is important for embryo selection blastocyst development rates are a limited measure of embryo competence. The study of embryo metabolism provides a quantifiable, objective measure of how ovarian stimulation could influence oocyte and subsequent embryo metabolic physiology and viability.

Study design, size, duration: We conducted a retrospective study to compare the impact of GnRH agonist and antagonist protocol on embryo development to blastocyst. In addition, we have measured non-invasively the metabolic activity of 779 IVF/ICSI embryos from 168 consecutive women who donated their surplus embryos to research, and have related the data retrospectively to their cycle outcome.

Participants/materials, setting, methods: In a clinical IVF setting, we compared oocyte number and recorded the development of supernumerary embryos collected from women receiving agonist protocol (n = 81 women, 401 embryos) and those from women receiving an antagonist (n = 87 women, 378 embryos). The non-invasive consumption/release of glucose, pyruvate, lactate and amino acids were measured on spent droplets of culture medium from day 5 to 7. Total triglyceride levels within individual embryos were also determined on day 7 of development.

Main results and the role of chance: Antagonist cycles led to significantly fewer oocytes per patient, higher rates of blastocyst formation on day 5 ($p < 0.01$) and lower implantation rates in treatment cycles, despite there being no differences in patient demographics including female age, AMH levels, BMI, cycle number and cause of infertility. Of those embryos donated to research, blastocyst rates were again significantly higher (42.6% compared to 31.2% on day 7) in the antagonist and these embryos had less endogenous triglyceride on day 7 (7.41 ng compared to 9.96 ng for the agonist group, $p < 0.01$). Glucose, pyruvate and amino-acid metabolism of day 7 blastocysts was not influenced by stimulation regimen, however lactate production was significantly higher in the antagonist group (122.97 & 100.42 pmol/embryo/hr respectively $p = 0.01$). The excess lactate production indicates that glucose is diverted into aerobic glycolysis, which may allow faster incorporation of carbon into biomass, facilitating rapid cell division. This may explain the higher rates of blastocyst development, however it could be speculated that, precocious development may be detrimental for implantation, hence the lower pregnancy rates in fresh cycles. Although statistical power has been achieved for metabolic primary end points, this is a retrospective study and further studies would be required to validate implantation findings.

Limitations, reasons for caution: The analysis has been performed on supernumerary embryos, originating from a single IVF unit and not selected for use in treatment. Endometrial interactions would also require further consideration.

Wider implications of the findings: The data indicate that ovarian stimulation is associated with distinct phenotypic changes in the embryo during the preimplantation period, highlighting the potential legacy of the ovarian conditions under which the oocyte has matured and influence on embryo developmental viability and potential likelihood of successful implantation.

Trial registration number: not applicable.

O-164 Noninvasive fluorescence lifetime imaging microscopy to detect metabolic differences in mouse embryos: safety and live birth analysis

T. Sanchez[1], D. Sakkas[2], E. Seidler[2,3], M. Venturas[1], D. Needleman[1]

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[2]*Boston IVF, Reproductive Endocrinology and Infertility, Waltham- MA, U.S.A.*
[3]*Harvard Medical School, Beth Israel Deaconess Medical Center, Boston- MA, U.S.A.*

Study question: Can noninvasive fluorescence lifetime imaging microscopy (FLIM) be used safely and does it detect different metabolic profiles in mouse embryos of the same morphology?

Summary answer: Metabolic imaging can sensitively detect differences in mouse embryo metabolism and does not decrease live birth rates when compared to non-exposed controls.

What is known already: Metabolic function changes as embryos develop, with both varying degrees of ATP synthesis and turnover at different developmental stages. As NADH and FAD+ are both autofluorescent and integral to cellular respiration, monitoring their fluorescence yields valuable information for characterizing cellular metabolism. Superior to intensity-only (single parameter) measurements, FLIM generates three additional parameters that are sensitive to metabolic shifts. As with other noninvasive assessment methods such as time lapse, FLIM does not require the introduction of additional markers or dyes. Thus, FLIM holds great potential as a new embryo assessment tool; however, its safety for embryos has yet to be validated.

Study design, size, duration: Control and FLIM illuminated blastocysts were transferred in groups of twelve to pseudopregnant females. In total, 96 illuminated embryos and 84 control embryos were transferred to 8 and 7 pseudopregnant females, respectively. At birth, the pups were counted and weighed

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to assess for any adverse effect of FLIM exposure. In addition, averaged FLIM data for 93 embryos was analyzed to evaluate for natural variation between embryos and reproducibility of the measurements.

Participants/materials, setting, methods: Mouse embryos were obtained from a commercial source. Embryos were cultured from the 2-cell until the blastocyst stage using a closed microscope chamber, with and without exposure to FLIM illumination. Comprehensive metabolic measurements (NADH and FAD+) were taken every two hours during the course of mouse embryo development using the on-stage incubation system. Control embryos were cultured in the same chamber but were not illuminated.

Main results and the role of chance: FLIM-based metabolic imaging was not detrimental to both live birth rate and pup weight when compared to control. The mean (+SD) number of pups per mouse for control and illuminated groups was 4.6 + 3.1 and 4.5 + 1.7, respectively. The pup weights (g) were 1.89 + 0.27 (Control) and 1.92 + 0.40 (FLIM), respectively. When we collated the metabolic FLIM signatures over the course of mouse embryo development from the 2-cell to the blastocyst stage we found that embryos exhibited distinct and consistent metabolic changes over the course of their development. In particular, NADH levels peaked around compaction and decreased significantly during blastocyst formation, likely corresponding with an associated change in energy demands. Additionally, we observed significant variation between embryos, compared to the error on our measurement, further indicating the potential of this technique to sensitively detect differences in metabolic state. Finally, we found a high degree of reproducibility from different days in the measurements. The ability to identify each embryo's distinct metabolic profile regardless of similar morphology validates the concept of using FLIM to generate a unique metabolic signature.

Limitations, reasons for caution: Although these findings represent an innovative mechanism of embryo evaluation, a defined standard for interpreting metabolic FLIM parameters remains to be developed. The hardiness of the mouse model may represent that caution is still needed if this method is to be used for testing human embryos.

Wider implications of the findings: This work represents a novel tool for assessing the metabolic health of embryos. Preliminary results indicate promise for both safety and its use as a potential embryo selection tool. Future in-clinic measurements on human embryo samples are planned and will investigate possible correlations between metabolic signatures and final pregnancy outcomes.

Trial registration number: Not applicable.

O-165 Non-invasive prediction of blastocyst implantation and live birth by day three embryo culture medium lipid fingerprinting

A. Setti[1], D.P.A.F. Braga[2], D.A. Montanni[3], E.C. Cabral[4], M.N. Eberlin[5], E.G. Lo Turco[3], E. Borges Jr.[2]

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[4]UNICAMP, Centro Pluridisciplinar de Pesquisas Químicas- Biológicas e Agrícolas – CPQBA, Campinas, Brazil
[5]UNICAMP, Laboratório ThoMSon de Espectrometria de Massas – Instituto de Química, Campinas, Brazil

Study question: Is the embryo culture media lipid fingerprinting, by mass spectrometry (MS), able to identify blastocyst with increased potential to implant and lead to live births?

Summary answer: The culture media lipidomics is a promising approach to identity blastocyst able to implantation and lead to live births.

What is known already: *In vitro* fertilization (IVF) success have been remarkably improved in the last decades. However, its efficiency, measured as the live birth rate, is still below 50%. Improvements to IVF depend on the ability to select the most viable embryos for transfer. Non-invasive approaches to access embryo development have been proposed, and it includes embryo metabolome and, more specifically, embryo lipidome. Modern approaches for lipidomic analysis are dominated by MS, which afford the study of intact lipid molecular species from very small amounts of samples. Therefore the embryo culture media lipidomics may be a promising tool to access embryo viability.

Study design, size, duration: For this case-control study 33 samples of embryo culture media were collected on day three for 22 patients undergoing intracytoplasmic sperm injection (ICSI) and day five embryo transfers. Samples were split into groups according to the implantation: Positive-Implantation-Group (n = 19) or Negative-Implantation-Group (n = 14). The study included exclusively samples from high-quality transferred blastocysts and the Positive-Implantation-Group included exclusively samples from patients with 100% implantation diagnosis. Subsequently to the implantation confirmation, culture medium samples were analyzed by MS.

Participants/materials, setting, methods: Samples were collected between January/2015 and November/2015 in a private university-affiliated IVF center. The lipids from culture medium were individually extracted using the Bligh and Dyer method. Mass spectra were obtained with a direct infusion of both the negative and positive ion modes into a Q-Tof mass spectrometer. Obtained data was analyzed by Principal Component Analysis (PCA) and Partial Least Square Discrimination Analysis (PLS-DA), combined with variable influence in the projection (VIP) scores.

Main results and the role of chance: All blastocysts from the Positive-Implantation-Group lead to live births. Concerning the PCA analysis, an increase difference was noted for lipidic characteristics between the Negative- and Positive-Implantation-Group, when compared with the negative mode.

Moreover, we could note a better clustering in negative implantation group for both, positive and negative modes. The PLS-DA showed satisfactory separations between the Positive- and Negative-Implantation-Groups for both, positive and negative modes.

The VIP plot of the PLS-DA provided a list of four ions, in the positive mode, with an area under the curve (AUC) of 73.5% and six ions, in the negative model, with and AUC of 72.0%. For both positive and negative modes, possible biomarkers for the negative implantation were identified by the lipidmaps: phosphoethanolamine, dicarboxylic acids, glycerophosphoglycerol, glycerophosphocholine, glicerophosphoinositol, phosphoethanolamine and unsaturated fat acids. The other ions were not identified. These lipids are involved in the GPI anchor biosynthesis and synthesis of glycerophospholipids and phosphate inositol.

Limitations, reasons for caution: For this study MS/MS, to confirm the presence of the lipids identified here, was not performed. In addition, the identification of the viable embryo may not guarantee the positive implantation and live birth outcome, since, besides proper embryo development, embryo implantation also depends on the acquisition of a receptive endometrium.

Wider implications of the findings: Mass spectrometry fingerprinting is a useful predictive tool for blastocysts that fail to implant, and therefore this technique could be incorporated in the laboratory routine, adjunct to morphology evaluation to identify embryos that should not be transferred.

Trial registration number: None.

SELECTED ORAL COMMUNICATIONS
SESSION 47: WHAT IS NEW IN AMH?
Tuesday 4 July 2017 Plenary 2 15:15–16:30

O-166 Intercyclic variation in AMH levels; challenges of using single-sample measurements for clinical predictions

L. Bungum[1], J. Tagevi[2], N. Tørring[3], C.Y. Andersen[4], N. Macklon[5], M. Bungum[6], A. Giwercman[6], S. Skouby[7]

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[5]University of Copenhagen and Southampton, Sjælland University Hospital, Roskilde, Denmark
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[7]University of Copenhagen Hospital, Gynecology and Obstetrics, Copenhagen, Denmark

Study question: To quantity the extent of long-term variability in Anti Müllerian Hormone (AMH) levels and potential differences between analytical assays

Summary answer: Long-term AMH measurements reveal a significant variation, probably caused by biological variation in the number of AMH-producing follicles

What is known already: AMH measurement has become increasingly used in fertility evaluation and prediction of response to ovarian stimulation and its interpretation may have major implications for clinicians' recommendations and patients' choice of treatment. While recognized clinical biomarkers are expected to express a low degree of variation, both biological and analytical variability exists. For AMH, reported intra- and intercyclic variability may be based on the dynamics in folliculogenesis with follicle activation and atresia providing a variable number of AMH-producing follicles at any time. Additionally, various assays will deliver diverse results depending on which molecular form of AMH being measured.

Study design, size, duration: Prospective cohort study covering three spontaneous menstrual cycles in ovulatory women

Participants/materials, setting, methods: A total of 27 healthy women, all non-smoking and with a regular menstrual cycle, volunteered to participate in the study. All study participants had blood drawn day 5-10-15-20-25 (30) in three consequtive cycles.All samples were analyzed using two platforms; the Immunotech EIA AMH/MIS manual kits from Beckman Coulter, Inc. and the automated Roche Elecsys Cobas e601. The difference in coefficiant of variation between assays was tested in a paired t-test.

Main results and the role of chance: The average number of consequtive AMH recordings per study subject (over three menstrual cycles) were 14.7 exposing a substantial degree of variation. The lowest differences in AMH recordings in one study subject were in absolute and relative form (%): Beckman Coulter 3.9 pmol/l (60%), Cobas 4.4 pmol/l (37.3%), and the corresponding maximum values were; Beckman Coulter 33.0 pmol/l (446%) and Cobas 31.4 pmol/l (274 %). There was no statistically significant difference in the coefficient of variation between assay platforms, neither by cycle, nor menstrual day

Limitations, reasons for caution: Number of participants limited

Wider implications of the findings: The findings question whether a single AMH measurement is sufficient for decision-making in assisted reproductive technologies.

Trial registration number: Lund Ethical review Board, Sweden Dnr. 539/2008.

O-167 High AMH in ovulatory women predicts high cumulative pregnancy rate after spontaneous conception

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Study question: Can AMH predict cumulative pregnancy rates and TTP in women attempting to conceive spontaneously and what is the lower AMH threshold compatible with spontaneous conception?

Summary answer: High AMH was associated with higher cumulative pregnancy rate compared with intermediate/low AMH. Spontaneous conceptions occurred with AMH levels down to 1.2 pmol/L.

What is known already: AMH is a quantitative marker of ovarian reserve and is successfully used in ART to predict ovarian response. However, AMH is a weak predictor of pregnancy after IVF-treatment. Few publications have investigated AMH in relation to spontaneous conceptions and the results are inconsistent possibly because some studies included only women that did conceive and thus had proven fertility; a few others used selected groups of patients/women.

Study design, size, duration: A cohort study of 260 women aged 25–42 years included at The University Hospital of Copenhagen, Rigshospitalet during 2008–2014. All included women initiated an attempt to conceive spontaneously or had an unplanned conception within two years after inclusion.

Participants/materials, setting, methods: The participants originated from two subcohorts: A) women aged 25–42 years consulting the Fertility Assessment and Counselling Clinic and B) health care workers aged 25–41 years employed at Rigshospitalet. Users of oral contraceptives at inclusion were excluded. All had AMH (Elecsys), ovarian sonography and extensive reproductive history. Follow-up on pregnancy attempts was collected through questionnaire.

Main results and the role of chance: Participants were stratified in low AMH (1st quintile): <9.5 pmol/L, intermediate AMH (2nd – 4th quintiles): 9.5 – 33 pmol/L, and high AMH (5th quintile): >33 pmol/L. Cumulative pregnancy rates within two years increased with increasing AMH: 60.1% (low) versus 70.0% (intermediate) versus 78.3% (high AMH) ($p = 0.03$). Pregnancy rates were reduced in women with high AMH and cycle length >35 days (pregnancy rate: 37.5%) compared with women with high AMH and regular cycles (pregnancy rate: 84.1%, $p = 0.01$). The incidence of unplanned pregnancies increased with AMH: low: 6.7%, intermediate: 20.4%, high AMH: 27.5%. In a univariate analyses the oldest quintile (>36.7 years) had longest TTP ($p = 0.03$). AMH was the only independent predictor of TTP in a multivariate analyses adjusted for female age, cycle regularity and previous conception. TTP was shorter among women with high AMH compared with intermediate or low AMH (HR: 0.63 and 0.55, $p \leq 0.01$). Trend test of AMH groups confirmed the stepwise trend ($p = 0.01$). Five of nine women with AMH value <3 pmol/L became spontaneously pregnant (TTP range 26–523 days). Spontaneous pregnancies were observed in women with AMH down to 1.2 pmol/L; three women had AMH below this value and none conceived spontaneously (attempted pregnancy for 104— 327 days).

Limitations, reasons for caution: Sperm analyses were unavailable.

Wider implications of the findings: Spontaneous conceptions occur over a wide range of AMH values but high AMH suggesting a low "biological age" was associated with high natural fecundity in ovulatory women. Nonetheless, TTP differ substantially within similar AMH levels and AMH therefore has limited value in prediction of TTP.

Trial registration number: Data collection was approved by the Ethical Committee of the Capital Region of Denmark (H-B-2007–129 and H-1-2011-081).

O-168 The impact of serum anti-Müllerian hormone (AMH) levels on clinical outcome of individualised follitropin delta dosing and conventional follitropin alfa dosing in controlled ovarian stimulation

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Study question: To evaluate ongoing pregnancy rate and the risk of early ovarian hyperstimulation syndrome (OHSS) in relation to serum AMH levels, following treatment with individualised follitropin delta or conventional follitropin alfa.

Summary answer: Individualised follitropin delta prevented ongoing pregnancy rates from declining in patients with high AMH and reduced the risk of early OHSS.

What is known already: In response to gonadotropin stimulation, there is a direct association between serum AMH and the number of oocytes retrieved, as well as between the number of oocytes retrieved and the risk for OHSS. An individualised dosing regimen of follitropin delta (FE 999049), targeting an adequate number of oocytes retrieved, significantly reduced the incidences of preventive interventions for early OHSS and preventive interventions and/or

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OHSS, while maintaining non-inferior ongoing pregnancy rates compared to conventional follitropin alfa treatment (Nyboe Andersen and Nelson et al, Fertil Steril, 2016).

Study design, size, duration: Randomised, assessor-blind, controlled trial including 1326 patients undergoing their first IVF/ICSI cycle. Patients were randomised 1:1 to individualised follitropin delta (fixed daily dose based on patient's AMH and body weight) or conventional follitropin alfa (starting dose 150 IU/day, with potential adjustments from day 6). Preventive interventions for early OHSS included cycle cancellation (≥25 follicles ≥12 mm), GnRH agonist triggering (25–35 follicles ≥12 mm, no fresh blastocyst transfer) and/or dopamine agonist administration (≥20 follicles ≥12 mm).

Participants/materials, setting, methods: Retrospective comparative analyses of ongoing pregnancy rates, early OHSS, preventive interventions for early OHSS and total FSH dose in subgroups defined by AMH cut-off levels of ≥5, ≥10, ≥15, ≥20, ≥25, ≥30 and ≥35 pmol/L, including 92%, 73%, 55%, 39%, 27%, 20% and 13% of the trial population, respectively. AMH was measured by Elecsys® AMH (Roche Diagnostics).

Main results and the role of chance: In the subgroup with AMH ≥5 pmol/L the ongoing pregnancy rate was 31.4% (193/615) following individualised follitropin delta dosing and 32.0% (194/607) following conventional follitropin alfa dosing. In both treatment groups, the ongoing pregnancy rate was stable with increasing AMH cut-offs up to the subgroup with AMH ≥20 pmol/L. Above this threshold, the ongoing pregnancy rate remained stable with individualised follitropin delta dosing, whereas conventional follitropin alfa dosing was associated with a decline in the ongoing pregnancy rate. This discordance was most striking for women with an AMH ≥35 pmol/L, where the ongoing pregnancy rate was 31.4% (27/86) for follitropin delta and 23.8% (20/84) for follitropin alfa. Early OHSS and preventive interventions for early OHSS increased with increasing serum AMH. In the subgroup with AMH ≥35 pmol/L, the incidence of early OHSS was 4.7% and 11.9% with follitropin delta and follitropin alfa, respectively, and the incidence of preventive interventions was 4.7% and 23.8%, respectively. The total follitropin delta dose declined with increasing AMH from 87.5 µg (AMH ≥5 pmol/L) to 63.7 µg (AMH ≥35 pmol/L), whereas the total follitropin alfa dose was AMH independent.

Limitations, reasons for caution: In this retrospective analysis the number of patients with high AMH (≥35 pmol/L) was limited.

Wider implications of the findings: In potential high responders, individualised follitropin delta dosing may improve ongoing pregnancy rates per started cycle and at the same time improve the safety of ovarian stimulation, in terms of lower incidences of OHSS and preventive interventions for OHSS.

Trial registration number: NCT01956110.

O-169 Towards the development of a WHO International Standard for immunoassays of Mullerian Inhibiting Substance/Anti Mullerian Hormone (MIS/AMH)

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Study question: Is formulated, lyophilised, recombinant human MIS/AMH suitable for the preparation of a WHO International Standard to calibrate AMH immunoassays?

Summary answer: A trial preparation exhibited a linear, parallel dose-response in the immunoassays tested. Between-method content estimates by commercially-available immunoassays varied with a GCV% of 26.67%.

What is known already: WHO has recognised the requirement for an International Standard to calibrate immunoassays of MIS/AMH. This requires sufficient MIS/AMH for the production of 2000–3000 ampoules, a stable, immunoreactive formulation and a strategy to define the content in mass units. Historical calibration exercises including the use of bovine, recombinant or native MIS/AMH calibrators and the calibration of new assays by serum value transfer present challenges both in the value assignment and in the eventual introduction of a reference material. The nature and extent of these challenges were explored by the measurement of a trial, lyophilised preparation, coded SS-581, by seven immunoassay methods.

Study design, size, duration: Five laboratories, provided with ampoules of SS-581, were asked to measure the MIS/AMH content in terms of their kit/method calibrators using the immunoassay methods currently in use in their laboratory and measuring a minimum of five concentrations in the linear part of the dose-response curve. In addition, participants were asked to measure concomitantly, a panel of six serum samples, coded S1 to S6, which contained between 0.1–13.0 ng/mL MIS/AMH.

Participants/materials, setting, methods: Trial ampoules coded SS-581 were prepared by lyophilisation of a 0.5 ml/ampoule volume of a formulation containing 0.24 % (w/v) bovine casein, 0.50 % (w/v) trehalose and nominally 1 µg/ml MIS/AMH. Human serum samples were obtained from a commercial source, aliquoted and stored at -80°C. Estimates of AMH content were determined in terms of kit calibrators by fitting a parallel-line model comparing log assay response to log concentration using CombiStats (Version 5.0, EDQM/Council of Europe).

Main results and the role of chance: Five laboratories returned data from seven methods coded A-G. In all methods, the recombinant, human MIS/AMH was recognised and the dose-response was parallel to kit standards, allowing estimation of MIS/AMH content. In terms of kit standards, the MIS/AMH content of SS-581 was variable with a GCV% of 39.95 % across all assay methods although it must be noted that this decreased to 29.08% for automated immunoassay platforms only and to 26.67% for the four commercially-available methods. Three assays are reported to use the same monoclonal antibodies (F2B 12/H and F2B 7/A). The variability of the estimates made by these methods was 29.8% (GCV), suggesting that epitope recognition may not be the sole contributor to the observed variation and that calibration, assay format and the kinetics of antibody-binding may also influence the AMH concentrations obtained. Variability in the reported AMH content of the serum samples ranged from 14.90 – 22.35 % (GCV) with the exclusion of serum S3 which had an MIS/AMH concentration near the lower limit of detection.

Limitations, reasons for caution: Three of the methods in the study were not commercially available at the time of the study.

It is not possible to draw conclusions regarding the commutability of a reference material prepared from human, recombinant MIS/AMH as considerably more serum samples would be measured in a full commutability assessment.

Wider implications of the findings: Lyophilised, recombinant MIS/AMH is suitable for the preparation of an International Standard. However, the introduction of such a reference material will require careful consideration. Furthermore, assignment of a mass value to the standard requires orthogonal physico-chemical data from SI-traceable reference methods, which will be challenging for a large, dimeric glycoprotein.

Trial registration number: N/A.

O-170 Applying data-driven approaches to develop a novel ovarian reserve score that leverages underlying relationships between distinct markers of ovarian reserve

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Study question: The objective of the study was to apply data-driven approaches to develop an ovarian reserve score leveraging underlying relationships between distinct markers of ovarian reserve.

Summary answer: A machine-learning approach identified distinct factors corresponding to follicular and pituitary components of ovarian reserve, which were algorithmically combined into a composite ovarian reserve score.

What is known already: Ovarian reserve is often estimated by indirect markers such as anti-Müllerian hormone (AMH) levels, basal antral follicle count (BAFC), or baseline levels of pituitary hormones, such as follicle-stimulating

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hormone (FSH) and luteinizing hormone (LH). These biomarkers are known to be interdependent and yet are still typically individually assessed as part of a standard diagnostic evaluation and optimization of ovarian stimulation protocols. Machine learning methodologies allow for combination of information from multiple correlated variables into summary (factor) scores, and hence reduce the impact of measurement error and improves results from prediction and correlation analyses.

Study design, size, duration: We conducted a multi-centre retrospective study of fresh IVF cycles at 13 clinics in the United States (2009–2015). After excluding cycles in which cleavage stage embryos were transferred or preimplantation genetic screening was performed, this analysis included a total of 31,263 cycles from 26,929 patients.

Participants/materials, setting, methods: Exploratory factor analysis (EFA) is used to group correlated variables that measure the same factor. We used EFA to determine the total number of distinct factors that AMH, BAFC, and basal levels of LH, FSH, and estradiol (E_2), E_2 at surge, total and metaphase II (MII) oocytes retrieved, and number of 2 pronuclear (2PN) and clinically usable embryos measure. Structural equation modeling (SEM) was used to explore relationships between the factors uncovered by EFA.

Main results and the role of chance: EFA/SEM analyses revealed that ovarian reserve was defined by two distinct factors: one measured to a similar degree by BAFC and AMH (Factor 1) and the other by FSH and the LH to FSH ratio (Factor 2). Interestingly, the two distinct factors that were automatically identified by the algorithms correspond to two biologically meaningful subgroups: ovarian follicle measures (Factor 1) and pituitary hormone-related measures (Factor 2). E_2 at surge, the number of total and MII oocytes retrieved, and the number of 2PN and usable embryos were all found to measure the same factor (ovarian response).

The ovarian reserve score decreased with maternal age and basal E_2 (both $p < 0.001$), but age had a larger impact. Higher ovarian reserve scores were correlated with lower total gonadotropin dose (GND, $p < 0.001$) and higher ovarian response ($p < 0.001$). Higher GND doses were correlated with higher ovarian response ($p < 0.001$). A correlation analysis revealed that the ovarian reserve score (R^2=0.55) was more than twice as powerful in explaining variation in ovarian response than any of the individual metrics alone: AMH (R^2=0.24), BAFC (R^2=0.22), basal FSH (R^2=0.06), the ratio between basal levels of LH and FSH (R^2=0.06), basal E_2 (R^2=0.007), or maternal age (R^2=0.07).

Limitations, reasons for caution: Our analysis included measurements performed at multiple laboratories. Our study was performed on retrospective data from the United States, and future studies are needed to investigate whether findings could extend to European practice patterns.

Wider implications of the findings: Although AMH, BAFC, and FSH measurements reflect different physiological components of ovarian reserve, this data-driven analysis revealed underlying relationships among them. We developed an ovarian reserve score based on these factors that reduced the impact of measurement error and offered better predictive accuracy than univariate measures alone.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 48: OUTCOMES AND OVARIAN STIMULATION
Tuesday 4 July 2017 Room A 15:15–16:30

O-171 Ovarian stimulation with Corifollitropin alpha (CFA) for 7 days leads to reduced progesterone levels on the day of trigger and to higher ongoing pregnancy rates

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Study question: Does hormonal stimulation with CFA-only result in lower progesterone levels and higher ongoing pregnancy rates (OPR) compared to CFA plus recFSH?

Summary answer: Stimulation with CFA-only leads to lower progesterone levels on the day of hCG-trigger and to higher ongoing pregnancy rates, compared to stimulation with additional recFSH.

What is known already: Premature progesterone rise during the late follicular phase of stimulation for IVF has a negative impact on the reproductive outcome of IVF / ICSI-cycles. Until now, the mechanism, by which elevated progesterone levels reduce pregnancy rates, is still not fully understood. Progesterone elevation has an impact on the endometrial receptivity by inducing endometrial advancement and therefore leading to asynchrony between the endometrium and the developing embryo. Recent studies suggest, that the cause of premature progesterone rise in ovarian stimulation for assisted reproductive techniques might be enhanced FSH stimulation.

Study design, size, duration: Post-hoc subgroup data-analysis of the previously published Ensure trial, which was a randomized, double-blind, double-dummy equivalence trial to compare the efficacy of 100 µg CFA with daily 150 IU recFSH for ovarian stimulation in patients ≤ 60 kg. Analysed were the data comparing CFA as a sole stimulation medication versus CFA and additional recFSH after day 8 of stimulation, comparing the incidence of elevated progesterone on the day of hCG-trigger and ongoing pregnancy rate.

Participants/materials, setting, methods: The ENSURE study included women aged 18–36 years, weighing ≤ 60 kg, a body mass index of 18–32 kg/m2, a menstrual cycle-length (24–35 days), access to ejaculatory spermatozoa and an indication for ovarian stimulation for IVF or ICSI. The Ensure study was a multicentre, multinational trial involving 14 centres in Europe and five centres in Asia.

Main results and the role of chance: The study included 396 patients. 268 patients underwent ovarian stimulation with 100 µg CFA and 128 patients with recFSH. Out of the 268 patients from the CFA-arm, 35 patients (13.1%) received the hCG-trigger after stimulation with CFA-only. 233 patients (86.9%) did not meet the criteria for final oocyte maturation on day 7 and needed therefore additional recFSH from day 8 onwards.

Of the 233 patients with additional recFSH after day 8, a total of 90 patients (38.6%) had progesterone levels of > 0.8 ng/ml, whereas in the group of the 35 patients, receiving CFA as sole medication, only 1 patient (2.8%) had a progesterone level of > 0.8 ng/ml ($p < 0.0001$).

The incidence for progesterone elevation in the CFA plus daily recFSH-group was 38.6% (90/233) for progesterone levels > 0.8 ng/ml, 7.3% (17/233) for > 1.5 ng/ml and 2.6% (6/233) for > 1.9 ng/ml respectively.

The OPR was 31.4% (11/35) for patients with CFA-only and 24.5% (57/233) for patients with additional recFSH after day 8 ($p = 0.378$). The OPR in patients with CFA and recFSH was 18.9%, 5.9% and 0% in the groups of progesterone levels > 0.8 ng/ml, > 1.5 ng/ml and 1.9 ng/ml respectively($P < 0.0001$).

Limitations, reasons for caution: The limitations of the data are the post hoc analysis of an earlier conducted prospective randomized controlled trial and the relatively small sample size of the patients who met the criteria of final oocyte maturation on day 8. Those points could limit the meaningfulness of the findings.

Wider implications of the findings: The pharmacokinetic profile of CFA mimicks a step-down-protocol. Reduction of FSH-dosage towards the end of the cycle releases stimulation pressure on the growing follicle. This results in lower incidence of premature progesterone rise and higher ongoing pregnancy rates.

Trial registration number: NCT 00702845.

O-172 Time interval between hCG administration and oocyte retrieval significantly influences IVF-ET results

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Study question: Do slight variations in the interval between hCG and oocyte retrieval (hCG-OR interval) influence IVF-ET results?

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Summary answer: Roughly within a 34–39 hour frame, our data indicated that the shorter hCG-OR interval, the poorer IVF-ET results.

What is known already: For decades, in controlled ovarian hyperstimulation (COH) cycles for IVF-ET, hCG-OR interval has been pragmatically set around 36 hours to mimic the physiological events occurring during the menstrual cycle. Yet, little attention has been paid, both in the literature and in the daily practice of IVF-ET clinics, on the possible impact of slight variations of such an arbitrary schedule on IVF-ET results. Indeed, the actual degree of flexibility offered to clinicians to properly schedule oocyte retrieval and the ideal hCG-OR interval remain to be set.

Study design, size, duration: We studied 616 COH cycles for IVF-ET. All patients received hCG (10,000 IU, IM) according to usual criteria of follicle maturation.

Participants/materials, setting, methods: Patients were sorted into 6 groups according to whether hCG-OR interval was 34.0–35.0 hours (n = 48), 35.1–35.4 hours (n = 89), 35.5–35.9 hours (n = 186), 36.0–36.4 hours (n = 180), 36.5–36.9 hours (n = 69), and 37.0–39.0 hours (n = 44).

Main results and the role of chance: As shown in the Table, whereas patients and COH characteristics were similar in all groups, we observed a remarkable stepwise increase in the number of mature oocytes and embryos obtained together with an increase in pregnancy rates from the 34.0–35.0 hours to the 37.0–39.0 hours groups. Incidentally, the prevalence of negative OR was comparable over the 6 groups.

	34.0–35.0 h	35.1–35.4 h	35.5–35.9 h	36.0–36.4 h	36.5–36.9 h	37.0–39.0 h	P
Ages (ys)	35.0 ± 0.7	35.0 ± 0.5	35.2 ± 0.3	34.5 ± 0.3	35.8 ± 0.5	35.7 ± 0.7	0.33
Serum AMH (ng/mL)	3.2 ± 0.4	4.3 ± 0.5	4.0 ± 0.2	3.9 ± 0.2	3.3 ± 0.5	3.9 ± 0.6	0.31
No. ≥16 mm follicles	6.0 ± 0.4	6.5 ± 0.2	6.1 ± 0.2	6.5 ± 0.2	6.0 ± 0.3	6.0 ± 0.4	0.62
No. mature oocytes	6.8 ± 0.5	8.5 ± 0.5	8.5 ± 0.3	9.5 ± 0.3	9.3 ± 0.6	9.6 ± 0.6	<0.007
No. cleavage embryos	4.7 ± 0.4	6.1 ± 0.4	6.3 ± 0.3	7.0 ± 0.3	6.7 ± 0.5	7.6 ± 0.6	<0.002
Ongoing pregn. rate (%)	25.0	21.3	33.9	41.7	46.4	50.0	<0.001

Limitations, reasons for caution: The present study could not address the question of the influence of larger hCG-OR intervals (>39 hours) neither discriminating possible outcome differences inside the 37.0–39.0 hours group due to limited sample size. Further prospective studies, including larger populations, are needed to confirm and expand present findings.

Wider implications of the findings: Our data indicate that the mere adjustment of the hCG-OR interval can lead to a noticeable improvement in IVF-ET output. Whether present results may be extrapolated or not to cases in which GnRH agonist is used instead of hCG to prime OR should be addressed in future studies.

Trial registration number: Not Applicable.

O-173 The negative effect of progesterone levels on hCG administration (Phcg) on live-birth rate (LBR) is not restricted to values above 1.5 ng/ml in IVF/ICSI cycles

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Study question: Is the negative effect of progesterone level limited to values above 1.5 ng/ml with a threshold effect, or already relevant for lower values?

Summary answer: The effect of P_{hcg} on LBR is non-linear, optimum at 0.80 ng/l and sharply decreasing for both lower and higher values. The 1.5 ng/ml threshold is arbitrary

What is known already: High P_{hcg} was commonly shown associated with lower LBR following IVF/ICSI cycles, irrespective of the use of analogue, antagonist, or type of gonadotropins for ovarian stimulation. As number of oocytes and estradiol level on the day of hCG administration are positively associated with progesterone levels, wether this negative effect persists after adjusting for ovarian response is still under debate. However, these effects were based in dichotomizing P_{hcg} following cut-off values, with the most known being 1.5 ng/ml. Models based on the continuous P_{hcg} value was never tested, thus linearity of the P_{hcg} effect and the validity of this threshold remain debatable

Study design, size, duration: Non-interventional, retrospective, observational, single-centre cohort study. No inclusion or exclusion criteria were applied. A sample size of 4300 cycles was calculated to provide a power of 90% to detect an odds ratio (OR) as large as .6 per Log(Pb) at p<.001 level (type I correction for multiple testing). Due to the expected correlation between cycles on the same patient, the analysis was conducted on last recent 5447 IVF/ICSI cycles performed on 2192 patients during 2009–2015

Participants/materials, setting, methods: Patients (median age 34y [IQ = 31–37]) underwent ovarian stimulation, with either GnRH long agonist, short, or antagonist protocol, and HP-hMG or rFSH, alone or combined with rLH, was used. We conducted a non linear mixed model featuring logistic regression assessing LBR as the dependent variable. The non linear effect of the log-transformed P_{hcg} was tested by polynomial regression, patient as random factor, patient mix, number of oocytes, time and total FSH dose used as fixed covariates

Main results and the role of chance: We found a highly significant non-linear effect featured by a linear (odds ratio OR = .58 per Log(P_{hcg}) ng/ml, 95% CI [.39-.85], p = .006) and quadratic (OR = .04, [.01,.13],p<.001) components, resulting in an optimal LB value at P_{hcg} = .8 ng/ml [.71-.86] sharply decreasing for both lower values and higher values. A significant effect was found in using the current threshold >1.5 ng/ml (OR = .32 [.21,.49], p<.001), however this threshold was not justified by a discontinuity and only 5.9% of sample were concerned. According to our model, a mean LB decrease of 34% (OR=.66, .56-.77) was found for patients out of the [.5,1.1] ng/ml P_{hcg} interval (33.8% of the sample). Sensitivity analyses successfully tested the robustness of our model: (a) P_{hcg} effect remains highly significant without adjusting on covariates; (b) The decreasing linear effect of P_{hcg} adjusting for the number of oocytes suggested in previous studies was found with a lower determination (OR = .83/log(Pf ng/ml), 95%CI [.66, .92]; (c) Results remain virtually unchanged in removing the number of occytes from the model; (d) P_{hcg} effects is independent of the number of oocytes (interaction test, OR=.98 per oocyte.ng/Ml, p=.67)

Limitations, reasons for caution: Data from a single-center study: the exact identification of the optimum value and its confidence interval may vary with different progesterone assay kits. In routine practice, P_{hcg} measurement is an important but very late information, raising the question as to whether earlier progesterone determination may also predict LBR

Wider implications of the findings: We demonstrate a non-linear effect indicating an optimal value and decreasing effect for both lower and higher values. The currently admitted threshold of 1.5 ng/ml is arbitrary and poorly sensitive. As this model is independent of retrieved oocytes number, it may represent a guidance in decision-making for cancellation or freeze-all policy

Trial registration number: unnecessary.

O-174 Cumulative ongoing pregnancy and live birth rates following repeated controlled ovarian stimulation (COS) cycles using individualised follitropin delta dosing compared to conventional follitropin alfa dosing

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[7]on behalf of the ESTHER-1 and ESTHER-2 trial group, Evidence-based Stimulation Trial with Human rFSH in Europe and Rest of World, Copenhagen, Denmark

Study question: To evaluate the clinical outcome of individualised follitropin delta per treatment cycle and the cumulative ongoing pregnancy and live birth rates following fresh blastocyst transfers after 3 treatment cycles.

Summary answer: The cumulative ongoing pregnancy and live birth rates after 3 treatment cycles were similar between individualised follitropin delta and conventional follitropin alfa treatment.

What is known already: An individualised dosing regimen of follitropin delta (FE 999049) resulted in non-inferior ongoing pregnancy and ongoing implantation rates, and significantly reduced the incidences of preventive interventions for early ovarian hyperstimulation syndrome (OHSS) and preventive interventions and/or OHSS, compared to conventional follitropin alfa treatment (Nyboe Andersen and Nelson et al, Fertil Steril, 2016). There were no reports of neutralising anti-FSH antibodies, neither after the first cycle nor following repeated cycles (Buur Rasmussen et al, Hum Reprod, 2016).

Study design, size, duration: Patients undergoing their first IVF/ICSI cycle were randomised to follitropin delta (n = 665) or follitropin alfa (n = 661). Patients who did not achieve ongoing pregnancy could continue with the same treatment in up to two repeated cycles. In cycle 2 and 3, 252 vs 261 and 95 vs 93 patients were included in the follitropin delta and follitropin alfa groups, respectively. Only blastocyst transfers were performed, but cryopreserved cycles were allowed between or after the fresh cycles.

Participants/materials, setting, methods: In the first cycle, follitropin delta was dosed based on the patient's anti-Müllerian hormone (AMH), measured by Elecsys® AMH (Roche Diagnostics), and body weight, while the follitropin alfa starting dose was 150 IU/day (11 µg). In subsequent cycles the starting doses were adjusted based on ovarian response in the previous cycle. In all cycles, the follitropin delta dose was fixed throughout stimulation, whereas the follitropin alfa dose could be adjusted from day 6 of stimulation.

Main results and the role of chance: The ongoing pregnancy rate with follitropin delta and follitropin alfa, respectively, was 30.7% vs 31.6% in cycle 1, 27.8% vs 25.7% in cycle 2, and 27.4% vs 28.0% in cycle 3. Excluding cryopreserved cycles, the cumulative ongoing pregnancy rate after 3 treatment cycles was 45.1% (300/665) with follitropin delta and 45.7% (302/661) with follitropin alfa, and the cumulative live birth rate was 43.9% (292/665) and 44.5% (294/661), respectively. The mean number of oocytes retrieved and good quality blastocysts was similar between the two treatment groups in each cycle. The average duration of stimulation was consistently 9 days regardless treatment group or cycle. The mean total dose in cycle 1 was 90.0 vs 103.7 µg ($p < 0.001$) with follitropin delta and follitropin alfa, respectively, 107.7 vs 121.7 µg ($p < 0.001$) in cycle 2, and 130.0 vs 132.7 µg in cycle 3.

Limitations, reasons for caution: Patients had the option to have one or more cryopreserved blastocyst transfers after each treatment cycle. The follow-up of these pregnancies is still ongoing and therefore not included in these data analyses.

Wider implications of the findings: Repeated cycles of follitropin delta treatment results in the same ongoing pregnancy and live birth rates as follitropin alfa treatment but the individualised follitropin delta dosing algorithm provides a safer treatment option.

Trial registration number: NCT01956110, NCT01956123.

O-175 Cumulative live birth rates following the first ovarian stimulation for IVF/ICSI. A European multicenter analysis of ~15.000 women using individual patient data

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Study question: What is the impact of ovarian response on fresh and cumulative live birth rates (LBR) in women undergoing their first ovarian stimulation cycle?

Summary answer: Although LBRs reach a plateau and subsequently decline when 20 oocytes are retrieved, cumulative LBRs steadily increase with the number of oocytes, exceeding 70%.

What is known already: Despite that controlled ovarian stimulation (COS) has been a milestone in modern ART, several studies supported that it may negatively affect oocyte quality and/or endometrial receptivity. Large registry analyses demonstrated that the highest LBR is associated with an oocyte yield of 10–15, beyond which success rates decline. Still, this was based only on fresh IVF cycle outcome. Taking into account the advancements in cryopreservation techniques and the increasing use of the 'freeze-all" policy, we set to evaluate the most meaningful outcome for patients, the cumulative LBR following the transfer of all fresh and frozen-thawed embryos, after their first ovarian stimulation.

Study design, size, duration: This is the largest multi-center analysis using individual patient data, conducted in 15 tertiary referral Hospitals in Europe. The study included women undergoing their t first ovarian stimulation cycle for IVF/ICSI in an antagonist protocol from 2009 to 2014. All patients were followed up for at least 2 years until using all their fresh and frozen embryos.

Participants/materials, setting, methods: A logistic model was fitted to predict live birth using fractional polynomials to handle the number of oocytes retrieved as a continuous independent variable. The prediction model, allowed the estimation of the probability of live birth in the fresh cycle, and also the cumulative live birth according to the number of oocytes retrieved. Cumulative live birth was defined as the delivery of the first liveborn after the transfer of fresh or all available frozen-thawed embryos.

Main results and the role of chance: In total, 14469 patients were analysed. The median number of eggs retrieved per cycle was 9 [inter-quartile range (IQR) 6–14]. Cumulative live birth rates steadily increased with the number of oocytes, reaching even 70% when >or=25 oocytes were retrieved. Interestingly, no plateau in cumulative live birth rates was observed but a moderate increase of 5.1% in average was detected beyond 27 oocytes. Regarding the fresh cycle outcome, the optimal number of oocytes was 13, as the likelihood of live birth was 36.5% when 13 eggs were harvested. Although no significant decrease was detected up the number of 20 oocytes, a drop in fresh live birth rates was identified thereafter. The drop in live birth rates could be attributed to the progressive increase in freeze-all cycle rate with the number of oocytes retrieved, exceeding 20% in patients with > 20 oocytes retrieved.

Limitations, reasons for caution: This is a cohort analysis based on retrospective data collection. Despite our robust methodological approach, the presence of biases related to retrospective design cannot be excluded.

Wider implications of the findings: This is the largest multicenter study, with individual patient data, evaluating for the first time the impact of ovarian response on cumulative LBR. The significant progressive increase of cumulative LBR with the number of oocytes, exceeding 70%, suggests that COS is unlikely to have a detrimental effect on oocyte/embryo quality.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS
SESSION 49: FACTORS AFFECTING SPERM FUNCTION
Tuesday 4 July 2017 Room B 15:15–16:30

O-176 Inherited diseases in donor conceived individuals -what can be expected

L. Bruhn Madsen, G. Koustas, O. Schou

Cryos International, Aarhus, Denmark

Study question: In gamete donor programs thorough efforts are made to avoid inherited diseases; but what can realistically be expected concerning inherited diseases in donor-conceived offspring?

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Summary answer: Evidence is provided, that risks of transmitting genetic diseases to donor offspring cannot be avoided. Therefore, informing all parties about this risk is extremely crucial.

What is known already: All humans carry several genetic diseases and are predisposed for various genetic conditions. Gamete donors at sperm and egg banks are currently assessed prior to acceptance by a thorough evaluation of donors personal and family medical history to screen for the risk of potential inherited diseases. However, only some inherited diseases can be elucidated, due to temporal factors, reduced penetrance or variable expressivity. Moreover, it is common for gamete donor banks to perform karyotyping and to screen for a variable number autosomal recessive diseases, using carrier screening to identify and reduce the incidence of a limited number of recessive diseases.

Study design, size, duration: During recent years, there has been a significant increase in the number of reported conditions on gamete donors even though donor screening programs are more thorough than ever. A retrospective review of reported conditions at the sperm and egg bank Cryos International has been carried out on all reported conditions in restricted donors from 2010–2016, representing a total of 124 sperm donors, corresponding to approximately 20% of the total donor cohort at the bank.

Participants/materials, setting, methods: All data were collected from reported conditions in either donor conceived offspring, supplemental tests performed on donors or new diagnoses reported in the gamete donors or in their family. Reports of conditions have been received and collected using standardized procedures throughout the time period in order to notify relevant parties upon specific concerns with respect to elevated genetic risk in offspring.

Main results and the role of chance: 124 donors were assigned a condition based on suspected or established diagnosis of the donor, his family members or in donor-conceived offspring.

32% of the conditions could be attributed to various or suspected recessive diseases and 78% of these were caused by reports from donor-conceived individuals. The remaining cases were discovered mainly due to additional carrier screening introduced, on previously accepted donors.

An autosomal dominant disease was assigned to 7 of the restricted donors despite thorough screening of the medical history at the time of acceptance, but could in some cases be explained by variable expression or penetrance. In a case of branchial fistula further questions to donor's medical history confirmed the finding in the offspring.

48% were assigned a condition due to an increased likelihood for a multifactorial disorder (MD). Congenital heart defects, and autism spectrum disorders, accounted for 21% and 25% of these, reflecting the general population frequency, as these are the most frequent congenital abnormalities. In some cases, it is not evidenced if the MD is associated to the donor since multiple factors are playing a role including preterm delivery, multifetal pregnancies, and advanced maternal age.

Limitations, reasons for caution: Quantification of reported conditions might be biased due to incomplete reporting, i.e. lack of reported conditions, or report of live births. Moreover, some recipients might over attribute medical concerns in a child to inherited factors from the donor.

Wider implications of the findings: This study contributes to the discussion among health care providers, authorities, and recipients to provide clear expectations towards the use of gamete donors as it is impossible to screen out genetic disorders and predispositions for genetic diseases, since all individuals are carriers of mutations for various diseases.

Trial registration number: Not applicable.

O-177 The role of sialyl-Lewis(x)-binding protein and sialidase in spermatozoa-zona pellucida Interaction in humans

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Study question: Are sperm Sialyl-Lewis(x) (SLeX)-binding protein and sialidase involved in human spermatozoa-zona pellucida (ZP) interaction?

Summary answer: SLeX-binding protein mediates the initial spermatozoa–ZP binding and the sperm sialidase desialylates the ZP glycans for further spermatozoa-ZP interaction and induction of acrosome reaction.

What is known already: Human fertilization begins when a spermatozoon binds to ZP, which induces acrosome reaction of ZP-bound spermatozoon. Defective spermatozoa-ZP binding leads to subfertility and it is an important cause of reduced fertilization rates in assisted reproduction. By using ultrasensitive mass spectrometry, we are the first to identify SLeX sequence as the most abundant terminal sequence on the glycans of human ZP and it is also the major carbohydrate ligand for human sperm-ZP binding. Molecules carrying SLeX can suppress spermatozoa-ZP binding but do not affect acrosome reaction, showing that other molecules are involved in the induction of acrosome reaction during spermatozoa-ZP interaction.

Study design, size, duration: The cellular localization of sperm SLeX-binding protein and sialidase were studied. The roles of SLeX-binding protein and sialidase on sperm functions and their association with capacitation were assessed.

Participants/materials, setting, methods: Spermatozoa were obtained from semen samples from normozoospermic men. Human oocytes were obtained from an assisted reproduction program. Affinity chromatography followed by mass spectrometric analysis was used to identify the SLeX-binding proteins on spermatozoa. The localization/expression of sperm SLeX-binding protein and sialidase were studied by immunostaining and Western blotting. Their functional roles were determined by functional blocking antibody, pharmaceutical inhibitors or competitive substrate. Sperm functions were assessed by standard assays.

Main results and the role of chance: C1orf56 was identified to be one of the SLeX-binding proteins in the membrane protein extracts of capacitated spermatozoa. The acrosomal region (a region known to bind ZP) of spermatozoa possessed C1orf56, sialidase-1 and -3 immunoreactivities and their intensities increased after capacitation in vitro, suggesting translocation of these molecules during capacitation. Blocking the functions of C1orf56 and sialidase-1/3 inhibited spermatozoa-ZP binding and ZP-induced acrosome reaction, but did not affect sperm viability, motility and spontaneous acrosome reaction. The role of sperm sialidase on sperm-ZP interaction was further demonstrated by the increased sperm binding capacity and acrosome reaction-inducing ability of the human ZP after exogenous sialidase treatment.

Limitations, reasons for caution: The functional relationship between sperm SLeX-binding protein and sialidase on spermatozoa-ZP interaction have not been depicted.

Wider implications of the findings: The results provide direct evidence that human spermatozoa-ZP interaction involves different proteins assembled during capacitation. The results also indicate the possible use of sperm surface SLeX-binding protein content and/or sialidase activity determination as sperm function test for prediction of the fertilization potential of semen samples.

Trial registration number: This work was supported in part by The University of Hong Kong Research Grant Council Grant 764512 M, 764611 M and 201109176154.

O-178 The impact of male partner age on cumulative incidence of live birth following in vitro fertilization

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Study question: How does male partner age impact the cumulative incidence of live birth among couples undergoing in vitro fertilization (IVF)?

Summary answer: There was an independent effect of male age on the cumulative incidence of live birth in couples with a male partner age >35.

What is known already: Increasing female age is clearly associated with declines in the cumulative incidence of live birth. However, these analyses have

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not accounted for the age of the male partner, and it is unknown whether similar declines are seen with increasing male age.

Study design, size, duration: This retrospective study analyzed up to six cycles from women age 18–<42 years of age whose first fresh autologous cycle began between January 2000 and March 2014; outcomes were assessed through December 2014. Women who used donor sperm (n = 801) and those without a male partner or whose male partner's age was not documented (n = 6,195) were excluded. In total, 18,802 cycles from 7,753 couples were analyzed.

Participants/materials, setting, methods: Patients were treated at a large, academically-affiliated center. A competing-risk regression model was used to estimate the cumulative probability and 95% confidence interval (CI) of the first live birth in up to 6 cycles within strata of male and female age, with IVF cycle number as the time metric. Age groups were defined as follows: <30, 35–<35, 35–<40, 40–<42, and (men only) ≥42 years of age.

Main results and the role of chance: Cumulative incidences of live birth were lowest among couples whose female partner was 40–<42 (range: 35.7–42.6); male age had no impact within this female age group (all p≥0.58). However, within the other strata of female age, the cumulative incidence of live birth declined with increasing male partner age. Among couples whose female partner was <30, male partner age 40–<42 was associated with a significantly lower cumulative incidence of live birth (45.5, 95% CI: 27.6–67.9) compared to male partner age 30–<35 (73.3, 95% CI: 68.9–77.4; p = 0.01). For couples whose female partner was 30–<35, male partner age ≥35 was associated with significantly decreased cumulative incidences (range: 61.9–63.7) compared to couples whose male partner was 30–<35 (70.4, 95% CI: 68.1–72.7; all p≤0.04). Among women 35–<40, the cumulative incidence was lower in couples whose male partner was 40–<42 (48.6, 95% CI: 43.8–53.7; p = 0.04) and higher for male partner age <30 (70.0, 95% CI: 55.6–83.2; p = 0.01) compared with male partner age 30–<35 (54.4, 95% CI: 49.9–59.0). Among couples whose primary diagnosis was male factor infertility, cumulative incidence of live birth tended to decrease with increasing male age; this decline was most pronounced among couples whose female partner was <30.

Limitations, reasons for caution: Using categories of age instead of continuous age could result in residual confounding; additional analyses will be conducted to assess this possibility. Additionally, some cells had small sample sizes, especially when stratifying on male factor infertility, which resulted in wide confidence intervals and potentially inadequate power to detect true differences.

Wider implications of the findings: These findings provide evidence of an independent effect of male age on the cumulative incidence of live birth following IVF. Together with previous work calculating the cumulative incidence by female age, these results can be used to provide more informative counseling for couples pursuing IVF.

Trial registration number: Not applicable.

O-179 Human seminal plasma-derived exosomes display canonical exosomal markers and are uptaken by spermatozoa

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Study question: Assessment of the exosomes role in the spermatozoa maturation process

Summary answer: Molecular characterization of exosomes isolated from seminal plasma revealed that they carry proteins involved in the spermatozoa maturation and fertilization capacity.

What is known already: Several organs throughout the male reproductive tract secrete exosomes that keep in contact with sperm cells during their maturation. In animal models, the acquisition of fertilization ability by spermatozoa during epididymal transit occurs in part by the transfer of molecules from membranous vesicles called epididymosomes. Prostasomes, extracellular vesicles released into prostatic fluid by prostate epithelial cells, have been proposed to regulate the timing of sperm cell capacitation and induction of the acrosome reaction, as well as to stimulate sperm motility. Exosomes are universally recognized as small extracellular vesicles (30-120 nm) carrying biological information involved in most physiological and pathological processes.

Study design, size, duration: Normozoospermic men between 18 and 55 years undergoing semen analysis, for their female partner's infertility, have been considered for the enrolment in the project. Patients with varicocele, genito-urinary inflammation, seminal tract infections were excluded. Seminal plasma was collected and process to purify spermatozoa and exosomes.

Participants/materials, setting, methods: Exosomes were isolated by sequential centrifugations and characterized by Nanoparticle Tracking Analysis, transmission electron microscopy and western blot. The uptake of labelled exosomes by spermatozoa was monitored by immunofluorescence and flow cytometry.

Main results and the role of chance: Seminal plasma contain exosomes displaying canonical protein markers such as CD9, CD63, Alix and TSG101. In addition, exosomes represent a discrete population and fall in the expected size range (30-120 nm). Qualitative analysis revealed that they derive from different tissues of the male reproductive tract like epididymis and prostate, carry proteins involved in the spermatozoa maturation and fertilization capacity and in the mechanism of anti-oxidative protection. After ejaculation, sperm cells are still receptive and can continue to receive vesicle-delivered cargos. Indeed, we demonstrated that spermatozoa could uptake exosomes derived from different sources.

Limitations, reasons for caution: This is an explorative analysis of a pilot study.

Wider implications of the findings: Exosomes play a strategic role in sperm maturation and capacitation along the male reproductive tract, but also after ejaculation, opening new perspectives for the assisted *reproductive* technology.

Trial registration number: -.

O-180 The impact of antenatal exposure to environmentally ubiquitous bisphenol A (BPA) on measures of male reproductive function at 20 years of age

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Study question: Does antenatal exposure to BPA influence testicular volume, semen parameters and serum reproductive hormone concentrations in adulthood?

Summary answer: There was no evidence that maternal circulating BPA levels, indicative of antenatal exposure, were associated with detrimental effects on adult male reproductive function.

What is known already: Despite the increasing prevalence of trace amounts of xenoestrogens, such as BPA, in the environment, domestic animal sperm production has not changed over 100 years. However, human studies of exposure to exogenous xenoestrogens suggest they may have adverse effects on spermatogenesis, and antenatal exposure to estrogenic phthalates is associated with subtle defects in male genital development. As Sertoli cell proliferation mainly occurs during fetal or early postnatal life, it is proposed that estrogenic environmental exposures during development may influence mature testicular function. We previously demonstrated that elevated cord blood oestrogen levels were associated with a reduced sperm output.

Study design, size, duration: Western Australian (Raine) Cohort men aged 20–22 years (born 1989–1991) underwent testicular ultrasound examination (n = 404) and provided semen (n = 365) and serum (n = 384) samples.

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Maternal serum collected at 18 and 34 weeks gestation (n = 156) was assayed to determine concentrations of total BPA (post-deconjugation).

Participants/materials, setting, methods: Testicular volume was calculated by ultrasonography, and semen analysis performed by WHO approved methods. Maternal serum (18 & 34 weeks' gestation) was stored at -80^0 C until thawed, when aliquots from both samples were combined to provide an estimate of overall antenatal exposure. Serum was analysed to determine concentrations of luteinising hormone (LH), follicular stimulating hormone (FSH), inhibin B by immunoassays and testosterone by liquid chromatography-mass spectrometry (LC-MS). Total BPA was measured by LC-MS after deconjugation.

Main results and the role of chance: Maternal serum was assayed for BPA from 247 males, 149 of whom underwent testicular volume examination; 136 provided a semen sample and serum hormones were measured in 141. The limit of detection (LOD) of BPA in serum was 0.001 μg/L; 11% of maternal Raine cohort samples had undetectable BPA levels. Blanks were included throughout the deconjugation, extraction and analysis steps to exclude external contamination. The significant number of non-detectable samples suggests systematic contamination was unlikely. Values ranged from ≤0.001 – 12.58 μg/L (median 0.32 μg/L). The 10th, 25th, 75th, 90th and 95th percentile concentrations were ≤0.001 μg/L, 0.12 μg/L, 0.76 μg/L, 1.64 μg/L, 2.64 μg/L, respectively.

After adjustment for time since last ejaculation, maternal serum BPA levels were associated with an increase in sperm concentration (0.18 p = 0.03), and sperm motility (0.18 p = 0.05), but not sperm output (p = 0.11). These associations were lost after adjustment for maternal smoking, and a personal history of smoking, drug use, and presence of a varicocele. No other associations with any measures of male reproductive function (testicular volume, sperm output, or serum testosterone, LH, FSH or inhibin B concentrations) in adulthood were detected. As only two men had cryptorchidism, no association with endocrine disrupter exposure could be examined.

Limitations, reasons for caution: BPA may have leached from the collection and storage vials over time, although this is unlikely as BPA was undetectable in many of the samples analysed. Total BPA was measured, representing both the free and conjugated forms. The study was limited by the sample size.

Wider implications of the findings: This is the first long term study of antenatal BPA exposure on adult male reproductive function; the reassuring findings should be viewed cautiously due to the study's limited sample size and the theoretical potential for external contamination of the analysed sample.

Trial registration number: Natioanl Health and Medical Research Council Project Grant number 634557

SELECTED ORAL COMMUNICATIONS

SESSION 50: MOSAICISM IN GAMETES AND EMBRYOS

Tuesday 4 July 2017 Room W+X 15:15–16:30

O-181 Comprehensive comparison of inner cell mass and trophectoderm reveals the complex nature of chromosomal mosaicism in human embryos

M. Popovic[1], A. Dheedene[2], C. Christodoulou[1], M. Van der Jeught[1], F. Van Nieuwerburgh[3], D. Deforce[3], P. De Sutter[1], E. Van den Abbeel[1], B. Menten[2], B. Heindryckx[1]

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Study question: To what extent does trophectoderm (TE) biopsy reliably reflect the genomic status of the inner cell mass (ICM) in human blastocysts?

Summary answer: Although concordance between TE and ICM was confirmed in most samples analysed, the very nature of mosaicism can confound diagnosis from a single TE biopsy.

What is known already: The optimisation of next generation sequencing (NGS) platforms for pre-implantation genetic diagnosis/screening (PGD/PGS) has recently led to an increase in reports of chromosomal mosaicism in TE biopsies. Its precise prevalence, however, particularly at the blastocyst stage of development, is controversial. To date, no comprehensive NGS analyses comparing ICM and TE have been performed. Recent studies have suggested that mosaic embryos may develop into viable pregnancies, indicating that, at present, some potentially euploid blastocysts are inadvertently being classified as clinically unsuitable. Although the ability of NGS to detect mosaicism has immense scientific value, its clinical relevance remains to be elucidated.

Study design, size, duration: We performed intra-embryo comparison of the isolated ICM and multiple TE portions. Overall, 60 samples, from 15 embryos were included in the study, which consisted of two groups. Primarily, biopsies (n = 32) were taken from blastocysts (n = 8), obtained from cryopreserved cleavage-stage embryos. These were donated following standard IVF/ICSI cycles. In addition, vitrified, genetically abnormal blastocysts (n = 7) from PGD/PGS cycles, were warmed and re-biopsied (n = 28). These embryos were selected based on their presumed mosaic chromosomal profile.

Participants/materials, setting, methods: Four biopsies were taken from good-quality blastocysts. Initially, ICMs were isolated using micromanipulation techniques, while the TE was further segregated into three portions. Sureplex whole genome amplification (WGA) followed by shallow whole genome sequencing, was performed on all embryo biopsies, at a ~5 Mb resolution. Genomic profiles were compared between samples from the same embryo, while results from pre-tested blastocysts were further correlated to the original report.

Main results and the role of chance: The overall concordance between ICM and all three TE samples was 73.3% in both embryo groups. Within the untested group, 37.5% (3/8) of embryos presented with normal profiles, while 25.0% (2/8) were abnormal, with consistent results in all four biopsies. Strikingly, a normal ICM was detected in 25.0% of the untested blastocysts, for which at least one TE sample was abnormal. Conversely, one embryo presented with an abnormal ICM, while normal profiles were observed in the respective TE samples. The trisomy 6 detected in this particular ICM, appeared to be mosaic and is not associated with a viable outcome. Evidence of mosaicism was also noted in the selected PGD/PGS blastocysts. Of these results, 42.9% (3/7) did not match the original TE biopsy. Specifically, re-analysis of an embryo with a mosaic 9q deletion, revealed a normal profile in the ICM and all TE samples. Similarly, for a further blastocyst, a mosaic monosomy 4 detected in addition to a balanced translocation in the original TE biopsy, was not observed in our analysed portions. Moreover, for one embryo we observed varying mosaic aberrations in the original profile and the biopsied segments, with additional aberrations detected in the re-biopsied portions.

Limitations, reasons for caution: To establish significance and thoroughly evaluate the occurrence and implications of mosaicism, expanding our study to include more blastocysts is necessary and currently ongoing. The known methodological artefacts of whole-genome sequencing, warrant careful interpretation.

Wider implications of the findings: Our results highlight the complex nature of chromosomal stability during early development. It is clear that a consensus regarding the clinical management of the diagnosis of mosaicism is difficult to attain. More precise quantification may enhance the embryo selection process, furthering efficiency, safety and ultimately improving IVF outcomes.

Trial registration number: N/A.

O-182 The extent of chromosomal mosaicism influence the clinical outcome of in vitro fertilization treatments

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Study question: Can the extent of chromosomal mosaicism influence the development potential of mosaic embryos?

Summary answer: Mosaic embryos with low aneuploidy percentage (<50%) have higher chances to result in healthy babies born compared to embryos with higher mosaicism levels (≥50%).

What is known already: Embryonic mosaicism is a phenomenon characterized by the presence of two or more genetically distinct cell lineages, typically

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one with a chromosome abnormality and the other possessing a normal chromosome constitution. In a recent published study, we have demonstrated that mosaic embryos hold the potential to implant and result in the birth of healthy babies. As a consequence, the transfer of these embryos is now offered as an option for women who undergo in vitro fertilization (IVF) resulting in mosaic embryos but no euploid embryos. We hypothesize that the extent of mosaicism affect the IVF success rate.

Study design, size, duration: The transfer of mosaic embryos at different aneuploidy percentage was offered to 73 women for whom IVF had resulted in no euploid embryos between May 2013-March 2016. The comparison of the clinical outcome obtained after transfer of mosaic embryos with low (<50%) and high (≥50%) aneuploidy percentage, was performed in order to assess a statistically significant difference in the development potential between the two groups.

Participants/materials, setting, methods: To obtain reference curves for determination of mosaicism percentage we assessed 114 diploid/aneuploid mosaic reconstructed samples (10–90% mosaicism) using both next generation sequencing (NGS) and array-comparative genomic hybridization (array-CGH) techniques. All embryos were cultured to blastocyst stage; trophectoderm biopsy was performed on Day-5 of development or Day6/7 for slow growing embryos. Comprehensive chromosome screening PGS was performed using either NGS or array-CGH methodologies.

Main results and the role of chance: Transfers of mosaic embryos with a high percentage of chromosomally abnormal cells (≥50%) resulted in a live birth rate of 16.7% and involved a miscarriage rate of 10%. In contrast, mosaic embryos with a lower aneuploidy percentage (<50%) resulted in a live birth rate of 39.5%, with a miscarriage occurring in 7.0% of the transfers. All pregnancies that went to term were confirmed, through sampling of the chorionic villi and/or amniotic liquid, to have a normal karyotype. A comparison of the clinical outcomes between the groups, with low and high aneuploidy percentage, showed a significantly higher ongoing clinical pregnancy rate/embryo transfer (39.5% vs 16.7%; $p = 0.036$), and baby born rate (41% vs 17%; $P = 0.027$) in embryos with aneuploidy percentage <50% compared to embryos with a mosaicism level >50%. The biochemical pregnancy rate and miscarriage rate were not significantly different between the two groups.

Limitations, reasons for caution: Additional clinical data must be obtained before this approach can be evaluated for routine integration into preimplantation genetic screening programs in women undergoing IVF. Transfer of mosaic embryos with purportedly "viable" aneuploidies should be considered with extreme caution.

Wider implications of the findings: The results of this study further confirm that mosaic embryos can develop into healthy euploid newborns. We demonstrated that the extent of mosaicism affects the IVF success rate. Priority for transfer should be given to mosaic embryos with low mosaicism levels.

Trial registration number: None.

O-183 Mosaicism across individual chromosome in trophectoderm biopsies by Next generation sequencing: changes with chromosome length

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Study question: To investigate the individual chromosome distribution of mosaicism by next-generation sequencing (NGS) platform.

Summary answer: The highest prevalence of mosaicism was observed within chromosomes 1, 7, 9 and 11; mosaicism of mitotic origin more likely occurs in the longer chromosomes.

What is known already: The aneuploid rate considerably increases with the maternal age, while the mosaicism rate stays constant at each age span (about 25%). The aneuploidy were mainly attributed meiotic errors increased along with gradually maternal age. However, the embryonic mosaicism mostly originated from mitotic errors occurring during the post-zygotic stage, regardless of the maternal age.

Study design, size, duration: A retrospective cohort study during 2015 to 2016, a total of 379 mosaic embryos screened by NGS.

Participants/materials, setting, methods: The involved blastocyst were biopsied on Day 5 and 6. PGS/NGS includes whole-genome amplification, sequencing (Miseq®), and analysis of BlueFuse Multi®. Aneuploidy, low-rate aneuploidy and euploidy were defined as the aneuploid ratio over 50%, 20%–50% and under 20%, respectively. Mosaicism was classified into two categories: "pure mosaicism" as diploid/aneuploid mixture (low-rate aneuploidy), and "aneuploid mosaicism" as an absolutely aneuploidy containing low-rate aneuploidy. Total mosaicism included the pure and aneuploid mosaicism.

Main results and the role of chance: Individual chromosomes were divided into three groups according to their lengths: Group A (>150 Mbp, including chromosome 1, 2, 3, 4, 5, 6, 7, X), Group B (100–150 Mbp, including chromosome 8, 9, 10, 11, 12, 13, 14, 15), and Group C (<100 Mbp, including chromosome 16, 17, 18, 19, 20, 21, 22, Y). The incidence of total mosaicism was significantly higher in the Group A and B than in the Group C (A: 39.3%, B: 34.1%, C: 26.7%, $p < 0.0001$). Pure mosaicism often occurred in chromosome 1, 7, 16, and aneuploid mosaicism mostly in chromosome 1, 6, 9. Combining together, the highest prevalence of total mosaicism was seen for chromosome 1 (6.9%), 7 (5.5%), 9 (5.7%) and 11 (5.5%). Unlike the meiotic aneuploidies, the observation indicated that mitotic mosaicism tends to occur in the longer chromosomes.

Limitations, reasons for caution: The characteristic of PGS is mainly to detect the variation of chromosome dosage, and is unable to identify the microdeletion or microduplication (<10 Mb), balanced translocation, inversion, recombination, uniparental disomy (UPD), polyploidy, monoploidy, and very-low percentage of mosaicism.

Wider implications of the findings: The present study suggested that the highest prevalence of mosaicism was mostly in the longer chromosomes, including chromosome 1, 7, 9, and 11. It implied that mosaicism of mitotic origin tends to occur in the longer chromosomes, whereas aneuploidy of meiotic origin are more likely in the shorter chromosomes.

Trial registration number: Not applicable.

O-184 Increased risk of aneuploidy in preimplantation embryos from heterochromatic chromosomal variants carriers

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Study question: Is there a correlation between heterochromatic variants in the karyotype of one of the members of a couple and increased risk of aneuploidy in their preimplantation embryos?

Summary answer: In this study, couples with heterochromatic variants in the karyotype show increased aneuploidy rate in preimplantation embryos compared to a control group.

What is known already: Polymorphism variations mainly refer to the variants in the length of the heterochromatic region in the chromosomes 1, 9, 16 and Y; or to the variants in the length of the short arm or the short-arm satellites and stalks of the acrocentric chromosomes (13, 14, 15, 21, 22). These are considered variants of a normal karyotype, without phenotypic effects in the carriers, but they seem to be overrepresented in infertile couples. During the last years, there have been published many articles with conflicting views on the clinical effect of chromosome variants.

Study design, size, duration: Comprehensive chromosome analysis was performed in embryos from couples undergoing IVF-PGD due to previous history of sterility and with one of the partners carrying a heterochromatic polymorphic variant in the karyotype. Results have been analyzed retrospectively. The abnormal karyotypes included in the study were: 46,XY,1qh+; 46,XY,13pss; 46,XY,22 ps+; 46,XY,9qh+; 46,XY,1qh+,9qh+; 46,XY,1qh+,21 ps+; 46,XX,21 ps+; 46,XY,21 ps+.

Participants/materials, setting, methods: 84 embryos (11 IVF-PGD cycles) from couples showing a polymorphism in the karyotype were studied. One blastomere was biopsied from each embryo on D+3 of development. Biopsied samples were amplified and processed for array CGH analysis (Illumina), except for one cycle that was analyzed by Karyolite (PerkinElmer).

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Results were compared with 82 embryos (13 IVF-PGD cycles) from a control group of patients (egg donation cycles, normal karyotypes no male factor associated) analyzed also by aCGH.

Main results and the role of chance: The group of patients with a polymorphisms in the karyotype have a maternal age mean of 30.27 y.o, and the control group of 30.07 y.o ($p = 0.9048$), allowing further comparisons.

In the study group, the percentage of euploid, aneuploid and other abnormalities (complex abnormalities with >12 aneuploid events and polyploidy) was 27.16%, 49.38% and 23.45%, respectively. The corresponding values observed for the control group were 58.53%, 37.80% and 3.66%.

A statistically significant increase in the percentage of abnormal embryos was observed in the studied group compared with the control one (72.84% vs. 41.46%, $p < 0.0001$). In 3 out of 11 cycles (27%) no euploid embryos were found.

The study group involved embryos from couples with heteromorphisms in the karyotype, showing increased heterochromatin at the chromosome centromere, telomere or at the short arm. It's been suggested that heterochromatin variation in these regions may cause partial asynapsis, defects in centromere function and kinetochore assembly, difficulty in homologous chromosome pairing, and impact on cell division. All of the above events could affect cell division and gamete formation and might cause the increased aneuploidy observed. Embryo aneuploidy consequences as implantation failure, miscarriage could explain the high percentage of patients with these altered karyotypes referring reproductive failures.

Limitations, reasons for caution: As per the difficulty in recruiting patients with this type of karyotype undergoing IVF-PGD cycle the sample size of this study is limited. More studies should be added.

Wider implications of the findings: This study shows high increase of aneuploidy in embryos from carriers of heterochromatin variant. This finding could explain the high percentage of polymorphisms carriers in infertile population. More attention must be directed to infertile couples with a karyotype revealing those chromosomal variants, and an IVF-PGS cycle should be considered.

Trial registration number: not applicable.

O-185 Reevaluation of the Frequency and Characteristics of Aneuploidy of Surgically Retrieved Spermatozoa in Light of Enhanced Molecular Genetic Techniques

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Study question: We challenged the notion that sampling epididymal and testicular tissues yields spermatozoa with a higher incidence of aneuploidy than those retrieved in the ejaculate.

Summary answer: With the implementation of advanced molecular genetic techniques, we confirmed that surgically retrieved spermatozoa have at least comparable incidence of aneuploidy than those ejaculated.

What is known already: Previous studies, including our own, evidenced, on about 100 testicular spermatozoa in 8 to 13 men and by only 4 chromosome probes, that these spermatozoa have a remarkably higher (13%) occurrence of aneuploidy. This notion, however, did not translate into a higher incidence of miscarriages nor a lower rate of pregnancy. Moreover, ICSI offspring generated from surgically retrieved gametes did not suffer from increased aneuploidy than those generated from ejaculated specimen. In light of the availability of more accurate molecular genetic techniques, we have decided to challenge this dogma.

Study design, size, duration: From December 2014 to November 2016, FISH aneuploidy was carried out on the specimens 2 donor controls, on the ejaculates of 67 men, and on surgical specimens of 6 azoospermic men. To confirm our findings, DNA sequencing technology was carried out on the ejaculates and surgical samples of 20 men. A simultaneous assessment was performed on non-azoospermic men with high DNA fragmentation in their ejaculate. ICSI pregnancy outcome was also analyzed and compared.

Participants/materials, setting, methods: Consenting men treated for infertility provided their specimens. FISH was performed on at least 1000 spermatozoa with a threshold of >1.6% with 2-3% FISH error. DNA was extracted and amplified from a comparable number of spermatozoa by PCR-based random hexamer amplification (average DNA concentration 610 ± 102 ng/ul and quality of 1.7 ± 0.1 nm). By Next Generation Sequencing (NGS), duplications and deletions by Copy Number Variants (CNVs) were then calculated for all chromosomes by CASAVA and VarScan2 software programs.

Main results and the role of chance: A total of 67 couples were included in our study (maternal age 99.9 ± 9yrs and paternal age 39.4 ± 3yrs). Sperm concentration of 9 ± 0.2x10^6/ml, 25 ± 21% motility, and 1.6 ± 2% normal morphology. Aneuploidy by FISH yielded 0.9% for the donor control but rose in the study group to 3.6% in the ejaculated, 1.2% for the epididymal, and 1.1% for testicular spermatozoa. There were no differences in autosomal or gonosomal disomies, nor nullisomies. In this group, the ejaculated spermatozoa yielded 22% clinical pregnancy rate and 50% with the surgically retrieved specimen.

NGS yielded 1.2% for the control while in the study was 11.1% for the ejaculated specimen and decreased to 1.8% in the epididymal and 1.5% for the testicular ($P < 0.0001$). The pregnancy rate for the ejaculated specimen was 47.2% and 50% for the surgically retrieved.

Simultaneous aneuploidy assessment on the ejaculated and testicular samples in the same individual evidenced a sperm chromatin fragmentation (SCF) of 20% in the ejaculate while on the testicular spermatozoa was only 8%. The pregnancy rate was 0% with ejaculated while 100% with the testicular spermatozoa. Aneuploidy assessment by FISH evidenced 2.8% in the ejaculated and 1.2% in testicular biopsy while with NGS became 8.4% and 1.3% in testicular biopsy ($P = 0.02$), respectively.

Limitations, reasons for caution: This is still a limited number of observations carried out on men screened for infertility. If confirmed, this study may suggest that testicular sampling may be beneficial even in non-azoospermic men where retrieving spermatozoa with lower SCF may also control for sperm aneuploidy.

Wider implications of the findings: This study challenges the dogma that testicular spermatozoa conceal a higher proportion of aneuploidy. This implies that testicular gametes do not contribute to chromosomally related pregnancy losses. Moreover, this may explain why offspring from testicular biopsy do not evidence higher autosomal or gonosomal aneuploidy than those resulting from ejaculated spermatozoa.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 51: TRAVELS THROUGH IMPLANTATION

Tuesday 4 July 2017 Room C 15:15–16:30

O-186 Sperm transcript dysregulation: role in recurrent pregnancy loss

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Study question: Do sperm transcripts have any relevance in recurrent pregnancy loss?

Summary answer: Paternal transcripts delivered to the oocyte at fertilization are responsible for regulating the critical processes involved in early embryonic development

What is known already: Substantial literature has previously been cited regarding the molecular and cellular events underlying fertilization and early embryonic development. Apart from the previous studies on extensive embryo-maternal interface, the role of paternal factors in embryonic development is being brought to surface. Sperm with disrupted DNA integrity do fertilize an oocyte but on account of being transcriptionally inert and limited repair mechanisms the damage exists post fertilization. It not only affects the outcome of pregnancy but also exert profound impact on the health of the future progeny.

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Study design, size, duration: A case control study of 75 male partners of couples experiencing RPL and 30 controls at AIIMS, New Delhi, India. Study duration was 1year.

Participants/materials, setting, methods: Semen samples from male partners of couples experiencing RPL were analyzed for assessing oxidative stress and DNA damage parameters by assessing ROS (reactive oxygen species) levels (RLU/sec/million sperm) and DNA fragmentation index (DFI) by chemiluminiscence assay and sperm chromatin structure assay (SCSA) respectively. q-PCR analysis was performed on the semen specimens after reverse transcribing the RNA isolated from the samples. The relative quantification of target genes was calculated with 2^{DDCt} method after normalization to β-actin.

Main results and the role of chance: The mean DFI was seen to be significantly higher (38.29 ± 9.0%) as compared to controls (27.4 ± 6.4%) ($P < 0.0001$) in cases as compared to controls. Seminal ROS levels were also seen to be significantly higher (356.9 ± 137.8 vs 26.7 ± 9.8) in cases with respect to controls. The odds of occurrence of RPL was 7.33 times higher, whose DFI>28 % (OR 7.33, 95% CI: (2.23–24.1), and was statistically significant ($p = 0.001$). While the odds of occurrence of RPL was 0.77 times higher, whose ROS>25 (OR 0.77, 95% CI: (0.3–2.0), and was not significant statistically ($p = 0.587$). The transcript levels of genes critical for embryonic development were analyzed by q-PCR and were correlated with DFI and ROS levels.

Limitations, reasons for caution: A potential limitation for this ongoing study is limitation in number of controls recruited for the current study. Also the role of maternal factors and other confounding factors which may affect implantation and embryogenesis cannot be negated.

Wider implications of the findings: The events underlying this extensive feto-maternal cross-talk for a successful pregnancy have the limitations of being directly studied in humans, the analysis of factors contributing to its failure will certainly provide insight into the critical steps involved in early embryonic development.

Trial registration number: N/A.

O-187 Trophoblastic spheroids derived from human embryonic stem cells as an early human embryo surrogate

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Study question: Is the human embryonic stem cells (hESCs) derived trophoblastic 3D spheroid model better than 2D monolayer culture, as embryo surrogate?

Summary answer: The trophoblastic spheroids differentiated in 3D structure expressed significantly higher levels of early trophoblastic markers, and can be used as embryo surrogate for functional study.

What is known already: Implantation failure is a cause of infertility. However, study of human implantation is restricted by lack of a proper model. Trophoblastic cells can be derived from monolayer hESCs by BAP (mouse embryonic fibroblast conditioned medium (CM) supplement with bone morphogenic protein 4 (BMP4), A83-01 and PD173074) treatment, but the in-well differentiated cells is difficult to be used as embryo surrogate in functional study. Recently, our group established a 3D hESC-derived trophoblastic spheroid (BAP-EB) implantation model. Yet, direct comparison of the trophoblastic markers in 3D and 2D models are lacking.

Study design, size, duration: The hESCs were induced to differentiate into trophoblast in 2D (monolayer) and 3D (spheroids) format by using BAP treatment for 4 days. The usefulness of the 3D model in functional attachment assay was further explored using antibodies against adhesion molecules during attachment on endometrial epithelial Ishikawa cells.

Participants/materials, setting, methods: Human ESCs were either cultured in monolayer (2D) or aggregated to form embryoid bodies (EB, 3D) before treated with BAP from 24 to 96 hours. The expressions of trophoblastic markers in the differentiated cells were studied by real-time PCR and compared to choriocarcinoma cell JEG3 spheroids and first trimester trophoblastic (PTB) cells. The effects of antibodies against adhesion molecules (E-Cadherin, beta3-integrin, Annexin A2) on the attachment of BAP-EB onto Ishikawa cells were studied.

Main results and the role of chance: Our results showed that the 2D trophoblastic cells and 3D BAP-EB had similar expression of the common trophoblastic markers like KRT7, beta-hCG and TFAP2C. However, compared with the 2D in well differentiation, the expression of pluripotent marker OCT4 was significantly lower in BAP-EB after 96 h of differentiation ($p < 0.05$, Mann-Whitney U test). On the other hand, the expression levels of the early trophoblastic markers CDX2 (at 48 h and 72 h) and ELF5 (at 72 h and 96 h) were significantly higher in BAP-EB. When compared with PTB cells, the expression level of trophoblastic markers like EOMES, ELF5, KRT7 and beta-hCG were similar in BAP-EBs at 72 h or 96 h; while the expression of OCT4, ELF5, KRT7 and beta-hCG were significantly lower in JEG3 spheroids ($p < 0.05$, Mann-Whitney U test). BAP-EB differentiated for 72 h was then used for the attachment assay. The attachment rate was reduced after blocking Ishikawa with E-Cadherin antibody (CDH1, 64.4% vs. control 83.3%, $p < 0.01$, chi-square test) but not with antibodies against other adhesion molecules like beta3-integrin (90.0%) or Annexin A2 (96.7%). The higher expression of CDH1 expression in receptive EEC lines (Ishikawa and RL95-2) than the non-receptive EEC lines (AN3CA and HEC1-B), supported a role of E-Cadherin on embryo attachment.

Limitations, reasons for caution: This is an in vitro study to mimic embryo implantation process, and the observations need to be confirmed with human blastocysts.

Wider implications of the findings: With the limited availability of human embryos, the 3D BAP-EBs could potentially be used as embryo surrogate to study implantation. The results of the functional study supported the role of CDH1 in the receptivity of endometrium during implantation.

Trial registration number: not applicable.

O-188 Altered miRNA-profile dependent on ART outcome in plasma of women at the time of embryo transfer and pregnancy test targeting Wnt-pathway

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Study question: Is there a change in microRNA (miRNA) profile of women undergoing ovarian hyperstimulation dependent on the ART outcome and which pathway is targeted?

Summary answer: The presented results show a significant change in miRNA profile dependent on the ART outcome, the most important changes target the Wnt-pathway.

What is known already: Molecular profiling is gaining traction in reproductive medicine. Small non-coding RNAs, so-called miRNAs, are key regulators in physiological but also pathophysiological processes that show a vast impact on fertilization. Recently, some miRNA-clusters targeting important processes during implantation in the embryo and the endometrium have been discovered, targeting genes like TGFbeta, Smad2, Smad 4, MUC 1 or Wnt-signaling. To deepen our knowledge on systemic molecular changes following embryo transfer (ET) we examined the genome-wide miRNA profile of women undergoing ovarian hyperstimulation.

Study design, size, duration: Blood samples were obtained after informed consent from women undergoing ovarian hyperstimulation. Changes in miRNA-expression in plasma of women, whose embryos implanted successfully ($n = 6$) vs. women who showed no implantation ($n = 6$) following day 5 embryo transfer (ET), were investigated at day of ET and pregnancy testing (PT). Protein expression to validate the finding was performed with an increased sample size of $n = 20$ (10 per group). Statistical analysis was performed using paired student's t-test.

Participants/materials, setting, methods: miRNA-analysis was performed using Human miRNA Microarray Kit Release 21.0. Enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses was performed using DIANA-miRPath v3.0 software based on predicted targets by DIANA-microT-CDS.

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Targets of miRNAs with a score of more than 0.8 were selected. Only KEGG pathways with a P value <0.05 and a false discovery rate (FDR) <0.05 were retained. Wnt3 and Wnt7 were measured by enzyme-linked immunosorbent assay (Cusabio Life Science®).

Main results and the role of chance: No significant differences concerning age, BMI or embryo quality were observed between the two groups. By comparing cases with clinical pregnancy after day 5 transfer with patients whose embryos did not implant, we observed, among others, miR-4327 as the most relevant marker. Systems biology analysis using DIANA-miRPath v3.0 of miRNA alterations highlighted 331 genes that have been experimentally validated as targets of altered miRNAs. Further analysis showed that the 11 miRNAs showing highest significant alterations in our analysis were all associated with regulation of Wnt 3 and Wnt7. Wnt 7 presented with a significant decrease between ET and PT in case of successful implantation (207.18 ± 6.15 pg/ml (ET) vs. 178.81 ± 10.21 pg/ml (PT), p = 0.03) while women with no implantation showed unaffected concentrations (190.37 ± 13.06 pg/ml (ET) vs. 192.48 ± 23.89 pg/ml (PT), p = 0.90). Wnt3 presented with a significant decrease in plasma levels in both groups. However, the loss of Wnt3 between ET and PT was significantly higher in patients with successful implantation compared to women without implantation (relative loss 2.06 ± 0.29 vs. 1.57 ± 0.16, p = 0.03).

Limitations, reasons for caution: Main limitation of this prospective study is its small sample size, defining it as a pilot analysis that requires further validation in a prospective clinical trail allowing to define the best biomarker among the altered miRNAs and possibly detecting even more miRNAs and related proteins predicting implantation success.

Wider implications of the findings: The presented results show a significant change in miRNA profiles dependent on the ART outcome affecting Wnt-pathway. These changes are most likely already visible at the time of embryo transfer. Our findings indicate potential downstream effects influencing pregnancies and a prospective use as biomarkers for implantation success

Trial registration number: Not applicable.

O-189 Attenuated pyruvate kinase M2 signaling pathway: the missing link in hyperhomocysteinemia-associated pregnancy loss

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Study question: The aim was to identify potential microRNA (miRNA) markers of placental origin for hyperhomocysteinemia associated pregnancy loss.

Summary answer: Ten accurate and reliable miscarriage pathways were identified for hyperhomocysteinemia associated pregnancy loss based on microarray data sets.

What is known already: Pregnancy loss is a multifactorial disorder that often involves impaired placental function due to overt oxidative stress. The mild-to-moderate degree of hyperhomocysteinemia is considered a possible threat to women with placental abruption or recurrent miscarriage. Pyruvate kinase M2 (PKM2) prevents oxidative stress-induced apoptosis, while suppression of PKM2 reduces apoptosis resistance by dephosphorylating bcl-2. miRNAs are putative biomarkers of implantation in early embryonic–endometrial communication, and the spectrum of miRNAs differs between the normal and pathologic conditions. A pathway–based microarray approach may, therefore, identify a distinct profile of miRNAs that carries signature to attest the pathway linking hyperhomocysteinemia-associated miscarriage.

Study design, size, duration: Four-month-old female rats, weighing approximately 150–160 g, were used. Hyperhomocysteinemia was induced in a group of pregnant rats (n = 15) using a pre-validated method (gavaging an aqueous solution of DL-homocysteine at a dose of 100 mg/kg body weight/day for D1 to D18 of pregnancy). The control set (n = 8) received the vehicle at the same volume. Placental gene expression was studied by microarray, and reconfirmed by quantitative real-time PCR (qRT-PCR) and immunohistochemistry (IHC).

Participants/materials, setting, methods: Placental tissues were isolated on gestational d19 and processed for hematoxylin-eosin staining. Tissue mRNA was extracted and subjected to microarray analysis by using Affymetrix U133 microarray platform to determine differential gene expression. Path analysis was done using Ingenuity pathway analysis. Recursive feature elimination (RFE) was used to evaluate the predictive ability. The significance of differences was evaluated by Pearson's correlation test and Student's t-test (two-tailed). P < 0.05 was considered to be significant.

Main results and the role of chance: Hyperhomocysteinemic rats developed thrombus in the chorionic plate vessels. Microarray analyses revealed alteration in a panel of candidate pathways including oxidative stress, inflammation, angiogenesis, apoptosis, and homocysteine metabolism. A novel pathway featuring PKM2 signaling was characterized. qRT-PCR validation identified a panel of 21 pathways, which were narrowed down to 10 using RFE that discriminated the treated placentas from controls. In the study cohort, the expression of selected genes associated with homocysteine metabolism (cystathionine-β-synthatase (CβS) and PKM2-involved pathways (PKM2, Akt) were down regulated by at least 3-fold while those associated with oxidative stress-mediated inflammation (hypoxia inducible factor-1α (HIF-1α), COX-2, nf-kβ), angiogenesis (sflt-1), and apoptosis (bax, caspase-9) were significantly up-regulated (p < 0.001). IHC scores using a scale from 0 to 3 based on the intensity of staining, correlated with qRT-PCR expression levels (CβS: r = 0.81, P < 0.001; HIF-1α: r = 0.82, P < 0.001; COX2: r = 0.74, P < 0.001; nf-kβ: r = 0.66, P < 0.001; bax: r = 0.62, P < 0.01; caspase 9: r = 0.76, P < 0.001; sflt-1: r = 0.83, P < 0.0001; PKM2: r = 0.79, P < 0.001; Akt: r = 0.66, P < 0.001).

Limitations, reasons for caution: Hyperhomocysteinemia is possibly one of many causes for placental abruption and pregnancy loss. The present study included no experimentally-induced threatened abortion group without involving hyperhomocysteinemia. The results, therefore, provide no evidence that attenuated PKM2 pathways were a direct attribute of hyperhomocysteinemia, rather than a consequence of placental abruption/pregnancy loss.

Wider implications of the findings: This is the first report that PKM2 is aberrantly expressed in miscarriage. The findings provide evidence that an oxidative stress-mediated placental damage perhaps represents the pathogenesis of hyperhomocysteinemia-associated pregnancy loss, which may pave the path towards development of pathway-based therapeutic options for recurrent miscarriage.

Trial registration number: Not applicable.

O-190 The accumulation of vitrified oocytes is a valid strategy to increase the number of euploid available blastocysts for transfer after preimplantation genetic testing

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Study question: The aim was to increase the number of viable euploid blastocysts in patients undergoing PGD-A using fresh oocytes together with previously accumulated vitrified oocytes.

Summary answer: Oocyte vitrification/warming do not generate aneuploidy in blastocysts. The number of viable euploid embryos is increased using for ICSI accumulated vitrified oocytes and fresh oocytes.

What is known already: In a preimplantation genetic diagnosis for aneuploidy (PGD-A) program, the more embryos available for biopsy, consequently increases the chances of obtaining euploid embryos to transfer.

Study design, size, duration: During 2 years, 69 patients with normal ovarian reserve candidates for PGD-A due to repeated implantation failure or recurrent pregnancy loss indication underwent several cycles of ovarian stimulation to accumulate and vitrify oocytes.

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Participants/materials, setting, methods: 591 accumulated vitrified oocytes and 463 fresh oocytes were micro-injected with the same partner's semen sample. PGD-A was completed on 134 blastocysts from vitrified/warmed oocytes and 130 blastocysts from fresh oocytes. PGD-A was performed using Next Generation Sequencing technology.

Main results and the role of chance: The euploidy and aneuploidy rates were comparable in blastocysts obtained from micro-injected vitrified/warmed oocytes and fresh oocytes (42.5% versus 40.8% and 57.5% versus 59.2%, $p>0.05$) from the same patients. Implantation rates of euploid blastocysts were comparable between the two sources of oocytes (56.0% from vitrified/warmed oocytes versus 60.9% from fresh oocytes, $p>0.05$).

Limitations, reasons for caution: The present strategy was applied on patients with a normal ovarian reserve. Its potentiality remains to be established for patients with reduced ovarian reserve.

Wider implications of the findings: The number of available blastocysts for biopsy and consequently the number of viable euploid blastocysts for transfer can be increased by accumulating oocytes to micro-inject produced from repeated ovarian stimulations. The present strategy is potentially applicable for all preimplantation genetic protocols.

Trial registration number: NCT0282041

SELECTED ORAL COMMUNICATIONS
SESSION 52: NEW INSIGHTS INTO SPERM ANALYSIS

Tuesday 4 July 2017 Plenary 1 17:00–18:00

O-191 The impact of semen parameters and number of dominant follicles on the outcome of intrauterine insemination

S. Chow, M. Irani, C. O'Neill, Z. Rosenwaks, G. Palermo

Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: What are the male and female partners' characteristics that can influence the success rate of intrauterine insemination (IUI)?

Summary answer: The number of total motile and morphologically normal spermatozoa (TMN) significantly influence IUI outcomes, particularly in patients with only one dominant follicle.

What is known already: Several factors are related to the outcome of IUI including female age, infertility diagnosis, and number of inseminated motile spermatozoa. There are conflicting data regarding the impact of strict sperm morphology on IUI outcomes. Previous studies comparing sperm morphology criteria often did not control for confounding factors that can influence IUI outcomes. Therefore, it is important to evaluate whether there is any value of normal concentration, motility, and morphology (0%, 1%, 2%, 3%, or 4%), or a combination of the above, that may affect the IUI outcomes taking into consideration the number of ovarian follicles.

Study design, size, duration: It is a retrospective cohort study at an academic medical center. Cycles were either natural or following ovulation induction with Clomiphene citrate, Letrozole, or gonadotropins. All patients who underwent IUI cycles between 2004 and 2013 were reviewed. To control for eventual concealed female factors, inclusion criteria consisted of women ≤35 years who have normal uterine cavity and patent fallopian tubes. Sperm parameters were evaluated according to WHO 2010 criteria.

Participants/materials, setting, methods: A total of 2929 IUI cycles were included. The clinical pregnancy rate (CPR) was compared between patients with different semen volume, concentration, motility, sperm morphology, and number of developing ovarian follicles. χ^2 and Fisher's exact tests were used for categorical variables. Values were expressed as mean +/− standard deviation.

Main results and the role of chance: The mean semen volume was 2.7 ± 1 mL, concentration: 48.5 ± 22x10^6/mL, motility: 50.3 ± 9%, and morphology: 3.8 ± 2%. Maternal age was 31.4 ± 3 years and number of dominant follicles ≥16 mm was 1.4 ± 1. Among each individual parameter, morphology affected IUI outcomes only at 0% normal forms with no clinical pregnancy versus a CPR of 12.9% for a morphology of 1% onward ($P = 0.02$). A combination of total number of motile spermatozoa (TM) at a threshold ≥25x10^6 was associated with a CPR of 13.8% when compared to < 25x10^6 at 7.3% ($P < 0.001$). Moreover, a total number of motile and morphologically normal (TMN) spermatozoa of <500.000 yielded only a CPR of 7.1% ($P < 0.001$). Couples whose female partner had ≥2 follicles had a higher CPR compared to those who had just one dominant follicle (16.9% vs. 12.2%; $P = 0.005$). Unless they were zero, TM and TMN spermatozoa did not affect IUI outcomes when the female partner had ≥2 follicles. On the other hand, with only one dominant follicle, the threshold of TM spermatozoa increased to ≥30x10^6 to yield a 13.6% CPR, compared to 7.4% for those below this threshold ($P = 0.01$). The TMN spermatozoa threshold also rose to ≥800.000, with a CPR of 13.8% above versus 7.2 below threshold ($P = 0.007$).

Limitations, reasons for caution: Although this is a retrospective study, this may serve to define the basis for generating criteria that combine semen characteristics and ovarian superovulation to predict a satisfactory IUI outcome in young women.

Wider implications of the findings: The total number of morphologically normal spermatozoa as well as the number of follicles contribute to IUI outcomes. The lower availability of TMN spermatozoa can be offset by increasing the number of dominant follicles. Couples with 0% normal morphology should undergo in vitro fertilization (IVF).

Trial registration number: Not Applicable.

O-192 Search for new predictive parameters of assisted reproduction outcomes through analysis of male gamete

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Study question: Are sperm chromatin maturity (by aniline blue and chromomycin A3) and expression of Catsper, a sperm-specific calcium channel, predictive of assisted reproduction technique (ART) outcomes?

Summary answer: Sperm chromatin maturity is associated with fertilization rate and embryo quality. CatSper expression is related to embryo quality and, in younger women, with pregnancy achievement.

What is known already: Infertility is a worldwide health problem affecting about 15% of couples. A widely used treatment option for couple infertility is ART. Despite recent improvements, ART success is still low and identification of predictive markers is one of the main goals of current research. Sperm chromatin status of spermatozoa is of fundamental importance for the fertilization process, embryo growth and pregnancy achievement. CatSper, sperm calcium channel, is required for hyperactivated motility, necessary for oocyte penetration. These data prompted us to evaluate chromatin maturity status and CatSper involvement in the fertilization process.

Study design, size, duration: We evaluated chromatin maturity status and expression of Catsper in spermatozoa from 206 male partners of couples undergoing ART within two years. Then, these parameters have been related to ART outcomes (fertilization rate, embryo quality and achievement of pregnancy) using SPSS statistical programme.

Participants/materials, setting, methods: Sperm chromatin status was evaluated by determination of histones persistence (Aniline Blue (AB) staining) and protamination degree (Chromomycin A3 (CMA3) staining) and reported as percentage of AB and CMA3 positive spermatozoa. CatSper expression was determined by immunofluorescence/ flow cytometric method and reported as mean fluorescence intensity.

Main results and the role of chance: Sperm percentage of AB and CMA3 positivity were correlated (r=0.5, p < 0.0001, n = 147). AB positive sperm were negatively associated with fertilization rate (r=-0.2, p = 0.004, n = 163; β=-0.2, p = 0.01 after adjustment for female age and female factor). CMA3 positivity was significantly lower in couples with higher embryo quality (EQ) (median and range values: 12.0 [6.00÷31.00] in EQ ≥0.5, n = 9 vs 23.0

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[7.00÷69.00] in EQ<0.5, n = 133, p = 0.005). Conversely, CatSper expression was significantly higher in high quality embryos (median and range values: 5.3 [3.05÷11.10] in EQ≥0.5 n = 16 vs 4.3 [2.00÷14.3] in EQ<0.5, n = 120, p = 0.02). All these results were confirmed in the subgroup of couples with women age ≤ 35 (median age of the cohort), where an association between CatSper expression and achievement of pregnancy was revealed (median and range values 5.2 [3.3÷11.1] in pregnant, n = 20 vs 4.1 [2.0÷12.7] in non-pregnant, n = 44, p = 0.02).

Limitations, reasons for caution: A higher number of recruited couples could strengthen our results, particularly concerning associations with EQ and results in the subgroup of young women.

Wider implications of the findings: AB and CMA3 tests are low cost, easy and rapid to perform and could be introduced in couple infertility work-up. This is the first study evaluating the association between CatSper expression and ART outcomes and suggests a role of the channel in the achievement of a good EQ and pregnancy.

Trial registration number: N.A.

O-193 Testicular versus ejaculated sperm in men without azoospermia: a systematic review and meta-analysis

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Study question: In couples without azoospermia, is the probability of pregnancy higher when testicular as compared to ejaculated sperm is used for ICSI?

Summary answer: In couples without azoospermia, the probability of pregnancy is higher when testicular as compared to ejaculated sperm is used for ICSI.

What is known already: Currently, testicular sperm extraction (TESE) is performed in patients with azoospermia, while in the presence of even a few spermatozoa in the ejaculate, this invasive procedure can be avoided.

Some couples, however, repeatedly fail to achieve pregnancy despite successful fertilization. In these cases, the use of surgically retrieved spermatozoa has been proposed. In fact, this concept has also been evaluated in couples with normal semen analysis but high DNA fragmentation index. However, it is not yet clear whether the probability of pregnancy is higher when testicular as compared to ejaculated sperm is used for ICSI in couples without azoospermia.

Study design, size, duration: A systematic review and meta-analysis was performed aiming to identify trials evaluating whether the probability of pregnancy is higher when testicular as compared to ejaculated sperm is used for ICSI in couples without azoospermia. For this purpose, a relevant literature search was carried out until December 2016. Primary outcome measure was achievement of pregnancy, expressed as clinical pregnancy or live birth. Secondary outcome measures were fertilization rate, number of 2-pronuclei (2pn) oocytes and miscarriage rate.

Participants/materials, setting, methods: One prospective and five retrospective comparative studies offering data to answer the research question were identified including a total of 754 couples who performed 839 ICSI cycles. Meta-analysis of weighted data using random and fixed effects model was performed. Results are reported as relative risk (RR) or weighted mean differences (WMD) with 95% confidence intervals (CI).

Main results and the role of chance: No significant differences were observed between the two groups compared regarding male age, male FSH, testicular volume and DFI, female FSH, antral follicle count, endometrial thickness, number of retrieved oocytes, number of MII oocytes, the number of embryos transferred and the proportion of patients reaching ET.

However, female age was significantly higher in the testicular group compared with the ejaculate group (WMD +1.56, 95%CI: +0.66 to +2.46), while female BMI was significantly lower in the testicular group as compared to the ejaculate group (WMD: -1.23, 95%CI: -2.07 to -0.39).

A significantly higher probability of live birth rate both per started cycle (RR: 1.72, 95%CI 1.28–2.31) and per embryo transfer (ET) (RR: 1.66, 95%CI 1.25–2.22) was present in the TESE as compared to the ejaculate group. Similarly, a significantly higher probability of clinical pregnancy, both per started cycle (RR: 1.51, 95%CI 1.20–1.91) and per ET (RR: 1.54, 95%CI 1.23–1.93) as well as a lower probability of miscarriage (RR: 0.38, 95%CI 0.21–0.71) were present in the TESE as compared to the ejaculate group.

No significant difference was present in the fertilization rate in the TESE as compared to the ejaculate group (WMD: -6.84, 95%CI -17.53 to +3.86).

Limitations, reasons for caution: The results of the current meta-analysis are based on a limited number of non-randomized studies that do not allow for additional subgroup analyses. In addition, differences have been observed in baseline characteristics between the populations compared that could affect the primary outcome measure assessed.

Wider implications of the findings: The findings of the current meta-analysis, if confirmed, might lead to important changes in the management of patients without azoospermia undergoing ICSI due to male factor. However, it is imperative prior to routinely adopting TESE for ICSI in these patients, to confirm the current results by appropriate randomized controlled trials.

Trial registration number: none.

O-194 The influence of ejaculatory abstinence intervals on semen quality - old concept, new evidences

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Study question: Do ejaculatory abstinence (EA) intervals influence semen quality and treatment outcomes in couples undergoing intracytoplasmic sperm injection (ICSI) as a result of male infertility?

Summary answer: Longer EA intervals result in higher sperm volume, concentration and TMSC. However, it increases sperm DNA fragmentation and decreases fertilization rate and blastocyst formation.

What is known already: There is no consensus in the literature as to the optimal period of EA for achieving maximum semen quality. Some studies have shown that increasing EA generally increases sperm concentration. Despite the known effects of EA on conventional semen parameters, the effects on general semen quality, such as total motile sperm count (TMSC), sperm DNA fragmentation index (DFI) and motile sperm organelle examination (MSOME) results remain unknown. Additionally, the subsequent effects of EA intervals on ICSI outcomes, such as fertilization, blastocyst formation, pregnancy, and implantation rates are yet to be elucidated.

Study design, size, duration: This prospective cohort study included 818 male patients undergoing conventional semen analysis for infertility investigation, in a private university-affiliated in vitro fertilization center, between October/2015 and October/2016. Regression analyses were used to investigate the influence of EA intervals on semen quality and ICSI outcomes. Results were expressed as regression coefficients (RC) and R-squared (R-Sq) for linear regressions. Odds ratios (OR) with 95% confidence intervals (CI) were used for binary regressions.

Participants/materials, setting, methods: The EA interval was recorded for each patient. Semen samples collected in the laboratory were evaluated for sperm concentration, motility and morphology, according to WHO recommendation. The TMSC was obtained by multiplying the volume of the ejaculate by the sperm concentration and the proportion of progressive motile spermatozoa divided by 100%. Two-hundred spermatozoa per sample were tested for DNA integrity, using the sperm chromatin dispersion test, and were analyzed by MSOME under 6,600×.

Main results and the role of chance: Mean male age was 38.2 ± 6.4 years. Increasing EA intervals positively influenced semen volume (RC: 0.1405, R-Sq: 5.28%, p < 0.001), sperm concentration/mL (RC: 3.1261, R-Sq: 2.59%, p < 0.001), total sperm concentration (RC: 18.941, R-Sq: 8.37%, p < 0.001) and TMSC (RC: 9.6396, R-Sq: 6.14%, p < 0.001), while sperm motility (RC: -0.3355, R-Sq: 0.23%, p = 0.212) and morphology (RC: 0.0227, R-Sq: 0.23%,

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p = 0.215) were not significantly influenced. The number of sperm cells affected by nuclear vacuoles, observed by MSOME, was not influenced by EA intervals (RC: 0.022, R-Sq: 0.08%, p = 0.463), however, the number of sperm cells presenting DNA fragmentation was negatively influenced by increased EA intervals (RC: 0.5355, R-Sq: 2.57%, p < 0.001). As for ICSI outcomes, negative influences of increasing EA intervals on fertilization rate (RC: -0.2123, R-Sq: 5.25%, p = 0.029) and blastocyst formation rate (RC: -0.1252, R-Sq: 4.47%, p = 0.012), however, increasing EA intervals did not influence implantation (RC: -0.0391, R-Sq: 0.01%, p = 0.950) and pregnancy rates (OR: 0.99 CI: 0.91–1.08).

Limitations, reasons for caution: The cut-off value for the EA intervals over which sperm DNA integrity, fertilization and blastocyst formation are negatively affected, is still to be elucidated. Additionally, the lack of information concerning social habits, occupation, and use of medication by men included in the study might have biased the results.

Wider implications of the findings: Lengthening of EA intervals may be associated with sperm DNA damage perhaps because the sperm are exposed to reactive oxygen species in the testicle for prolonged time. Shortening of EA intervals could optimize sperm quality, fertilization and blastocyst formation. Accurate EA intervals should be considered when managing infertile couples.

Trial registration number: None

SELECTED ORAL COMMUNICATIONS
SESSION 53: FERTILITY PRESERVATION
Tuesday 4 July 2017 Plenary 2 17:00–18:00

O-195 Spontaneous activation of human primordial follicles and subsequent follicular development to the antral stage during matrix-free three-dimensional culture

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[2]*School of Medicine- Oregon Health & Science University, Department of Obstetrics and Gynecology, Portland, U.S.A.*
[3]*School of Medicine- Oregon Health & Science University, Department of Pathology, Portland, U.S.A.*

Study question: Can human primordial follicles be activated spontaneously *in vitro* for subsequent follicular development and function during long-term three-dimensional culture in a matrix-free system?

Summary answer: The two-step matrix-free culture supported human primordial follicle activation and development to the small antral stage, as indicated by steroid hormone and autocrine/paracrine factor production.

What is known already: Mouse primordial follicles were activated and grew to the antral stage during two-step culture in a matrix-encapsulated three-dimensional culture system. Human primordial follicles were activated and grew to the pre-antral (primary and secondary) stage, but rarely to the antral stage, during long-term matrix-encapsulated ovarian tissue culture.

Study design, size, duration: The two-step matrix-free culture was performed using ovarian tissue from adult, female patients (26–47 years; n = 6). Ovarian cortex containing primordial follicles was processed into 0.3 mm × 0.3 mm × 0.3 mm pieces (> 5 follicles/piece) and cultured for 3 weeks. Secondary follicles were isolated from cultured ovarian issue for subsequent 6 weeks of culture. Data were analyzed using a one-way ANOVA.

Participants/materials, setting, methods: Ovarian cortical pieces (1 piece/patient) were cultured at 20% O_2 in alpha minimum essential medium supplemented with recombinant human follicle-stimulating hormone and insulin. Secondary follicles (1–5 follicles/patient), mechanically isolated from cultured ovarian tissue at week 3, were cultured individually in the ultra-low-attachment dish. Follicle survival, growth, oocyte size and steroidogenic enzyme expression were assessed. Culture media were analyzed weekly for estradiol, progesterone (mass spectrometry), anti-Mullerian hormone (AMH) and vascular endothelial growth factor (VEGF) (ELISA) concentrations.

Main results and the role of chance: Primordial follicles (diameter 35 ± 0.8 μm) in ovarian cortical pieces were activated spontaneously and grew to the secondary stage during 3 weeks of tissue culture. AMH became detectable in the media at culture week 2 (0.2 ± 0.1 ng/ml). A total of 85% isolated secondary follicles survived additional 6 weeks of individual culture. About 97% of survived follicles formed an antrum at week 3 with diameters increased at week 6 (145 versus 514 μm; $P < 0.05$). Granulosa and theca cells developed in cultured follicles produced aromatase (CYP19A1) and 17α-hydroxylase (CYP17A1), respectively, as indicated by immunohistochemical staining. Concentrations of estradiol, progesterone and VEGF secreted by *in vitro*-developed follicles increased in the culture media after antrum formation and peaked at week 6 (121 versus 2472 pg/ml; 2 versus 348 ng/ml; 0.2 versus 3.3 ng/ml; $P < 0.05$). Media AMH concentrations increased during secondary follicle culture, peaked at week 3 (0.6 versus 5.5 ng/ml; $P < 0.05$), and plateaued after antrum formation. Healthy germinal vesicle oocytes were harvested from *in vitro*-developed antral follicles. Oocyte diameters at week 6 were greater than those of secondary follicles at the beginning of culture (90 versus 65 μm; $P < 0.05$).

Limitations, reasons for caution: In the current study, functional assessment of *in vitro*-developed follicles was limited to steroid hormone and autocrine/paracrine factor production. Oocyte competence following prolonged follicle culture needs further evaluation. Culture conditions could be improved in future studies with increased sample size.

Wider implications of the findings: This study developed a novel culture strategy supporting human primordial follicle activation and development to the antral stage *in vitro*. The model offers an opportunity to study the process and regulation of folliculogenesis. By achieving the goal of producing competent oocytes, this technique may contribute to fertility preservation in women.

Trial registration number: Not applicable.

O-196 Multicolor flow cytometry: a promising tool for ovarian tissue qualification and minimal residual disease detection in human ovarian cortex

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[5]*Centre Hospitalier Régional Universitaire, CIC 1431, Besançon, France*

Study question: How could fresh or cryopreserved ovarian tissue be qualified from a functional and carcinological point of view?

Summary answer: Multicolor flow cytometry (MFC) is useful to evaluate the presence of leukemic cells and different ovarian cell subpopulations in the ovarian cortex.

What is known already: Ovarian tissue cryopreservation (OTC) is a fertility preservation option for women before gonadotoxic chemo- and/or radiotherapy treatments. However, autograft of cryopreserved ovarian tissue must be performed with caution in women suffering from malignancies that may metastasize to the ovaries. For this purpose, minimal residual disease (MRD) detection in ovarian cortex with sensitive methods is a crucial step. Furthermore, ischemic tissue damage occurring after autograft is currently another important issue to be resolved for successful ovarian transplantation.

Study design, size, duration: We developed an automated ovarian tissue dissociation method to obtain ovarian cell suspensions. Then, we used MFC for MRD detection in the ovarian cortex from leukemia patients and for the identification of different cell subpopulations in the cell suspension thus obtained.

Participants/materials, setting, methods: Human ovarian tissues from patients undergoing surgery for polycystic ovary syndrome (reference tissue) or ovarian tissue cryopreservation (tissue for OTC) were used in this study. Ovarian cortical biopsies (fresh or frozen-thawed) were dissociated using an automated dissociation method. We used 7-AAD and SYTO13 markers to

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gate viable ovarian cells by MFC. Variable markers were then chosen to differentiate and identify leukemic cells or cell subpopulations among the viable ovarian cells.

Main results and the role of chance: The dissociation yield was on average $1.58 \pm 1.22 \times 10^6$ and $1.70 \pm 1.65 \times 10^6$ viable ovarian cells per 100 mg of reference ovarian cortex (n = 60) and tissue for OTC (n = 18), respectively (p = 0.781). No significant difference was observed for cell viability after ovarian tissue dissociation between reference ovarian tissue (31 ± 17 %, n = 60) and tissue for OTC (24 ± 20 %, n = 18) (p = 0.088). Ovarian cell subpopulations were isolated from reference ovarian tissue by MFC: on average, 28 ± 10 % of $CD34^+$ cells (n = 30) and 11 ± 6 % of $CD31^+$ cells (n = 20) were identified among viable ovarian cells. In regard to MRD detection in cryopreserved ovarian tissue from leukemia patients, one T-acute lymphoblastic leukemia and 3 acute myeloid leukemia patients showed a positive MRD by MFC among the 12 leukemia patients tested, while no molecular markers were available for these 4 patients.

Limitations, reasons for caution: We do not know if the percentage of $CD34^+/CD31^+$ ovarian cells is a factor associated or not with time for ovarian function recovery after autograft and if disease-free ovarian cortex strips are absolutely safe.

Wider implications of the findings: Functional and carcinological qualification of fresh or frozen-thawed ovarian tissue can be performed by MFC. MRD detection in ovarian cortex by MFC could be applied to all leukemia patients contrary to molecular MRD detection methods. MFC is a promising tool for ovarian cortex qualification before autograft of cryopreserved ovarian tissue.

Trial registration number: Not applicable.

O-197 Glycogen synthase kinase 3 (GSK3) inhibition decreases cisplatin-induced oocyte death in vitro

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[3]*University of Edinburgh, MRC Centre for Reproductive Health, Edinburgh, United Kingdom*

Study question: Are pharmacological GSK3 inhibitors able to protect the ovary against cisplatin-induced damage?

Summary answer: GSK3 inhibitors (from GlaxoSmithKline library or commercially available) provide significant protection to oocytes against cell death induced by cisplatin (Cs) in vitro.

What is known already: Several studies have focused on the identification of compounds that, given with the chemotherapeutic drugs, may reduce gonadal toxicity. In particular, we focused on substances able to protect oocytes from Cs-induced damages. Since Cs resistance of some cell types is attributable to reduced activity of certain kinases, in particular of GSK3, we addressed the hypothesis made in the study question

Study design, size, duration: The GlaxoSmithKline library contains 63 inhibitors against the GSK3 pathway; we tested three of such compounds (GW809885X, GW827099X, GW827105X) and also CHIR99021, a commercially available inhibitor. Ovaries obtained from P4 GFP/c-Kit mice, were sliced into 7–8 pieces and cultured for 4 days in α-MEM plus 10% FBS and ITS at 37°C in 5% CO2 in air, until ovarian fragments form individual thin tissue layers containing easily to score GFP-oocytes enclosed within follicles.

Participants/materials, setting, methods: Ovarian fragments were pretreated for 1 h with increasing concentration GlaxoSmithKline inhibitors (0,003-3 μM), and CHIR99021 (3 μM, 5 μM, and 7,5 μM), before adding 10 μM Cs for 24 h plus inhibitors. In each layer, the number of morphologically healthy GFP-positive oocytes was scored before and after treatments.

Main results and the role of chance: At the higher concentration (3 μM), GW809885X and GW827105X but not GW827099X, exerted significant oocyte protection against death induced by Cs (healthy oocytes = 9885X: 55% ± 6,5%, n: 328; 827105X: 54% ± 3%, n: 160; 827099X: 59% ± 18.5%, n: 240; Ctrl: 95% ± 1,3%, n: 369 and Cs: 25%± 2,1%, n: 257; P < 0.05, Anova); CHIR 99021 showed a similar ovoprotection effect at all three tested concentrations.(3 μM = 50% ± 3,9%, n: 528, P < 0,01; 5 μM = 59% ± 4,1%, n: 379, P < 0,001; 7,5 μM = 51% ± 4,6%, n: 384, P < 0,01).

Limitations, reasons for caution: This screening study has been performed using short term in vitro assay in mice. Results need to be confirmed in vivo and at least in vitro in human and against other chemotherapy drugs. The molecular mechanisms underlying GSK3 ovoprotection against Cs is not known at this point.

Wider implications of the findings: The results outlined here confirm the value of our culture system for rapid and simple drug screening effects on ovarian tissues. The use of GSK pathway inhibitors may provide a novel approach to protect female fertility against chemotherapy.

Trial registration number: Not applicable.

O-198 Effect of inhibitor and activators of PI3K/Akt/mTOR pathway on human primordial follicles activation and growth in vitro: to slow down for better quality?

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Study question: How can biochemical inhibition and activation of PI3K/Akt pathway reduce the spontaneous or induced activation of primordial follicles in vitro, and improve subsequent follicular development?

Summary answer: PI3K/Akt activators triggered a massive follicular recruitment, whereas inhibitor partially safeguarded the follicular reserve. Growing follicles expressed markers of healthy development, but displayed structural abnormalities.

What is known already: Activators of the PI3K/Akt pathway promote activation of human primordial follicles in vitro before grafting in patients with premature ovarian insufficiency, leading to the delivery of few healthy babies. However, rapid and massive follicular activation raises concerns about the quality of growing follicles and the impact on oocyte competence. Recent data challenge their benefit on follicle survival. Here, we hypothesized that everolimus, an mTORC1 inhibitor, might reduce spontaneous massive follicular activation in vitro and slow down growing process, improving subsequent follicular development, through its interaction with PI3/Akt pathway and its antiapoptotic effects observed during organ transplantations.

Study design, size, duration: Fifty-six frozen-thawed ovarian cortex fragments (4x2x1 mm) from 4 patients between 19 and 29 years old were used. Fragments were exposed to either DMSO (control vehicle), everolimus (inhibitor) or bpV (HOpic) and 740Y-P (activators) during the first 24 and 48 h respectively, and cultured for additional 5 days. After 0, 1, 3 and 6 days of culture, early activation, follicular viability, proliferation and development were evaluated by immunohistology, western blot and PCR on isolated follicles (ongoing).

Participants/materials, setting, methods: Early activation was assessed by evaluation of follicle number and developmental stage in sections of ovarian cortical tissue. The developmental potential of follicles was assessed after 6 days of tissue culture, by using GDF9 and Kit Ligand (KL) (development) and Ki67 (proliferation) immunohistochemical markers. Apoptosis was studied by detection of phosphorylated histone H2A.x and by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) analysis.

Main results and the role of chance: A total of 11403 follicles were analyzed. Spontaneous activation of human primordial follicles occurred during the culture period: primordial follicles constituted 90% of the follicular pool at Day 0 (D0) while 80% were activated at D6. The exposure to activators triggered an additional significant drop of the percentage of primordial follicles compared with control (p < 0.05 at D1 and D3; p < 0.001 at D6), whereas culture with inhibitor partially reduced quiescent follicles recruitment (p < 0.01 at D6). Follicles viability was preserved whatever the group and the culture period, and their capacity to proliferate was demonstrated by Ki67 positive staining of the granulosa cells. GDF9 and KL were expressed by the oocyte of in vitro grown follicle, reflecting their ability to develop, although morphological irregularities

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were observed in all groups. We hypothesized these defects may be due to the rapid growth, as in vitro folliculogenesis is still highly accelerated compared to in vivo physiology. The assessment of the efficiency of activators and inhibitor to disrupt PI3K/Akt/mTORC1 pathway is currently ongoing by western blot and PCR, as well as the evaluation of follicular quality.

Limitations, reasons for caution: Impact of in vitro culture might cover up the potential benefit of the everolimus on growing follicles morphology after 6 days. It should be later verify in vitro using longer exposure time and in vivo using xenograft model.

Wider implications of the findings: To our knowledge, this is the first study aiming to regulate follicular activation in vitro by slowing down the follicular recruitment and the developmental time frame. The results question the use of accelerated growth systems, and provide new approach to improve in vitro culture of primordial follicle.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 54: SAFETY AND QUALITY ISSUES
Tuesday 4 July 2017 Room A 17:00–18:00

O-199 Randomized controlled trial comparing dydrogesterone versus micronized vaginal progesterone for luteal support in IVF: Effect of day of embryo transfer and treatment on pregnancy rate

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[5]*Abbott GmbH & Co. KG, Clinical Development & Medical Affairs, Ludwigshafen, Germany*

Study question: Day of ET is a known prognostic factor for pregnancy. Was this also observed in this study and was there a difference between treatment arms?

Summary answer: Age, site, and day of ET are significant prognostic factors that influence ongoing pregnancy rate. Treatment arm and day of ET do not significantly interact.

What is known already: Oral dydrogesterone is non-inferior (ongoing pregnancy rate at 12 weeks of gestation) to micronized vaginal progesterone (MVP) in IVF when used for luteal support. Dydrogesterone may be an attractive treatment alternative for patients because of the ease of oral administration. Day of ET can vary between day 2 and day 6 after oocyte retrieval following local clinical practice, which can significantly differ between clinical sites and countries where IVF occurs. The day of ET relative to luteal phase support treatment can have an influence on pregnancy rate.

Study design, size, duration: Lotus I was an international Phase III randomized controlled trial, performed across 38 sites, in 7 countries from August 2013 to March 2016. Subjects were premenopausal women (>18 to <42 years) with a documented history of infertility who were planning to undergo IVF. A centralized electronic system was used for randomization to oral dydrogesterone 10 mg tablets with placebo intravaginal capsules three times daily (TID) or MVP 200 mg capsules with oral placebo tablets TID.

Participants/materials, setting, methods: In total for Lotus I, 1031 subjects were randomized to receive either oral dydrogesterone (n = 520) or MVP (n = 511). Luteal support was started on the day of oocyte retrieval and continued until 12 weeks of gestation if a positive pregnancy test was obtained at 2 weeks after ET. Following local clinical practice, ET was performed on different days after oocyte retrieval. Intake of other progesterone products was not permitted during the study.

Main results and the role of chance: In the full analysis set, 497 and 477 subjects in the oral dydrogesterone and MVP groups, respectively, underwent ET. Non-inferiority was demonstrated, with pregnancy rates at 12 weeks' gestation of 37.6% and 33.1% in the dydrogesterone and MVP treatment groups, respectively (4.7% difference; 95% CI: -1.2%–10.6%). Pregnancy rates (%) at 12 weeks gestation varied depending upon the day of ET and treatment group: (dydrogesterone group [95% CI] vs MVP [95% CI], difference [95% CI]): Day 2, 25.0 (16.0, 35.9) vs 18.9 (10.8, 29.7), difference 11.0 (-1.7, 23.7); Day 3, 36.9 (31.2,43.0) vs 35.0 (29.2, 41.3) difference 1.3 (-6.7, 9.3); Day 4, 57.7 (36.9, 76.7) vs 45.2 (27.3, 64.0), difference 16.0 (-7.1, 39.0); Day 5, 42.6 (33.4, 52.2) vs 34.8 (26.1, 64.0), difference 9.4 (-2.9, 21.8). For both groups, the maximum pregnancy rate was achieved on ET Day 4. Dydrogesterone was well tolerated and had a similar safety profile to MVP. Multivariable logistic regression resulted in the following known significant prognostic factors for pregnancy rate: age ($p = 0.009$), site ($p = 0.001$), day of embryo transfer ($p = 0.0077$) and also the treatment group was a significant prognostic factor ($p = 0.0427$). There was no statistically significant interaction between treatment and day of ET.

Limitations, reasons for caution: Result analysis was powered to demonstrate non-inferiority of oral dydrogesterone vs MVP for the overall clinical pregnancy rate, not for each separate day of ET. Although pregnancy rates differed by ET day in both treatment groups, conclusions relating to the differences between treatments should be made with caution.

Wider implications of the findings: Oral dydrogesterone may replace MVP as the standard of care for luteal phase support in IVF, due to its convenience of administration and efficacy. The treatment group and day of ET were significant prognostic factors, after adjusting for other factors, with a numerically higher pregnancy rate observed with oral dydrogesterone.

Trial registration number: NCT01850030 (clinicaltrials.gov).

O-200 Liquid nitrogen sterility in sperm, oocyte and embryo banking

I. Molina Botella[1], J.V. Martinez Sanchis[1], E. Novella-Maestre[2], J.L. Lopez-Hontangas[3], J. Frasquet[3], J.M. Rubio[1], J. Peman[3]

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[3]*Hospital Universitari i Politecnic La Fe, Department of Microbiology, Valencia, Spain*

Study question: To evaluate the potential hazard of disease transmission through cryopreserved and banked gametes and embryos with special attention to the survival of pathogens in liquin nitrogen (LN)

Summary answer: Saprophytic bacteria were found in all of the containers at oocyte-embryos bank and in the 5 oldest containers container at the sperm bank

What is known already: The risk of disease transmission in humans during gamete and embryo cryopreservation and banking has been described. During assisted reproductive procedures cryostorage is the only situation where large quantities of biological materials of patients are kept together in a common liquid medium. Although the temperature of liquid nitrogen is -196°C, it may transmit infective agents from one sample to the other. Also cryoprotectants used during cryopreservation protocols protect bacteria and viruses. Contamination present in LN can be due to exposure to environmental agents during the management and transport of samples as well as to impaired sealed or cracked straws

Study design, size, duration: Retrospective study of the oocyte-embryo and sperm banks LN sterility performed from June 2014 to November 2016 in the Assisted Reproduction Unit in La Fe Hospital. The LN supplied by the company, the storage container, and the LN in the 25 containers at the oocyte-embryo and sperm bank were evaluated for bacteria, fungi and human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV)

Participants/materials, setting, methods: LN samples were obtained in closed 120 ml sterile bottles. Vapours LN protocol and straws heat sealed on both sides and 2 ml tubes were used for sperm freezing and storage. Cleavage embryos were stored using closed containers (CryoTip ®). For oocytes and blastocyst open containers (Cryotop®) were used. Vitek2 automated system (BioMerieux) were used for the bacteria identification. Fungus detection was performed by colonies morphology evaluation and their microscopic characteristics. Viruses were evaluated by PCR

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Main results and the role of chance: No fungi or virus were observed in any LN samples tested at the sperm, oocyte and embryo banks. Only 5 of the 20 containers of sperm bank showed bacterial contamination which correspond to the oldest containers which have been used over 25 years. Saprophytic bacteria such as Bacillus spp, megaterium and circulans and Sphingomona paucimobilis were found. Bacterial contamination was observed in all of the containers of oocyte-embryos bank. Saprophytic bacteria such as: *Stenotrophomonas maltophilia, Enterobacter spp; Bacillus spp* were found. The number of stored samples was not related to the type of pollution obtained

The air in the room, the operators by contact during processing samples or handling cryogenic tanks could be the cause of bacterial contaminations in oocyte-embryo bank. Therefore it has been demonstrated that cryopreservation technology used nowadays for fertility preservation in men is effective and all methods used to collect, cryopreserve and store human sperm do not increase the risk of cross contamination through LN. Nevertheless, LN manipulation during vitrification of oocytes and embryos could lead to the bacterial contamination observed in the oocyte-embryo bankLimitations, reasons for caution:

The fact that micro-organisms can survive in gametes and embryo cryobanking is not only important for the potential of disease transmission but also for testing for health certification for national and international gamtetes and embryo movement. Periodic chemically sterilization in LN containers is specially recommended for oocyte and embryo banking

Wider implications of the findings: The use of sealed straws and closed tubes in addition to the poor LN handling in sperm freezing decreases the risk of bacterial contamination in sperm banks. LN manipulations in the process of vitrification would be responsible for the high bacterial contamination observed in the oocyte and embryo bank

Trial registration number:

O-201 Lost oocytes and embryos in the ART laboratory

V.N. Bolton[1]

[1]*Guy's hospital-, Assisted Conception Unit, London, United Kingdom*

Study question: What is the incidence and nature of accidents in the laboratory resulting in the loss of oocytes or embryos, and what are the contributory factors?

Summary answer: In the UK, during 2015, 73 such incidents were reported during >72,000 treatment cycles, representing an incident rate of around 1 per 1000 cycles.

What is known already: Each of the numerous procedures carried out in the ART laboratory poses technical challenges inherent in the manipulation of microscopic oocytes and embryos, whether moving them between culture dishes, from dish to catheter or between dish and cryopreservation device. Moreover, during multiple procedures, dishes containing oocytes or embryos are moved between microscope stage and incubator, and cryopreservation devices between containers of liquid nitrogen. Each process holds the risk of accidental catastrophic loss of gametes or embryos.

Study design, size, duration: All incidents resulting in loss of oocytes or embryos that were reported to the UK Human Fertilisation and Embryology Authority (HFEA) during 2015 were collated and examined for similarities, trends and contributory factors.

Participants/materials, setting, methods: The HFEA is the body responsible for licensing and regulating ART and embryo research in the UK. It is a regulatory requirement that licensed clinics submit reports, within a prescribed timeframe, of all incidents that result in damage to, or loss of gametes or embryos. The incidents reported to the HFEA by licensed clinics during 2015 were analysed.

Main results and the role of chance: The 73 reported incidents can be divided into 3 broad categories, relating to (i) moving oocytes and embryos between dishes (n = 28); (ii) failure to follow process (n = 26); and (iii) accidents (n = 19). In the first category, there were incidents where oocytes/embryos were not recovered from micropipettes (n = 18), were damaged whilst using micropipettes (n = 4), where the pipette tip had not been secured (n = 2), where oocytes were lost during denudation (n = 2), or because of difficulty with a cryopreservation device (n = 1) or other technical difficulty (n = 1). Failure to follow process included gametes/embryos discarded in error (n = 6), oocytes not inseminated (n = 2), error during cryopreservation (n = 3), exposure to incorrect reagents (n = 5), error in preparation of culture dishes (n = 3), oocytes/embryos not accounted for (n = 4), and other process errors (n = 3). Accidents occurred where dishes were knocked (n = 4), dropped (n = 6), cryopreservation devices dropped (n = 3) and micropipette tips knocked (n = 5). A single incident reported gamete/embryo loss through bacterial contamination of culture medium.

Limitations, reasons for caution: Differences between licensed centres in interpretation of the definition of an adverse event, and possible variations in levels of adherence to regulatory requirements mean that the number of incidents may be under-reported.

Wider implications of the findings: Detailed SOPs, encompassing all essential confirmation, checking, witnessing and documentation, must be drafted and adhered to rigidly to minimise risk of loss of gametes or embryos. Experience of incidents should be shared widely, within and between clinics, in order that lessons may be learned and steps taken to minimise risk.

Trial registration number: Not applicable.

O-202 Pregnant after using the simplified Walking Egg IVF culture system: Perinatal outcome of the first 60 babies

P. Jacobs[1], **W. Ombelet**[2], **M. Janssen**[3], **I. Van der Auwera**[3], **K. Vandenborne**[2], **N. Dhont**[2], **G. Mestdagh**[2], **R. Campo**[2]

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[3]*Ziekenhuis Oost-Limburg, Department of Fertility, Genk, Belgium*

Study question: What is the perinatal outcome of pregnancies resulting from a new simplified IVF procedure, the so-called Walking Egg IVF culture method?

Summary answer: 60 healthy babies are born after using the Walking Egg simplified IVF system. Results are reassuring with a low figure for prematurity and low birthweight.

What is known already: To assess the potential health risks for the offspring after assisted reproduction (ART) is important, even more crucial if a new technique of IVF culturing is used. Multiple pregnancies are undoubtedly associated with a poorer perinatal outcome but also ART singletons have an increased risk of birth asphyxia, perinatal mortality, low birth weight and preterm birth.The reason why perinatal health problems occur more frequently in ART- singletons compared to naturally conceived singletons is still unclear and probably multifactorial.

Study design, size, duration: We prospectively investigated the perinatal outcome of 60 newborns which were the result of using a new simplified culture system for clinical IVF, the so-called Walking Egg culture system. The results were compared with the perinatal outcome data from all ART (IVF/ICSI) babies born in Belgium in 2014 and the previously published IVF perinatal outcome results in Flanders.

Participants/materials, setting, methods: We studied the perinatal data of all babies born after using the Walking Egg simplified IVF system (fresh and cryo cycles). Pregnancy duration, birth weight, admission to the neonatal care unit, congenital malformations and method of delivery were noted. 60 babies are born, data of this group were compared with the 2014 perinatal outcome data of ART (IVF/ICSI) pregnancies in Belgium and IVF outcome results from the Study Center for Perinatal Epidemiology of Flanders (SPE).

Main results and the role of chance: Perinatal data of 60 newborns (study group) were compared with the perinatal data of 3265 babies born after IVF/ICSI in Belgium in 2014 (BELRAP) and 3974 babies born after IVF (SPE). We found 3 twin and 54 singleton pregnancies, 36 after fresh embryo transfer, and 18 after cryo/thawing. Considering fresh singletons: mean birth weight was 3378 +/− 468 grams, compared to 3211 +/− 588 grams (BELRAP) and 3328 +/− 520 grams (SPE). Prematurity (< 37 weeks) was found in 2.7 % (1/36) compared to 14.2 % and 9.1 % (SPE and BELRAP). Birth weight < 2.5 kg was observed in 2.7 % compared to 11.6 % and 10.4 % in the SPE and BELRAP group respectively. The Caesarean section rate was 20.1 %, 23.8 % and 33.7 % in the study group, SPE and BELRAP group respectively. Cryo babies (18) had a mean birth weight of 3827 grams, comparable with the BELRAP data.

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Prematurity and low birth weight was also found in only 1/18 of cases. Twin pregnancies delivered at 34, 37 and 38 weeks, with a birth weight between 2245 and 3170 grams. No congenital malformation could be detected.

Limitations, reasons for caution: Up to December 2016, only 60 babies were born after using this new simplified IVF method. We surely have to enlarge this series to draw firm conclusions. 43 Pregnancies are still ongoing (> 14 weeks), more data can be expected very soon.

Wider implications of the findings: According to our preliminary results, the perinatal outcome of babies born after using the simplified Walking Egg IVF culture system is reassuring. Nevertheless a strict follow-up of all babies born after using this new method remains mandatory.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 55: ENVIRONMENT MATTERS
Tuesday 4 July 2017 Room B 17:00–18:00

O-203 Public perception of In-Vitro Fertilization (IVF) and fertility preservation: assessed by the Listening IVF and Fertility in Europe (LIFE) survey

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[1]*University Medical Center Utrecht, Department of Reproductive Medicine & Gynaecology, Utrecht, The Netherlands*
[2]*Teva Pharmacetuical Europe, Europe Fertility Medical Manager, Amsterdam, The Netherlands*

Study question: To assess the perception of in-vitro fertilization (IVF) and fertility preservation using oocyte-freezing among European men and women.

Summary answer: LIFE survey, conducted in six European countries and over 6000 individuals, indicated wide acceptance of IVF and oocyte-freezing for both lifestyle and medical reasons.

What is known already: Europe has low fertility rates and is amongst the lowest in the world. Biological and lifestyle factors may contribute to the decreasing fertility rates. Although cost and reimbursement of infertility treatment vary in Europe, there is wide access to fertility techniques and services for individuals experiencing infertility. Likewise, oocytes cryopreservation for fertility preservation is generally available throughout Europe to women diagnosed with cancer or other medical conditions that could affect fertility and to those that choose to delay parenthood for lifestyle reasons. Few local studies indicated public support for infertility treatment and fertility preservation.

Study design, size, duration: LIFE survey was conducted via an online questionnaire in September 2016 and was distributed to 8682 individuals. The survey was conducted in six European countries: France, Germany, Italy, Spain, Sweden and the United Kingdom. The survey consisted of twenty-two multiple choice and open-ended questions assessing European men and women's attitudes and opinions toward IVF and oocyte-freezing. Respondents were screened by country of origin, sex, sexual orientation and age.

Participants/materials, setting, methods: European men and women amongst five age groups (16–24 years, 25–34 years, 35–44 years, 45–54 years and >/= 55 years) were provided the online questionnaire via an independent research company. IVF questions assessed the beliefs surrounding the treatment and its success, the need for public funding and the use of IVF among different lifestyles. Oocyte freezing was evaluated in terms of social acceptance and funding.

Main results and the role of chance: A total of 6,110 (70% of total) men and women responded to the LIFE survey. Among the respondents, 55% would or have considered IVF in case of infertility for themselves or their partner or have had IVF treatment. According to the respondents' opinion, the mean maximum age for IVF should be 42 years old. The survey demonstrated support for IVF in single women (61%) and same sex couples (64%). Although 93% of all respondents believed that IVF treatment should be publically funded to some extent, 78% were willing to pay for IVF. Oocyte freezing was supported by a majority of respondents for both medical reasons (84%) and lifestyle decisions (60%), such as starting a family later in life. Respondents believed the mean maximum age for oocyte freezing should be 39 years old. However, unlike with IVF, 50% of respondents felt the funding for oocyte freezing for medical reasons should not be provided by the government. Overall, it was generally expected that there would be an increase over the next five years in IVF treatment (76%) and oocyte freezing (69%).

Limitations, reasons for caution: With the LIFE survey conducted via an online questionnaire, individual willingness to respond might have been influenced by exposure to infertility (personal, relatives or friends) and over-representation of such individuals cannot be ruled out. Likewise, the very large sample of the individuals surveyed cannot ensure representation of the whole population.

Wider implications of the findings: The survey results provide a general acceptance of IVF and oocyte-freezing for both lifestyle or medical reasons among Europeans. These findings could potentially drive discussions among patients and prescribers to explore either IVF treatment and/or oocyte-freezing and among legislators and payers for the funding of these procedures.

Trial registration number: N/A.

O-204 Apps/calendar methods for trying to conceive – can they accurately predict ovulation?

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[2]*SPD Development Company Ltd., Statistics and DataManagment, Bedford, United Kingdom*

Study question: Women are only fertile for a limited number of cycle days – in women seeking to conceive, can these days be accurately predicted using calendar methods?

Summary answer: True day of ovulation varies considerably for each cycle length, therefore it is not possible for Calendar/App methods to give accurate prediction.

What is known already: Menstrual cycle characteristics are well published but are based on dated studies of the general population. Women seeking to conceive will commonly use Apps to monitor their menstrual cycle and help them to time intercourse within their fertile period. These Apps utilise historical study data to predict ovulation (assumed to be day 14 for a 28 day cycle). This may not be applicable to the population trying to conceive because they tend to be older and may include a higher incidence of subfertility (referenced by their failure to conceive necessitating use of ovulation prediction) than the general population.

Study design, size, duration: This was a home based observational study. Demographic data was self-reported. 850 volunteers seeking to conceive naturally (>18 years old) were recruited via internet advertisements from across the UK. Volunteers collected daily urine samples for one entire month and completed a daily diary characterising their menstrual cycle.

Participants/materials, setting, methods: Volunteers mean age was 32 years (range: 18–50) and mean body mass index was 26.67 (range: 14.8–63.8). Urinary luteinising hormone was measured using AutoDELFIA (Perkin Elmer, Waltham, MA, USA). Day of ovulation was determined as the day after the LH surge. A probability curve was created for each cycle length to determine likelihood of ovulation on any given day for each cycle length.

Main results and the role of chance: Mean cycle length was 28 days (range: 17–53 days); 34% of volunteers had a 28-day cycle and >90% of volunteers had a cycle length of between 25 and 30 days. On average, women had been trying to conceive for 15 months (range: 1–162 months), had had one previous live birth (range: 0–10) and reported an average of one miscarriage/stillbirth. 3% of women had endometriosis and 7% had polycystic ovary syndrome.

Women actively trying to conceive with a standard cycle length of 28 days (n = 119), only have a 14% probability of ovulating on day 14. Day 16 had the highest probability of being the day of ovulation (21%), but the probability of ovulating was spread across a range of days: day 11 (1%), day 12 (3%), day 13

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(7%), day 15 (19%), day 17 (17%), day 18 (10%), day 19 (5%), day 20 (2%). A similar broad spread of probable ovulation days was observed in cycle lengths that were both shorter and longer than 28 days. Apps based on menstrual cycle lengths only, therefore cannot accurately determine ovulation day.

Limitations, reasons for caution: The study was for one menstrual cycle, thus variation in ovulation days between cycles in individual women could not be determined. Despite a large data set, some of the cycle length cohorts only consisted of a small number of women (range: 119 [28-day cycle] to 13 women [23-day cycle]).

Wider implications of the findings: Women seeking to conceive and using calendar-based smartphone apps to time intercourse with fertile days are unlikely to be using accurate information. For a smartphone app to accurately predict when ovulation is possible, it would need to provide women with a very large window during which to have intercourse.

Trial registration number: NCT01577147.

O-205 Children born prior to paternal cancer diagnosis have an increased risk of congenital malformations: a study using Swedish national registries

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[2]*Lund University, Department of Occupational and Environmental Medicine, Lund, Sweden*
[3]*Lund University, Department of Translational Medicine, Malmö, Sweden*

Study question: Is paternal cancer associated with increased risk of congenital malformations in the offspring, when offspring birth precedes cancer and cancer treatment?

Summary answer: Children of men having cancer have a statistically significant increased risk of congenital malformations, independently of birth occurring before or after paternal cancer diagnosis.

What is known already: The growing number of male cancer survivors and the potential mutagenic effects of cancer therapies have prompted studies into the health of their offspring. Previously observed increases of birth abnormalities for children born to male cancer survivors have been attributed to the mutagenic effects of cancer therapies. However, the risk of congenital malformations in children fathered by men prior to diagnosis is not known and it is therefore unclear if it is the treatment that is causing the increase in malformations or if this category of men has an inherent increased risk e.g. due to genomic instability.

Study design, size, duration: This registry based study sourced data from the Swedish Medical Birth Register, the Swedish Cancer Register and the Swedish National Quality Register for Assisted Reproduction. All children born in Sweden between 1994 and 2014 (n = 2 108 574) and their fathers (n = 1 181 492) were included and their diagnoses were identified using the registers. The risk of congenital malformations of the children conceived before the father's cancer diagnosis was calculated and compared to counterparts without cancer.

Participants/materials, setting, methods: Paternal cancer diagnoses were retrieved from the Swedish Cancer Register. Similarly, the Swedish Medical Birth Register supplied congenital malformation diagnoses (ICD-8:740–759 & ICD10:Q00Q99). Associations between paternal cancer and birth abnormalities of children conceived prior and after paternal cancer diagnosis, as well as those of healthy fathers, were investigated using binary logistic regression model. The model yielded Odds Ratios (ORs) with 95% Confidence Intervals (CIs). All statistical tests were two-sided and $p < 0.05$ adopted as statistically significant.

Main results and the role of chance: In our cohort of 1 181 492 fathers, 24 338 (2.1%) had received a cancer diagnosis. Of the n = 28 902 children born to fathers who developed cancer after conception, 3.9 % (n = 1 132) had a congenital malformation, as opposed to 3.5% for the children born to healthy fathers (OR = 1.08, 95% CI = 1.01 to 1.15, p = 0.023). The children (n = 10859) born to fathers who had previously had cancer, had a comparable increase though not statistically significantly so (OR = 1.06, 95% CI=0.944 to 1.19, p = 0.324, 3.7% vs 3.5%). The national cancer registry is a mandatory registry of all cancers in Sweden with an approximated completeness of registration of 99%, ensuring a complete assessment of cancer diagnosis. The role of chance is hence very low.

Limitations, reasons for caution: The degree of detail of registry data was limited due to ethical considerations; therefore detailed information regarding congenital malformation and cancer diagnosis was not available at the start of this study. This data is expected to be released shortly and data thereafter reanalyzed.

Wider implications of the findings: The increase in congenital malformations for children to cancer patients cannot solely be attributed to cancer therapies, indicating that cancer *per se* is causing the observed increase in birth anomalies or that there is an underlying paternal factor, e.g. genomic instability, that gives rise to both conditions.

Trial registration number: N/A.

O-206 In-utero cigarette smoke exposure and the risk of earlier menopause

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[3]*University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands*

Study question: Is there an association between in-utero smoke exposure and age at menopause? We hypothesized that in-utero exposed women reach menopause earlier compared to non-exposed women.

Summary answer: In-utero smoke exposure was not associated with earlier menopause. There was no interaction between in-utero smoke exposure and previous or current smoking of participants themselves.

What is known already: Menopause occurs when the follicle pool is exhausted. Cigarette smoking is toxic to female germ cells and smoking women enter menopause approximately one year earlier than their non-smoking peers. Since the follicle pool is prenatally determined, harmful early life events might interfere with timing of menopause. Adult mice that were exposed to smoke in-utero show reduced follicle numbers. Likewise, exposed human embryos and fetuses show fewer germ and granulosa cells in the ovaries. It is expected that in-utero smoke exposed women are born with fewer follicles and therefore reach menopause earlier, but the few epidemiological studies available show conflicting results.

Study design, size, duration: This is a cohort study within the Avon Longitudinal Study of Parents and Children (ALSPAC). Pregnant residents (participants) of the Avon County, expected to deliver between April 1st 1991 and December 31st 1992 were included. Participants characteristics for current analysis were obtained using questionnaires from annual follow-up assessments. In 2010, questionnaires were sent to 9028/14541 (62.1%) participants of the initial cohort, 4168/9028 (46.2%) returned the questionnaires, of which 3551 were illegible after 617 exclusions.

Participants/materials, setting, methods: There were 2629/3551 (74.0%) premenopausal and 922 (26.0%) postmenopausal participants. Participants who ever had hormonal therapy (n = 154) or ovarian/uterine surgery (n = 148) were censored at event. Postmenopausal women due to chemotherapy or radiotherapy, other reasons or participants who had missing information were excluded (n = 617). When not available, age at natural menopause was estimated (481/620) by age at filling in the questionnaire minus one year. Cox proportional hazards was used to estimate hazard ratios of menopause.

Main results and the role of chance: Participants were on average 47.6 (± 4.4) years. Average age at menarche was 12.9 ± 1.5 years, 78.5% (2733/3482) of the participants had regular periods at baseline, 94.7% (3305/3491) used oral contraceptives at least once during follow-up and 39.1% (1339/3429) were ever (current or previous) smokers. Age at natural menopause was 50.6 ± 3.7 years. Of all participants, 20.1% (580/2882) were exposed to

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cigarette smoke in-utero. In-utero exposure was not associated with age at menopause (HR 1 .02 95%CI 0.85–1.24 p-value=0.81). Participants who were smokers themselves had higher hazards of menopause(HR 1.49 95%CI 1.20–1.85 p < 0.0001). Sensitivity analysis in participants with available age at menopause only did not change the results (HR 1.03 95% CI 0.78–1.35 p-value=0.84). Ever smokers tended to have earlier menopause, both for smokers who had been exposed in-utero (HR 1.45 95%CI 0.87–2.42 p = 0.16) and smokers who were not exposed (HR 1.37 95%CI 0.99–1.89 p-value=0.06) though non-significant. Non-smokers who were exposed in-utero did not have higher hazards of menopause (HR 0.95 0.78–1.17 p-value=0.64).

Limitations, reasons for caution: Exposure period and number of cigarettes smoked during pregnancy by the mothers of participants and their age at menopause were unavailable. The effect of high levels of smoke exposure cannot be excluded. The 18 years follow-up resulted in high drop-out rates and could lead to attrition bias.

Wider implications of the findings: Age at menopause is strongly programmed. Despite reduction of follicles being associated with in-utero smoke exposure shown in previous studies, little variance in age at menopause was observed in our cohort. Mechanisms to spare follicles may counterbalance the effect of in-utero smoke exposure and maintain the predetermined age at menopause.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 56: REPRODUCTIVE SURGERY

Tuesday 4 July 2017 Room W+X 17:00–18:00

O-207 Hysteroscopic metroplasty for T-shaped uterus: 23 years of a single-center experience

G. Ducellier-Azzola[1], L. Lecointre[2], M. Hummel[2], O. Garbin[2]

[1]*Pôle Mère-Enfant- Centre Hospitalo-Universitaire, Loire-Atlantique, Nantes, France*
[2]*Centre Médico-Chirurgical et Obstétrical, Alsace, Strasbourg, France*

Study question: The primary objective of this study is to evaluate the effect of hysteroscopic metroplasty for T-shaped uterus on living births's rate.

Summary answer: Hysteroscopic metroplasty is a well established surgery. It improves the obstetrical prognosis of patients with T-shaped uterus.

What is known already: T-shaped uterus is due to myometrium excess inducing an under cornual striction. It is related with higher rates of obstetrical complications (miscarriages, tubal pregnancies, premature delivery) and infertility. This is often related with an in-utero diethylstilbestrol exposure.

Study design, size, duration: This is an observational study about 112 patients treated by hysteroscopic metroplasty for T shaped uterus between 1992 and 2016 in a university hospital of Strasbourg, France. Among this patients, 55 were treated for repeated miscarriages and 57 for infertility.

Participants/materials, setting, methods: The population average age was 33,2 years and the average desire pregnancy was about 56 months for the repeated miscarriages group and 42,2 months in the infertility group. Before surgery, 161 pregnancies had been obtained resulting in 2.5% of living births, 787.3% of early miscarriages, 1.9% of late miscarriages and 14.3% of tubal pregnancies.

Main results and the role of chance: After surgery, 51 pregnancies were obtained in the repeated miscarriages group with a significant increased rate of living births (1.4% vs 58.8%, p < 0.02) and lower rate of miscarriages (85.7% vs 19.6%, p < 0.02). In the infertility group, 49 pregnancies were obtained, spontaneously for the half, with a higher rate of living births (14.3% vs 61%, p < 0.02) and a lower rate of tubal pregnancies (71.4% vs 10.2%, p < 0.02). Anatomical results for uterus size and shape were good for respectively 85.9% and 83.5% of cases. Sixty-one percent of the patients had a caesarean section. No case of uterine rupture has been reported.

Limitations, reasons for caution: The obtained pregnancies were still high risk pregnancies with 10% of premature birth in the miscarriages group and 30% in the infertility group. This uterus were considered as scarred uterus. No uterine rupture has been reported in this study.

Wider implications of the findings: Our results are consistent with those of the literature. This surgery is sparsely responsible of complications and should be proposed for every symptomatic patient with T-shaped uterus before medically-assisted procreation.

Trial registration number: not applicable.

O-208 Expectant management may reduce overtreatment in women affected by unexplained infertility confirmed by diagnostic laparoscopy

V. Tagliaferri, S. De Cicco, L. Selvaggi, D. Romualdi, V. Immediata, C. Di Florio, A. Lanzone, M. Guido

Università Cattolica del Sacro Cuore, Gynaecology and Obstetrics, Roma, Italy

Study question: Does the mini-invasive surgery still play a role in the diagnostic work-up and in the management ofthe couples affected by unexplained infertility?

Summary answer: Combined laparoscopy and hysteroscopy allow to select women with a diagnosis of unexplained infertility and to better address these patients to a correct management.

What is known already: At present, the management of unexplained infertility represents a controversial matter. Laparoscopy is the only method able to identify specific peritoneal factors, such as minimal endometriosis and pelvic adhesions, which may impair fertility. ESHRE-ASRM guidelines indicate surgery for infertile women with symptoms, or risk factors, or abnormal hysterosonography/ultrasonography, with no other medical indications to undergo ART. However, the diagnostic and therapeutic role of laparoscopy in asymptomatic women with normal imaging is debated, as few studies previously evaluated the effect of this procedure in terms of reproductive outcome.

Study design, size, duration: Retrospective study. Size: 270 women affected by unexplained infertility (170 women underwent combined surgery, 100 control patients). Duration: 2008–- 2013

Participants/materials, setting, methods: 170 infertile women (age range 25–38 years) with documented normal ovarian, tubal and uterine function underwent combined hysteroscopic and laparoscopic surgery; 100 women refused surgery or ART treatment (control group) choosing expectant management. A retrospective assessment questionnaire was proposed to enrolled women in order to collect the rate of spontaneous or ART-induced pregnancies.

Main results and the role of chance: In the whole group of 270 patients the mean age was 33.84 ± 3.05 years, the mean BMI was 23.5 ± 2.2 kg/m2. The mean infertility duration was 42.05 ± 5.03 months. The combined surgery revealed pelvic pathologies in 49.4% of patients, confirming the diagnosis of unexplained infertility only in 86 of studied patients. In this group of 86 selected women, 28 of them achieved a spontaneous pregnancy and 23 women obtained pregnancy after ART. The chi-square analysis shows that the pregnancy rate was not influenced by the employment of ART. The rate of spontaneous pregnancy was 32.5% in the 86 subjects with "sine causa" infertility, whereas only 14% of the 100 control women achieved a pregnancy after 18 months of expectant management. The Odds Ratio to obtain a spontaneous pregnancy in women affected by unexplained infertility who underwent surgery compared to control women resulted 2.96 (confidence interval 95%: 1.43–6.11, p < 0.01).

Limitations, reasons for caution: It is a retrospective study performed in a single unit. Although we found an high percentage of spontaneous pregnancies in the surgery group compared to control patients, it cannot be ruled out that factors other than surgery may play a role.

Wider implications of the findings: Diagnostic laparoscopy should be considered in the infertility work-up to screen couples that can be really considered affected by "sine causa infertility", providing possible changes in the management plan. Our results in terms of pregnancy outcome could justify an expectant management as first approach for these patients, avoiding overtreatment.

Trial registration number: N/A.

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O-209 The endometriosis fertility index (EFI) is useful for predicting the ability to conceive naturally after laparoscopic surgery, regardless of endometriosis

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[2]Sugiyama Clinic, division of Reproductive Medicine, Tokyo, Japan
[3]Nippon Medical School, Center for Reproductive Medicine and Endoscopy, Tokyo, Japan

Study question: We investigated the utility of the endometriosis fertility index (EFI) for predicting the ability to conceive naturally after laparoscopic surgery.

Summary answer: The EFI possesses greater predictive power for a successful pregnancy with natural intercourse or IUI in infertile patients, regardless of endometriosis, than the (r-AFS) classification.

What is known already: The EFI was developed in 2010 as a scoring system for estimating the rate of natural conception after laparoscopic surgery for endometriosis. The EFI includes a detailed evaluation of the appendix (fallopian tubes, fimbriae of fallopian tubes, and ovaries) and accounts for any fallopian tube dysfunction after operation. The EFI was recently indicated to have greater predictive power than the r-AFS for predicting a successful pregnancy with ART in endometriosis patients. However, there have been no reports on the relationship between the EFI and successful pregnancy without ART (natural intercourse or intrauterine insemination) with or without laparoscopic surgery.

Study design, size, duration: From July 2011 through December 2012, 133 infertile patients were recruited. Forty-eight of these patients had unilateral or bilateral endometriomas. All patients underwent diagnostic laparoscopy or laparoscopic ovarian cystectomy. Patients with a history of gynecological operations, other ovarian masses, tubal obstruction or male infertility were excluded from this study, in order to focus on evaluating the correlation between the EFI and its relationship with the reproductive outcomes. Informed consent was obtained from all patients.

Participants/materials, setting, methods: Three-port laparoscopy was performed with an umbilical 3-mm scope port and two additional 3-mm operating ports. The EFI accounts for the following clinical and surgical factors: the patient's age, duration of infertility (years), pregnancy history, r-AFS and least-function (LF) score. Patients who achieved pregnancy naturally after laparoscopy were classified into the pregnant group (P group); the others were classified into the not-pregnant group (NOP group). Several clinical factors were compared between these two groups.

Main results and the role of chance: Fifty-five patients successfully achieved pregnancy after laparoscopic surgery and were therefore classified into the P group; the others ($n = 78$) were classified into the NOP group. The age and duration of infertility in the P group were significantly lower and shorter than those in the NOP group ($p < 0.05$). Although there were no significant differences in the proportion of patients with severe r-AFS between the P and NOP groups (30.9% and 39.7%, respectively), the EFI in the P group (7.6 ± 1.6) was significantly higher than in the NOP group (6.8 ± 1.6, $p = 0.0063$).

A univariate linear regression analysis, we found that a younger patient age, shorter duration of infertility, and higher EFI were positively associated with a successful pregnancy without ART after laparoscopic surgery ($p < 0.05$).

According to the ROC curve, the cut-off EFI value for predicting a successful natural pregnancy was 7 (AUC=0.713); thus, the patients were divided into two groups: patients with an EFI of 8–10 were classified into the high-EFI group, while those with an EFI ≤ 7 were classified into the low-EFI group. The pregnancy rate of the high-EFI group was 52.5%, which was significantly higher than that of the low-EFI group (31.9%, $p < 0.05$).

Limitations, reasons for caution: The EFI scoring system has unique characteristics, incorporates historical findings such as patient's age, duration of infertility, and pregnancy history. Patients showing disadvantageous findings among these factors had a lower EFI and the prognosis for achieving pregnancy was poor, regardless of whether they showed a normal pelvic anatomy.

Wider implications of the findings: The average durations between laparoscopic surgery and pregnancy in the high- and low- EFI groups were 4.8 ± 3.8 and 5.0 ± 3.4 months, respectively. The patients were unable to achieve pregnancy within nine months after laparoscopic surgery regardless of their EFI, if they started ART treatment.

Trial registration number: This study does not have RCT status. As a result, therefore, it did not receive a trial registration number.

O-210 Resectoscopic plasty of uterine cervical canal stenosis is effective to enable the post-surgical passage and yields high pregnancy rate

T. Fujiwara[1], F. Matsumura[2], T. Kurosawa[2], N. Watanabe[2], M. Mitsunami[2], M. Noma[2], C. Tabata[2], M. Kusumi[2], M. Murai[2], Y. Tanaka[2], O. Tsutsumi[2]

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[2]Sanno Hospital, Center for Human Reproduction and Gynecologic Endoscopy, Tokyo, Japan

Study question: Can endoscopic intervention improve uterine cervical canal stenosis and contribute to successful pregnancy after the surgery?

Summary answer: Resectoscopic plasty of uterine cervical canal stenosis was successfully performed and made the passage of the canal much easier, which achieved high post-surgical pregnancy rate.

What is known already: There were several case reports with relatively small number of subject to attempt various approaches to smoothen uterine cervical canal stenosis which disturbed inserting an intra-uterine insemination(IUI) or an embryo transfer(ET) catheter, which include using a stylet with the catheter, preceding dilation of the cervix using dilators and pre-treatment with laminaria. However in some severe cases those methods cannot be applied and thus there were also some reports to surgically improve the condition using hysteroscopy, which seems effective, but the preceding reports included only small number of subject and histological finding was lacking.

Study design, size, duration: This is a retrospective study and the clinical data were picked up from the records. A total of twelve patients was included in the study, whose surgeries were done between 2010 and 2016.

Participants/materials, setting, methods: The infertility patients who had uterine cervical canal stenosis that was obstacle to IUI or ET were indicated to plastic surgery. In doing the surgery written informed consent was obtained from each patients and using a resectoscope mass in the cervical canal was cut or angulation of the canal was smoothened. After the surgery the condition of the canal was evaluated and pregnancy rate was observed.

Main results and the role of chance: The median age and the duration of infertility of the subjects were 38 years old and 34 months, respectively. In all patients the surgery using small sized resectoscopy was performed successfully without any complication. All patients could leave the hospital on the following day. After the surgery either IUI or ET catheter was inserted without difficulty in all patients and there was no case of recurrence of cervical canal stenosis. In all cases resected tissue was examined histologically; three cases had cervical polyp, four cases had mayoma and five cases had unpathological cervical tissue and there was no case of malignant disease. Following the surgery a total of eight patients got pregnant; six were conceived within six months, one within one year and one after four years and six months. All patients but one got pregnant with ET. All patients reached full term delivery without miscarriage, in which three had vaginal delivery and five had cesarean section.

Limitations, reasons for caution: This is a retrospective study and the patients who were estimated to have an indication of the surgery were all recruited to it and thus there was no control.

Wider implications of the findings: Surgical improvement of the uterine cervical canal stenosis by resectoscopic plasty is a powerful and effective tool for further treatment of infertility especially in cases with difficulty to reach intra-uterine cavity, achieving high pregnancy rate without any negative effect during pregnancy.

Trial registration number: not applicable

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SELECTED ORAL COMMUNICATIONS
SESSION 57: ETHICS AND LAW
Tuesday 4 July 2017 Room C 17:00–18:00

O-211 Stem cell derived gametes: a slippery slope towards designer babies?

S. Segers[1], H. Mertes[2], G. Pennings[2]

[1]*Bioethics Institute Ghent- Ghent University, Department of Philosophy and Moral Sciences, Gent, Belgium*
[2]*Bioethics Institute Ghent- Ghent University, Department of Philosophy and Moral Sciences, Ghent, Belgium*

Study question: Is the fear for designer babies a convincing argument against the development of stem cell derived (SCD-) gametes?

Summary answer: Although SCD-gametes can facilitate the creation of designer babies, this need not undermine the entire enterprise of *in vitro* gamete derivation.

What is known already: The dawn of new reproductive techniques is often accompanied by fears for eugenic practices, the creation of so-called designer babies in particular. The reproductive use of SCD-gametes feeds similar worries, in view of the possibility to select and design embryos with desired non-disease related traits. While such practices have a negative moral connotation, it should be investigated whether or not this moral worry is justified, both in terms of the scientific possibilities (state of the art), and the moral wrongness of selecting and or editing embryos in function of non-disease related traits.

Study design, size, duration: A literature study was performed to delineate how the terms 'eugenics' and 'designer baby' are used and how they relate to each other. Next, claims in the scientific and ethical literature about how SCD-gametes may be used for eugenic purposes were inventoried. These claims were critically evaluated for scientific accurateness. Finally, we question whether the claimed possibility of selecting or genetically designing future offspring based on non-disease related traits is necessarily a morally bad thing.

Participants/materials, setting, methods: Literature study, conceptual analysis, normative analysis.

Main results and the role of chance: A first possibility is to produce large numbers of gametes (especially oocytes) and embryos to select genetic traits. Second, stem cells could be edited via CRISPR/Cas9 and differentiated into gametes. Third, SCD-gamete technology could be used to recombine SCD-gametes with other gametes and derive gametes from the resulting embryos (and so on) to shape the genome through selective breeding, by combining desirable traits that arise in different embryos. Fourth, at present somatic cell nuclear transfer (SCNT) is hindered by the short supply of human oocytes. This could be overcome by means of SCD-gametes. By facilitating SCNT, SCD-gamete technology could ease the creation of embryos with the same genome as someone with a desirable genotype. A last possibility would be to create gametes from persons with desired traits (e.g. via induced pluripotent stem cells) and make these available via gamete banks. Each of these scenarios is premised upon further scientific developments. Given the rapid advance in CRISPR/Cas9, it might become possible to edit embryos so that SCD-gamete technology will be neither a sufficient nor a necessary condition to genetically design offspring. If SCD-gamete technology would become safe, it might nevertheless facilitate eugenic purposes, of which the moral wrongness remains contested.

Limitations, reasons for caution: Gamete derivation from human stem cells is still in the research phase. The question of the moral wrongness of enhancement and eugenics is explored but not 'answered', as it is a fundamentally normative question. Similarly, 'designer baby' is a stipulative concept, as it is contested which interventions amount to 'designing'.

Wider implications of the findings: The causal link between SCD-gamete technology and eugenics/designer babies is weak and speculative. Moreover, the wrongness of such an evolution is contested.

Trial registration number: n/a.

O-212 Are fertility clinics and sperm banks under a moral obligation to perform comprehensive genetic screening of their gamete donors?

H. Mertes

Bioethics Institute Ghent BIG Ghent University, Department of Philosphy and Moral Sciences, Gent, Belgium

Study question: Are fertility clinics and sperm banks under a moral obligation to perform comprehensive genetic screening of their gamete donors?

Summary answer: Although there are good reasons to screen gamete donors more thoroughly than reproductive partners, this moral obligation is limited in scope.

What is known already: There is a trend towards more comprehensive genetic screening of gamete donors, sometimes coupled with preconception carrier testing for the recipient. It appears that many perceive it to be the responsibility of the fertility clinic to preclude the transmission of genetic disorders from donor to child.

Study design, size, duration: Arguments for and against more comprehensive gamete donor screening were gathered and assessed for scientific correctness and normative legitimacy.

Participants/materials, setting, methods: Literature research; normative analysis

The method that is used to bring empirical data (as found in literature research) and normative ethics together is the 'Wide Reflective Equilibrium', the most commonly used method in bioethics.

Main results and the role of chance: While fertility clinics and sperm banks are partly responsible for the wellbeing of the children they help conceive, this responsibility is limited in scope. A first line of argument questioning the ethical requirement of comprehensive screening is based on the analogy with natural reproduction. The arguments that donors should be screened more thoroughly than reproductive partners due to the donor's replaceability and higher number of offspring are flawed. Other morally relevant differences which do place a higher degree of responsibility with the fertility clinic/sperm bank can be translated into a limited screening program and into providing more risk information, rather than guaranteeing a healthy baby.

A second, consequentialist, line of argument looks at the practical implications of more thorough donor screening. A first concern is the raise in cost to screen out limited risks. This negatively effects access. Also, the more screening is applied, the less donors will be found 'fit' to be used in reproduction (as we are all carriers of several harmful mutations). As a response to this, donor and recipient might be screened as a couple to prevent carriership of the same mutation in both parties. This again increases costs and impedes access.

Limitations, reasons for caution: This is a normative analysis, not an empirical study.

Wider implications of the findings: Despite the fertility clinics' and sperm banks' shared responsibility for the health of the children they help conceive, it is overly demanding to state that they have the responsibility to rule out all genetic 'defects' in the offspring. This also has implications for liability claims.

Trial registration number: N/A.

O-213 Ethics of oocyte banking: a qualitative interview study with donors, recipients, social freezers, and professionals on fair collection and distribution of donor oocytes

E. Kool[1], R. Van der Graaf[1], J. Pieters[2], I. Custers[3], B. Fauser[4], A. Bos[4], A. Bredenoord[1]

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Study question: What are the opinions of oocyte donors, recipients, social freezers and professionals of Dutch oocyte banks on fair collection and distribution of donor oocytes?

Summary answer: The main themes discussed regarding fair collection and distribution were: (financial) compensation for donors, selection of donors and recipients, waiting list procedures and treatment guarantees.

What is known already: Since 2012, three donor oocyte banks in The Netherlands have been set up. However, the demand for donor oocytes exceeds the supply leading to discussion on what entails fair collection and distribution. Suggested approaches are to 1) increase financial compensation for donors, 2) widen the selection criteria for donors or 3) narrow the selection criteria for recipients. This qualitative interview study aims to collect the moral considerations of all parties involved in order to enrich the ethical discussion on fair collection and distribution of donor oocytes in time of scarcity.

Study design, size, duration: The project has been set-up as a qualitative interview study and has taken place from October 2016 to April 2017. Hitherto, 18 semi-structured in-depth interviews were conducted. We will continue inclusion until saturation is reached, which is expected at approximately 30 interviews. The interviews have been audiotaped and transcribed verbatim. Discrete pieces with relevant data were labelled and compared between the transcripts. By means of a constant comparative analysis key themes were distilled.

Participants/materials, setting, methods: The respondents were recruited in three clinics which currently provide assisted reproduction by the use of donor oocytes in the Netherlands: University Medical Center Utrecht, Medical Center Child Wish Leiderdorp and Academic Medical Center Amsterdam. The study population consisted of four groups: oocyte donors, recipients of donor oocytes, women who froze their oocytes for future use and thus have experienced the procedure and professionals engaged with the practice of oocyte donation (gynaecologists, psychologists, social workers).

Main results and the role of chance: Hitherto, interviews(n = 18) were conducted with donors (n = 4), recipients (n = 4), social freezers (n = 3) and professionals (n = 7). In the interviews we aimed to discuss what entails fair collection and distribution of donor oocytes. First, the main themes addressed by the respondents regarding fair collection were: a suitable (financial) compensation and responsible selection criteria for donors. As a reaction to questions on financial compensation respondents addressed issues such as the demand of altruistic motivation to donate, the importance of voluntary donation and the invasiveness of the donation procedure. Moreover, different justifications for the financial compensation were discussed. By questioning the selection of donors the respondents addressed the appropriate age limits for donation, necessity of a completed family and the acceptable risks for genetically transferable diseases. Second, when asking about fair distribution of donor oocytes respondents addressed the importance of proper selection of recipients, fair treatment guarantees and acceptable waiting list procedures. Additionally, respondents discussed the acceptability of distributing donor oocytes with possible increased risks for minor disabilities. Along the importance of organizing the collection and distribution of scarce donor oocytes fairly, respondents expressed the need of increasing public awareness of the possibility of treatment with donor oocytes and the serious shortage of donors.

Limitations, reasons for caution: Donors and recipients who had a positive attitude and experience regarding their treatment were more likely to participate in the study which may have caused a potential bias in the results.

Wider implications of the findings: The findings contribute to the international discussion of the need and actual appearance of altruistically motivated oocyte donation and the justification of a financial compensation. Moreover, this study provides insight in the experienced challenges in case of scarcity of donor oocytes and indications for fair collection and distribution.

Trial registration number: NA.

O-214 How should the field of assisted reproduction respond to calls for banning animal research?

V. Jans, W. Dondorp, G. De Wert

Maastricht University, Department for Health Ethics and Society, Maastricht, The Netherlands

Study question: How should the field of assisted reproduction respond to calls for banning animal research?

Summary answer: To reduce possible health risks of children conceived through assisted reproduction, the field should continue with animal research, while working on optimizing animal research models.

What is known already: There is a growing awareness of the importance of preclinical safety research on new assisted reproductive technologies (ARTs), including animal research using both so called "lower" (e.g. rodents) and so called "higher" animals (e.g. non-human primates, like macaques and baboons). At the same time, there is international support for the three R's (Replace, Reduce, Refine), and the European Union even aims at the full replacement of animals for research.

Study design, size, duration: A literature study was performed to identify and analyze the normative conditions for animal research on ARTs. Interviews with key figures in reproductive medicine were held. Furthermore, a case study on animal research for Stem Cell Derived (SCD) gametes research was performed.

Participants/materials, setting, methods: Articles have been selected from scientific journals focusing on normative aspects of ARTs and animal research. Additionally, legislative reports considering animal research were studied. Finally, for the ethical analysis, the widely accepted method of "wide reflective equilibrium" was used.

Main results and the role of chance: Two tensions were identified; (a) between the importance of safety research and reasons for reducing the use of animals for research, (b) between optimizing research protocols for specific questions and reasons for avoiding the use of non-human primates. Taking into account both the imperative of reducing avoidable reproductive risks of ARTs and the lack of adequate alternatives, at least for the time being, the use of animals in preclinical safety research on ART meets the criteria of proportionality and subsidiarity. Although the proportionality criterion seems to suggest that the use of "lower" animals is always to be preferred, paradoxically, the use of non-human primates may be more proportionate if and in so far this generates more relevant and robust data. Given the importance of the 3 R's, the main challenge is and remains to evaluate each research proposal case by case. At the same time, a further debate about the societal value of new ARTs remains important.

Limitations, reasons for caution: In the highly dynamic field of reproductive biomedicine, new developments may require reconsideration, adaption and fine-tuning of current normative guidance. The possible future availability of new options and alternatives for preclinical safety research may lead to other conclusions than the ones drawn in this article.

Wider implications of the findings: Animal research is, and may always be, necessary for responsible innovation in ARTs, aiming at risk reduction for children thus conceived. We should scrutinize currently used animal models and be open for change, allowing, on conditions, also the use of non-human primates.

Trial registration number: Not applicable

INVITED SESSION

SESSION 58: COCHRANE SESSION: EVIDENCE BASED DECISION MAKING IN FERTILITY TREATMENTS

Wednesday 5 July 2017 Plenary 1 08:30–09:30

O-215 Evidence based decision – are we there yet or have we lost our way?

S. Bhattacharya

University of Aberdeen, Obstetrics and Gynaecology, Aberdeen, United Kingdom

Abstract text

Evidence based decision making – are we there yet or have we lost our way?

Prof Siladitya Bhattacharya, University of Aberdeen, Scotland, U.K.

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Evidence based medicine (EBM) remains the accepted way of delivering high quality effective care. Both the quality of the underlying research and the precision of the findings are important factors which can refine the clarity and strength of evidence based guidelines. In recent years the application of EBM has evolved into a more personalised approach based on individual estimates of the risk of benefit and harm.

Systematic reviews of high quality primary studies remain the currency of EBM. In their absence, there is a need to utilise the best available evidence for clinical decision making. The speed of discovery in assisted reproduction has challenged standard pathways of evidence generation which can be slow and unlikely to yield a definitive answer before new techniques are incorporated into routine clinical practice.

Interventions of unproven effectiveness are not necessarily the same as those which have been proven to be ineffective. It can be argued that the former can be considered in the presence of strong biological plausibility where randomised trials are unethical (e.g. IVF for bilateral salpingectomy), or in situations where the proposed treatment is innocuous and cheap. There is, however, no justification for offering interventions which have been shown to be ineffective.

Successful implementation of EBM needs to overcome a number of recognised barriers including access to, and awareness of, the evidence base, organisational and personal resistance to change, strongly held beliefs and reverence for tradition. Implementation science research has addressed many of these factors but there is room for further improvement. The need to generate a strong evidence base remains critical but we also need effective pathways of transmitting key messages to all stakeholders including patients, clinicians, managers and funders of health services.

O-216 Cochrane hot topics: evidence based laboratory practice

S. Mastenbroek

Academic Medical Center - University of Amsterdam, Center for Reproductive Medicine, Amsterdam, The Netherlands

Abstract text

As many as one in six couples experience subfertility at least once during their reproductive lifetime. IVF has rapidly evolved as the intervention of choice to help these couples, and has established itself firmly in modern day society. More than 700.000 IVF treatments are conducted worldwide each year. But IVF treatments are not always successful, as about 30 percent of treatments result in the birth of a child. Improvements are sought in every step of the procedure. In the IVF laboratory many things have changed in recent years with the idea of improving IVF success rates. Changes in culture conditions, fertilization methods, embryo selection methods, timing of transfer, and freezing protocols amongst many others. But have these developments actually helped in achieving the goal of increasing IVF effectiveness? And how should we evaluate this? Embryologists naturally focus on embryo development and implantation rates, but what are the outcomes to evaluate in case of IVF effectiveness? And how to deal with all new upcoming technologies? Responsible innovation in reproductive medicine requires to have an answer to these questions. Cochrane evidence provides a powerful tool to assist in this quest. Cochrane evaluates and summarizes evidence from research on for example culture media, ICSI, assisted hatching, preimplantation genetic screening, time-lapse, freeze-all, and the day of transfer. This talk will discuss how, but also to what extent, Cochrane evidence can help to improve the results of the IVF laboratory.

INVITED SESSION

SESSION 59: NEW SOURCES OF GAMETES

Wednesday 5 July 2017 Plenary 2 08:30–09:30

O-217 In vitro generation of germ cells from pluripotent and somatic cells

J.V. Medrano Plaza

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Abstract text

Up to 15% of people worldwide at reproductive age are affected by infertility, thus representing a significant impact to patients' quality of life. Despite the great progress of IVF treatments, the lack of functional gametes is a major handicap for their success, and usually end up with the need of gamete donation. In the search to offer patients the possibility of being the genetic parents of their children, basic approaches from Regenerative Medicine that allow the creation of functional gametes have gained attention in the last years. Important advances in this field have occurred from the first reports describing the ability of mouse embryonic stem cells to spontaneously give rise to oocyte-like structures fourteen years ago, to the latest ones, describing the complete process of in vitro oocyte generation starting from mouse iPSCs. Importantly, all these findings in animal models have helped to move forward the study of human germ line and nowadays we have evidence for robust in vitro models to differentiate human iPSCs into immature germ cells, and even transdifferentiate somatic cells into a germ cell-like phenotype by genetic modification. Thus, we can say, that today we are closer than ever to translate the in vitro gamete generation technology for future clinical applications. Here, I will try to update and discuss the most recent breakthroughs that research in Regenerative Medicine has achieved in the last years in response to the challenge offered from Reproductive Medicine.

O-218 Ethical concerns related to artificial gametes

H. Mertes

Bioethics Institute Ghent BIG Ghent University, Department of Philosphy and Moral Sciences, Gent, Belgium

Abstract text

The ethical concerns in the context of in vitro gametogenesis (IVG) are related to the goal (research vs reproduction), the source (embryonic stem cells vs induced pluripotent stem cells) and the methods (e.g. embryo research, 'therapeutic cloning' and animal research).

When it comes to the prospect of reproductive use, the main concern is safety, especially when 'personalized' gametes, resulting in genetic parenthood, are produced. This objective not only requires the procedure of deriving gametes from stem cells to become safe and reliable, but also the procedure of producing induced pluripotent stem cells (iPSCs) or somatic cell nuclear transfer (SCNT). Attempts to produce egg cells for a male patient or sperm cells for a female patient will be faced with additional technical barriers, resulting in additional safety concerns. The acceptability of different safety concerns should be evaluated in an absolute and in a relative manner. In absolute terms, the high risk of serious harm standard could be put forward. This means that a substantial amount of safety testing (genomics, epigenomics etc) will be required before clinical implementation and that a thorough follow-up should be installed after clinical implementation. In addition, however, we should consider whether the risks for the welfare of the future children are sufficiently outweighed by the benefits. This is not an easy exercise to make in the context of IVG as there is much controversy over the importance of genetic parenthood. While there is generally little room for additional risks when prospective parents would want to build in (or select for) preferred physical or psychological characteristics in their children, additional risks, costs and efforts are commonly accepted in reproductive medicine in order to establish a genetic connection. The cause of this discrepancy in moral judgement is however underexplored.

In the context of reproductive use, questions are also raised about access and resource allocation. This is an example of very high tech personalized medicine, which is unlikely to become accessible to a broad public anytime soon. Therefore, on the level of public funding and public policy, the question whether society should fund this research seems legitimate.

Regardless of whether IVG would be performed for research purposes, to produce donor gametes or to establish genetic parenthood, embryo research and in many cases the creation of embryos for research purposes will be involved. Acceptance of embryo research varies greatly between countries, given the great divergence in moral status that is attributed to the *in vitro* embryo. This means that research into IVG will necessarily be barred in countries attributing a high moral status to human embryos.

Besides these larger issues, there are also a number of specific applications which have received a lot of attention, such as same-sex couples becoming the

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genetic parents of a mutual child, postmenopausal or prepubertal reproduction, involuntary (genetic) parenthood, single reproduction and genetic engineering of future children. Concerns over these applications are oftentimes a mix of an intuitive response (the so-called 'yuck-factor') and legitimate concerns over safety or the welfare of the child.

In conclusion, although the advent of 'artificial gametes' is widely anticipated by many, many ethical concerns line the road to research and especially clinical applications.

INVITED SESSION

SESSION 60: PARAMEDICAL INVITED SESSION: THE OOCYTE AND BEYOND

Wednesday 5 July 2017 Room A 08:30–09:30

O-219 Reprogramming of the oocyte epigenome in early embryonic development

J.A. Dahl[1], I. Jung[2], G. Greggains[3], H. Aanes[1], A. Manaf[1], M. Lerdrup[4], I. Jermstad[5], M. Indahl[3], M. Bjørås[1], K. Hansen[4], K.T. Dalen[5], P. Fedorcsak[3], B. Ren[2], A. Klungland[1]

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[4]*University of Copenhagen, The Biotech Research and Innovation Centre and Centre for Epigenetics, Copenhagen, Denmark*
[5]*University of Oslo, Norwegian Transgenic Centre, Oslo, Norway*

Abstract text

Maternal-to-zygotic transition is an essential process in early embryonic development. Embryos undergo a minor wave of genome activation shortly after fertilisation, followed by a major wave at late 2-cell stage in mouse, and 4–8 cell stage in human. However, the epigenetic regulation of gene expression in early embryonic development is incompletely understood.

We developed and validated a μChIP–seq method for detection of histone H3K4me3 in as few as 500 cells. Using this technique, we were able to produce histone modification maps for mouse oocytes, 2-cell and 8-cell embryos. H3K4me3 normally displays narrow promoter-specific localisation in active genes of somatic cells. Surprisingly, we found that oocytes contain broad H3K4me3 domains covering ~22% of the genome. These broad domains can exceed 10 kilobases, and can be located far away from transcription start sites.

It has previously been shown that H3K4me3 is established during oocyte growth by histone methyl transferase, MLL2, and is necessary for generation of mature oocytes (Andreu-Vieyra 2010, PLoS Biol 8). We demonstrate that H3K4me3 broad domains are established during oocyte growth, but are lost by 8-cell-stage.

We identified two histone demethylases, Kdm5a and Kdm5b, which are responsible for H3K4me3 demethylation during the 2-cell stage. Knockdown of these two histone demethylases resulted in maintenance of H3K4me3 levels, downregulation of zygotic genome activation genes, and poor embryo development.

Our findings suggest that careful regulation of epigenetic marks is crucial for gene expression in the early embryo development, and abnormalities in these processes can lead to developmental failure. This raises the possibility for a role for epigenetic regulation in infertility.

O-220 How successful is the use of oocytes with Smooth Endoplasmic Reticulum aggregates in ICSI cycles?

I. Mateizel

UZ Brussel, IVF labo CRG, Jette- Brussels, Belgium

Abstract text

Smooth endoplasmic reticulum (SER) is one of the most abundant organelles in the mature oocyte, represented by an interconnected network of membrane-enclosed sacs or tubules. Under the electron microscopy, they appear surrounded by mitochondria, forming structures located in the cortical ooplasm. These structures are involved in the storage and release of Ca2+ during the fertilization process, in the synthesis of lipids and triglycerides, and in energy production.

In gonadotropin-stimulated cycles, the SER may cluster forming SER aggregates (SERa) visible as a round flat disk in the cytoplasm under inverted microscope. The oocytes presenting SERa are considered dysmorphic and have been associated with reduced embryological outcome and an increased risk of congenital anomalies. Therefore, in 2011 the Alpha/ESHRE Consensus strongly recommended not to inseminate mature oocytes (MII) displaying SERa dysmorphism (SERa+). Not all the laboratories followed this recommendation and as a consequence, some of them reported results on the use of dysmorphic oocytes in ICSI/IVF cycles. In this sense, a retrospective analysis of all ICSI cycles carried out between 2009 and 2011 in our centre showed that SERa+ and SERa- oocytes have similar developmental competence after ICSI. Moreover, the transfer of embryos originated from SERa+ oocytes may lead to healthy newborns. Some of the subsequent reports confirmed these results. Nevertheless, data concerning the use of oocytes displaying this dysmorphism are still scarce and a follow up of the children born after the transfer of these embryos is encouraged. Studies should be performed in order to investigate the origin and possible effects of SERa on the molecular status of the oocytes and embryos.

SELECTED ORAL COMMUNICATIONS

SESSION 61: EMBRYO IN MOTION

Wednesday 5 July 2017 Plenary I 10:00–11:45

O-221 Harnessing the potential of time-lapse microscopy reveals novel aspects of human fertilization and suggests new morphokinetic parameters

G. Coticchio[1], M. Mignini Renzini[1], P. Novara[1], M. Lain[1], E. De Ponti[2], D. Turchi[1], R. Fadini[1], M. Dal Canto[1]

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Study question: Can more accurate application of time-lapse microscopy (TLM) lead to the observation of novel or neglected aspects of human fertilization?

Summary answer: Intensive harnessing of TLM reveals novel or previously poorly characterised phenomena of fertilization, such as a cytoplasmic wave preceding pronuclear formation or pronuclear chromatin polarization.

What is known already: In recent years, preimplantation development has been the object of TLM studies with the intent to develop morphokinetic algorithms able to predict blastocyst formation and implantation. Regardless, our appreciation of the morphokinetics of fertilization remains rather scarce, currently including only times of polar body II (PBII) emission, pronuclear appearance and fading, and first cleavage. This is not consistent with the complexity and importance of this process, calling for further TLM studied aimed at describing previously unrecognised or undetected morphokinetic events and identifying novel developmental biomarkers.

Study design, size, duration: The study involved a retrospective observation by TLM of the fertilization process in 210 oocytes utilised in consecutive ICSI cycles carried out in 2016. Maximum five fertilized oocytes per patients were included in the analysis to reduce possible patient-specific biases. Oocytes of patients with different diagnoses of infertility where included in the analysis, while cases involving cryopreserved gametes or surgically retrieved sperm were excluded.

Participants/materials, setting, methods: Microinjected oocytes where assessed by a combined TLM-culture system (Embryoscope). Crucially, oocytes not amenable to TLM assessment, due to excess of residual corona cells or inadequate orientation to observe polar body II (PBII) emission, were not analysed. Twenty-eight parameters were identified and monitored, relevant to

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meiotic resumption, pronuclear dynamics, chromatin organization, and cytoplasmic/cortical modifications. Times (T) were expressed as mean±SD (hours) and analysed, where appropriate, by Paired T Student or Fisher's exact tests.

Main results and the role of chance: PBII emission (T = 3.1 ± 0.8) was rarely followed (4.3% of cases) by abortive attempt of emission of a third polar body. Pronuclear formation was always preceded by a radial cytoplasmic wave (T = 5.3 ± 1.1), whose site of origin corresponded to that of male pronucleus (PN) formation. The female PN always formed cortically, adjacent to the PBII, while the male PN formed at different central (52.9%), intermediate (33.3%) or cortical (13.8%) cytoplasmic domains. Female and male PN appearance was almost synchronous (T = 6.0 ± 1.1 and T = 6.2 ± 1.2, respectively). PN juxtaposition involved rapid (up to 40 μm/hour) and straight movement of the female PN toward the male PN. Female pronuclear chromatin, i.e. nucleolar precursor bodies, became polarized (T = 8.1 ± 2.7) toward the area of pronuclear juxtaposition during displacement of the PN from the site of origin, while male chromatin polarization (T = 11.5 ± 4.4; P < 0.0001) was successive to contact between PNs. PN juxtaposition (T = 8.5 ± 2.5) occurred at different cytoplasmic domains (central 48.1%, intermediate 40.5% or cortical 11.4%) and was never followed by separation of PNs. A cortical cytoplasmic halo (T = 11.5 ± 2.4), in most cases (72,7%) asymmetric, formed always after PN juxtaposition and its disappearance (T = 22.9 ± 3.5) shortly preceded PN breakdown (PNBD). PNBD (female, T = 23,9 ± 4.0; male, T = 24.0 ± 4.0; P = 0.04) was rarely asynchronous (7.6%) and occurred concomitantly with chromatin fading.

Limitations, reasons for caution: Possible inter-operator variability in annotation of the novel morphokinetic parameters described in this study should be assessed.

Wider implications of the findings: To our knowledge, these data represent the most detailed description of human fertilization. Many of the illustrated parameters are novel and should be tested to develop more potent embryo implantation algorithms. Interestingly, localization and timing of the cytoplasmic wave are reminiscent of a key fertilization event, i.e. sperm aster formation.

Trial registration number: Not applicable.

O-222 Morphokinetic differences between male and female embryos

J. Zhang[1], J. Robins[2]

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Study question: Are there morphokinetic differences between male and female embryos that are produced by intracytoplasmic sperm injection and cultured un-interruptedly for 5-6 days in Embryoscope incubators?

Summary answer: more female embryos reach blastocyst stage by day 5 than male embryos

What is known already: in vitro fertilization and development may alter sex ratio of human embryos

Study design, size, duration: Retrospective analysis of sex ratio of blastocysts on Days 5-6 and morphokinetics during the first two days of development following intracytoplasmic sperm injection (ICSI) for patients who elected preimplantation genetic screening (PGS) for aneuploidy during the calendar year of 2016.

Participants/materials, setting, methods: Patients who elected PGS at Northwestern Fertility and Reproductive Medicine, a university affiliated outpatient facility for assisted reproduction, were included for this study. Trophoblast biopsy of 3–5 cells was performed in blastocysts on days 5 or 6, following ICSI on day 0. Biopsied embryos were vitrified and transferred in later cycle after PGS results were known.

Main results and the role of chance: During calendar year 2016 a total of 395 blastocysts from 121 patients were biopsied for PGS. No PGS results were obtained in 12 blastocysts from 11 patients. Gender information was not reported in 12 blastocysts from 6 patients at patients' request. This study analyzed 371 blastocysts from 105 patients. Mean patient age was 30.8 +/− 2.8 (mean +/− std). There were 273 blastocysts biopsied on Day 5 and 98 on Day 6. Aneuploidy rates were 56.7% or 67.3% (p = 0.053, chi squared test), for blastocysts biopsied on Day 5 or Day 6, respectively. Among blastocysts biopsied on day 5, 38.4% were males. This is compared with 52% for blastocysts biopsied on day 6 (p < 0.05). Time (hours) needed for pronuclear fadeing after ICSI, 2-cell division, and 4-cell division were 23.7 +/2.9 (mean +/std), 26.3 +/−3.2 and 38.5 +/−4.1 for these 371 blastocysts, and were similar between male and female embryos, and between aneuploid and euploid embryos. However, time for 4-cell division was 37.2 h for embryos reaching blastocysts by Day 5 and 40.5 h for those by Day 6 (p < 0.05, Student's t-test).

Limitations, reasons for caution: These observations need to be verified by further studies with larger sample sizes.

Wider implications of the findings: There are no sex related morphokinetic differences between embryo during the first two days of development. However, after embryonic gene activation begins, female embryos exhibit faster growth rates than male embryos and are more likely to become blastocysts. This suggests that IVF may alter sex ratios of human embryos.

Trial registration number: not applicable.

O-223 Morphokinetic variables and ploidy: is there still a chance?

F. Pennetta[1], C. Lagalla[1], R. Sciajno[1], N. Tarozzi[1], M. Nadalini[1], M. Di Santo[1], M.A. Bonu[1], A. Borini[2]

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Study question: Despite recent experimental evidence, is it still possible to find a relationship between morphokinetic variables and human blastocyst ploidy status?

Summary answer: Since aneuploid human blastocysts showed a delay in the timing of pronuclear appearance (tPNa), an association could exist.

What is known already: Implantation failure is the major cause of patient drop out during infertility treatment so many efforts are directed towards the establishment of methods that can help in the selection of the best embryo. Preimplantation genetic screening (PGS) was introduced to identify genetically healthy embryos and time-lapse microscopy (TLM) was introduced as a non invasive tool for embryo evaluations based on their developmental kinetics. TLM main applications include: prediction of embryo implantation potential, prediction of blastocyst development and prediction of aneuploidy. Nevertheless, a debate is still open to understand whether TLM may or may not predict the genetic health of embryo.

Study design, size, duration: Retrospective cohort study. The normal distribution of data was verified using the Kolmogorov-Smirnov test with the Lilliefors' amendment and the Shapiro-Wilk test. Morphokinetic variables were expressed as median and quartiles (Q1, Q2, Q3, Q4). Mann-Whitney U-test was used to compare the median values of parameters. Univariate and multivariate logistic regression models were used to assess relationship between ploidy and kinetics. Several confounding factors have been also assessed. ROC analysis was employed for the identified variable.

Participants/materials, setting, methods: From May 2013 to November 2015, a total of 93 patients were enrolled in a PGS program and 287 blastocysts underwent trophectoderm (TE) biopsy on day 5 or 6 followed by array comparative genomic hybridization (aCGH). All blastocysts were cultured in a time-lapse incubator and kinetics examined. For each blastocyst, absolute and relative morphokinetic parameters were collected and compared between euploid and aneuploid.

Main results and the role of chance: Mean female age was 38.4 (95% CI 37.5–39.3, range 26–48). The overall euploid rate of the blastocysts population was 50.2% (144/287; 95% CI 44.4–56.0), while the aneuploidy rate was 49.8% (143/287; 95% CI 44–55.6). First we performed univariate logistic regression and significant parameters were: time of pronuclear appearance/disappearance (tPNa/tPNf), time to two/ five/nine cells (t2, t5, t9), start blastulation (tSB), synchronization of cell divisions (s2=t4-t3), synchronization of cleavage pattern (s3=t8-t5). Taking into account these results a multivariate logistic regression model was created. To test the strength of the prediction model, the effect of

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potential confounder factors (female age, BMI, causes of infertility, protocol of stimulation) were also considered. In the prediction model only time of pronuclear appearance (tPNa) (OR=1.295; 95% CI 1.121–1.498) had a good relation to ploidy outcome. The median value of tPNa was statistically different (P=0,02) between euploid and aneuploid blastocysts (euploid blastocysts = 8,85 h; aneuploid blastocysts = 10,34 h). Optimal range is defined by the Q2-Q3 consecutive quartiles (7.76 h-9.8 h) with highest euploid blastocysts probability. The model could be considered strong because the area under the ROC curve was 0.73 with a 95% confidence interval (0.64, 0.75).

Limitations, reasons for caution: Even if there is a good correlation between tPNa and ploidy outcome, it is not so strong.

Wider implications of the findings: We believe that embryo selection by time-lapse microscopy should not be considered as substitute for PGS but as a complement tool, especially for good-prognosis patients without indications for PGS and to reduce the number of patients that undertake PGS.

Trial registration number: None.

O-224 Selection of single blastocysts for transfer with time-lapse alone and with next generation sequencing to reduce multiple pregnancies: a prospective randomized pilot study

Z. Yang[1], J. Lin[2], S. Zhang[3], Y. Kuang[4], J. Liu[5]

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[5]*Jiaen Hospital, ART, Beijing, China*

Study question: Can time-lapse (TL) and next-generation sequencing (NGS) be efficiently used for the selection of single blastocysts for transfer in IVF patients to reduce multiple pregnancies?

Summary answer: The combined use of TL and NGS for selecting single blastocysts for transfer results in significantly higher clinical and ongoing pregnancy rates for IVF patients.

What is known already: Selection of single blastocysts with the highest potential of implantation for transfer still remains a challenge. There are obvious shortcomings with traditional methods of evaluation and selection of single blastocysts for transfer based on morphological assessment alone. Recent advances in time-lapse monitoring and next-generation sequencing have provided new methods for selecting competent embryos for transfer in IVF treatments. However, there is still very limited information about the effects of time-lapse monitoring and NGS testing on clinical and ongoing pregnancy outcomes of single embryo transfer for IVF patients to reduce multiple pregnancies in a randomized study.

Study design, size, duration: A total of 229 IVF patients who met the inclusion criteria (under 35 years old, no prior miscarriage and normal karyotype) were enrolled in this prospective randomized pilot study in our multiple IVF clinics from March 2015 to April 2016. Following IRB approval, patients singed the informed consents for selecting single blastocysts for transfer with TL and NGS. Sample size was calculated using GraphPad StatMate (San Diego, USA) to ensure power of study.

Participants/materials, setting, methods: The enrolled patients at mean age 31.4 ± 3.5 years were prospectively randomized into two groups: 1) Group A: patients (n = 115) had embryos cultured in a time-lapse system (EmbryoScope, Denmark) and tested with NGS (Yikon Genomics, China) and 2) Group B: patients (n = 114) had embryos cultured in the time-lapse system only. The statistical analyses were performed using GraphPad InStat (San Diego, USA). A two-tailed value of $P < 0.05$ was considered statistically significant.

Main results and the role of chance: This is the first randomized clinical study on the combined use of TL and NGS for selecting single blastocysts for transfer in IVF patients. There were no significant differences in female patient's mean age, Day 3 FSH, AMH, E2, antral follicle number between Group A and Group B ($p > 0.05$). The fertilization and blastocyst rates were also comparable between the two groups ($p > 0.05$). However, there was significant difference in clinical pregnancy rates per transfer between Group A and Group B (72.5% vs. 52.6%, respectively, $P < 0.05$). The observed ongoing pregnancy rate per transfer was also significantly higher in Group A compared to Group B (71.6% vs. 50.8%, respectively, $p < 0.05$). There were no multiple pregnancies in both groups.

Limitations, reasons for caution: Although the combined use of TL and NGS brings distinct clinical benefits for many IVF patients, the approach is not for all patients, especially those with diminished ovarian reserve. Further randomized studies with a larger sample are needed to define the role of TL and NGS for all age groups.

Wider implications of the findings: With the resulting high clinical and ongoing pregnancy rates following single embryo transfer, the combined use of time-lapse and NGS has demonstrated an efficient means for selecting single competent blastocysts for transfer to reduce multiple pregnancies for IVF patients.

Trial registration number: No applicable.

O-225 Single embryo transfer on cleavage-stage(D3) using Time-lapse selection VS on blastocyst(D5) using traditional morphological selection in patients with good prognosis: a prospective randomized controlled trial

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Study question: Does single cleavage-stage embryo(D3) selection using the results from the cleavage-stage embryo hierarchical classification model achieved the equal pregnancy outcome compared to single blastocyst(D5) selection based on traditional morphological method?

Summary answer: D3 single embryo transfer using time-lapse selection model could achieve equal clinical and ongoing pregnancy rates when compared to D5 by morphological selection.

What is known already: Single embryo transfers(SETs) has been advocated as a strategy to reduce the multiple births after assisted reproductive technology(ART). Blastocyst culture and single blastocyst transfer has been widely accepted to perform elective single-embryo transfer(eSET), but the longer in vitro culture period may raise potential risk for embryos. Previous trial on single cleavage-stage embryo transfer had negative result when compared to single blastocyst transfer. Recently time-lapse provide multiple parameters for better selection of cleavage-stage embryos. But little RCT results are available to demonstrate time-lapse selection could provide equal efficiency on pregnancy outcome in eSET when compared to blastocyst morphological selection.

Study design, size, duration: A prospective randomized controlled study was conducted in single embryo transfer patients undergoing IVF/ICSI at the Reproductive and Genetic Hospital of CITIC Xiangya between October 2015 and October 2016. This sample size achieves with a power of 80%, at a significance level(α) of 0.05, we would need to randomize 492 patients, 246 in each group. A sample size of 600 patients, 300 randomized to each group, was chosen to allow for those 20% dropped out.

Participants/materials, setting, methods: Patients were required to meet the inclusion criteria: ≤36 years of age, ≤2 failed IVF attempts using fresh cycles, ≥6 normally fertilized embryos(2PN), undergoing autologous IVF or ICSI.

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Randomization was done 1:1 using random numbers from sealed envelopes. Patients fulfilled the criteria on D1 were randomly assigned to conventional morphological blastocyst selection(D5,control group) or the cleavage-stage embryo hierarchical classification model for selection(D3,study group) cultured in the Primo Vision(Vitrolife;Budapest,Hungary). The primary outcome was ongoing pregnancy rate.

Main results and the role of chance: 334 patients were randomized (167 D3 vs 167 D5), 50 dropped out (23 D3 vs 27 D5) ,284 patients (144 D3 vs 140 D5) available for analyzed between 1 October 2015 and 27 October 2016 (The experiment is continuing). There were no differences in the demographic variables and cycle statistics between the two groups.The clinical 95/144 (66.0%) vs 102/140 (72.9%)(P >0.05) and ongoing pregnancy rates 93/144 (64.6%) vs 101/140 (72.1%)(P>0.05), early miscarriage rate 4.17% vs 5.71% and twin pregnancy rate 2.1% VS 1.4% respectively were not statistically different between D3 and D5.

Limitations, reasons for caution: The main limitation in this trial is the inclusion criteria were quite strict in favor of good-prognosis patients; in consequence, the potential value of the trial for improving clinical outcome might only be relevant to good-prognosis patients.

Wider implications of the findings: Time-lapse might help to establish efficient selection model for single cleavage-stage embryo transfer and it is worth to try in unselected patients to reduce multiple pregnancy rate.

Trial registration number: ChiCTR-ICR-15006600.

Trial registration date: 16 June 2015.

Date of first patient's enrolment: 1 October 2015.

O-226 Time-lapse (TL) morphokinetic parameters do not predict the outcome of fresh In-Vitro Fertilization/Single Embryo Transfer (IVF/SET) cycles

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Study question: Is there an association between early- and/or late-morphokinetic parameters and either pregnancy establishment or viability following IVF/SET?

Summary answer: Neither early- nor late-morphokinetic parameters predicted either pregnancy establishment or pregnancy viability in IVF/SET cycles.

What is known already: Certain morphokinetic parameters obtained from preimplantation human embryos cultured in TL- monitored incubators have been evaluated as markers of i) embryonic quality and ploidy status, and ii) embryo's potential for blastocyst formation and implantation. These include either static or interval morphokinetic variables from the early cleavage stage and/or later stages of embryonic development and blastocyst formation. However, it still remains unclear whether blastocysts resulting in live births differ in all or some of these morphokinetic parameters from those appearing morphologically normal but failing to either implant or establish a viable pregnancy.

Study design, size, duration: Design: Retrospective cohort.

Size: 223 women undergoing IVF/SET at a major academic center.

Duration: 09/2013-04/2016.

Participants/materials, setting, methods: Embryos cultured to blastocyst in a TL-monitored incubator and selected for fresh-SET.

Conception cycles were compared to non-conception ones, and viable pregnancy cycles were compared to pregnancy failures, in regards to the following early- and late-morphokinetic parameters: time period from i) pronuclei fading (tPN*f*) to 1st cytokinesis (P1), ii) 2- to 3-cells (P2), iii) 3- to 4-cells (P3), and iv) tPN*f* to early blastulation (PSB).

Statistics: *t*-test, chi-square, *one-way ANCOVA* controlling for potential confounders.

Main results and the role of chance: Groups did not differ in baseline characteristics (basal FSH, AMH, antral follicle counts, AMH). Results are presented as marginal means adjusted for maternal age (<38 vs. ≥38 years), basal-FSH, AMH, antral follicle counts and fertilization method (ICS vs. conventional insemination). In 60.5% (135/223) of cycles ICSI was utilized. Following IVF/SET, 64.1% (143/223) of the women conceived. Of those, 77.6% (111/143) had a viable pregnancy, progressing over 20 weeks gestation. When conception cycles were compared non-conception ones, no difference was noted in any of the morphokinetic parameters [marginal means(95%CI): 2.53(2.44–2.62) vs. 2.50(2.39–2.61), p = 0.66; 10.65(9.99–11.31) vs. 10.52(9.70–11.33), p = 0.81; 1.19(0.77–1.62) vs. 1.13(0.60–1.67), p = 0.86; 68.63(67.27–70.00) vs. 69.48-(67.64–71.32) hours, p = 0.48), for P1, P2, P3, and PSB, respectively]. Similarly, when viable pregnancy were compared to pregnancy failure cycles, morphokinetic parameters did not predict the outcome of the pregnancy [marginal means (95%CI): 2.57(2.46–2.66) vs. 2.44(2.23–2.66), p = 0.31; 10.57(10.00–-11.02) vs. 10.73(8.76–11.94), p = 0.83; 1.15(0.81–1.57) vs. 1.52(0.61–2.83), p = 0.63; 69.28(67.85–71.12) vs. 66.66(63.32–69.85), p:0.23), for P1, P2, P3, and PSB, respectively].

Limitations, reasons for caution: Limitations include the retrospective design, the evaluation of only those morphokinetic parameters thought to predict blastocyst formation but not pregnancy, and the potential effect of the fertilization method. Timeswere normalized to a common starting time-point, thus making it unlikely that the latter introduces bias affecting cycle outcomes.

Wider implications of the findings: In a group of unselected infertile patients undergoing fresh-IVF/SET, early-cleavage morphokinetic parameters and time to early-blastulation did not predict reproductive outcomes.

Trial registration number: Not applicable.

O-227 Comparison of morphokinetic markers which predict blastocyst formation and implantation potential from two large clinical datasets; CRM, WCM, U.S.A. and IVI, Spain

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Study question: To demonstrate whether the morphokinetic markers used for embryo selection have a similar relationship to blastocyst formation and implantation in two large clinical datasets.

Summary answer: Morphokinetic intervals for early cleavages were differently distributed between clinics. 2/3 morphokinetic markers in WCM and one in IVI data set were informative for implantation.

What is known already: The ability to correctly select the best embryo for transfer is a highly desirable capability as implantation is the ultimate goal of a successful IVF treatment. Time-lapse technology has assisted in determining whether key embryological events and temporal hallmarks are associated with embryo development or clinical outcome. Nevertheless, not all published algorithms based on morphokinetic markers has been found to be applicable in all clinic. The establishment of key developmental hallmarks in large datasets would demonstrate whether the published algorithms could be universally applied.

Study design, size, duration: Retrospective cohort study on two datasets of embryos cultured until the blastocyst stage (BL) (n = 27316) and/or implanted following single embryo transfers (I) (n = 816).

Participants/materials, setting, methods: Embryos in both clinics were cultured in a time-lapse system (EmbryoScope, Vitrolife, Sweden); IVI-Valencia (BL=11,414, I= 479) and Weill Cornell Medicine (WMC) (BL=15,902; I=337). Variables studied included: t2, t3, t4, up to t9 as well as the transition among all described cleavages. Two different datasets were compared using quartile plots with 95% CI on the quartile limit and the quartile average value, as well as AUC of the parameter against the relevant outcome.

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Main results and the role of chance: A detailed graphical analysis was performed for t3, t5, cc2 (t3-t2) and the ratio (t5-t3)/(t5-t2). In relation with our best defined marker (t5), timings were not affected between clinics. However, WCM proportions were significantly affected by the definition of achieving BL vs. not achieving BL, when compared to IVI data. A significant decrease in the proportion of blastocysts with longer times to t5 was observed for WCM. Meanwhile, t5 were more informative in the IVI data set in relation to implantation. Similar results were observed in cc2 and the ratio (t5-t3)/(t5-t2). Although similar, t3 timings were significantly higher in IVI data than WCM for the proportion of implanted embryos in the 2nd quartile (within the confidence interval).

Limitations, reasons for caution: Although validated throughout a large datasets of two experienced time-lapse user clinics, the retrospective nature of the analysis is less than ideal.

Wider implications of the findings: These embryo selection algorithms may be suitable among two different and independent large datasets. The parameters are sensitive to the specific attributes of the data, and should not be universally applied. Evaluation of the outcomes depends on parameters used and should be evaluated before the incorporation of any selection algorithms.

Trial registration number: 1407-MAD-053-NB

SELECTED ORAL COMMUNICATIONS

SESSION 62: MUTATIONS AND PRIMARY OVARIAN INSUFFICIENCY

Wednesday 5 July 2017 Plenary 2 10:00–11:45

O-228 Health and morbidity of women with POI due to FSH resistant ovaries

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Study question: Do inactivating FSH receptor mutation and hypoestrogenism have long-term effects on health and morbidity, and can these be overcome by an adequate hormone therapy (HT)?

Summary answer: Early-onset HT with median duration of twenty years seems to be effective in preventing major health problems and morbidity in the genetic form of POI.

What is known already: Adverse long-term health outcomes of POI have been reported in several epidemiological and observational studies. FSHRO (FSH resistant ovaries), caused by mutations in FSH receptor, is a genetic form of primary ovarian insufficiency (POI). FSHRO patients have low endogenous estrogen levels and arrest of follicle development at antral stage. We have previously studied the long-term health morbidity of the FSHRO cohort by a health questionnaire. Here we report the results of a detailed clinical examination.

Study design, size, duration: The cardiovascular and metabolic health was assessed by the analysis of the lipid profile and oral glucose tolerance test with insulin measurements. Bone mineral density and whole body composition was analysed with DXA. Psychological and sexual well-being was assessed with BDI21, GAD-7 and FSFI questionnaires. The data were compared to the results of a national, age- and area-stratified population risk factor monitoring survey carried out in 5-year intervals (FINRISK).

Participants/materials, setting, methods: We identified 26 women with an inactivating A189V FSH receptor mutation. Twenty-two gave their written, informed consent to participate in the study. Fourteen (63.3%) were examined at Helsinki University Hospital and Oulu University Hospital.

Main results and the role of chance: All FSHRO women had been on HT, and the median time of use was 20 years. The FSHRO women had an elevated median A/G (android/gynoid) ratio. However, no metabolic syndrome or diabetes was detected. Based on the lipid profile analysis, there was not elevated risk to cardiovascular diseases as compared to age-matched controls. Osteoporosis was rare (one patient), but eight women (63.4%) were diagnosed with osteopenia despite the HT use. 69% of the FSHRO women were at risk, and 31% were at high risk, for sexual dysfunction irrespective of their relationship status.

Limitations, reasons for caution: FSHRO is a rare genetic form of POI, and thus the study cohort is small, which leaves a possibility for chance.

Wider implications of the findings: According to our findings, twenty years of HT in POI seems to be sufficient in preventing major cardiovascular and metabolic morbidity. However, bone health and the prevention of bone mineral density reduction may require a longer period of HT, which would better correspond to the natural duration of fertile age.

Trial registration number: The Hospital District of Helsinki and Uusimaa Ethics Committee for gynaecology and obstetrics, pediatrics and psychiatry 333/13/03/03/2013.

O-229 POR mutations as novel genetic causes of female infertility due to partial 17 alpha-hydroxylase deficiency

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Study question: Can *POR* mutations underlie female infertility without apparent congenital malformations?

Summary answer: *POR* mutations likely represent a rare cause of infertility without other clinical features through partial 17α-hydroxylase deficiency (17OHD).

What is known already: *POR* encodes an electron donor for all microsomal P450 enzymes including CYP17A1. *POR* mutations typically cause a congenital syndrome characterized by skeletal dysplasia, adrenal insufficiency, genital abnormalities, gonadal dysfunction, and infertility. Although *POR* mutations have been identified in undervirilized males diagnosed with non-syndromic 17OHD, such mutations have not been detected in infertile women without other clinical features.

Study design, size, duration: Clinical and molecular analyses of two unrelated Japanese infertile women without other clinical features besides menstrual abnormality.

Participants/materials, setting, methods: Two women visited our hospitals because of infertility. We examined clinical features of these patients. Serum steroids and urine steroid metabolites were measured by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry, respectively. Sequence analysis was performed for cording regions of *CYP17A1* and *POR*. The functional significance of identified substitutions was assessed by *in silico* analyses.

Main results and the role of chance: Both patients had a history of menstrual irregularity and presented with borderline hypertension, but had no genital or skeletal abnormalities. Serum and urine analyses showed decreased CYP17A1 activity in both patients. Urine analysis also showed slightly decreased CYP21A2 activity only in one patient. Both patients had no mutation in *CYP17A1*; however, they harbored compound heterozygous mutations in *POR* (p.R457H;[G413S;R550W] and p.Q201X;Y607C). PolyPhen-2 predicted impaired function of these mutants. Structural analysis predicted that p.R550W disrupts hydrogen bonds between amino acids and p.Q201X eliminates more than half of the amino acids of the POR protein. Structural analysis predicted that p.R457H and p.Y607C disrupts the interaction of POR with FAD and NADPH, respectively. These results suggest that the POR mutations are responsible for partial 17OHD and resultant infertility in these women.

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Limitations, reasons for caution: Our conclusions are based on the observation of only two individuals, and await further validation.

Wider implications of the findings: Various *POR* mutations may result in partial 17OHD without other clinical features. The frequency of *POR* mutations among women with non-syndromic infertility needs to be clarified in future studies.

Trial registration number: not applicable.

O-230 Serum levels of the oocyte-secreted factors GDF9 and BMP15 are associated with reproductive potential in women

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Study question: Are the oocyte-secreted growth factors (GDF9 and BMP15) detectable in human peripheral blood and are they possible biomarkers of female reproductive function?

Summary answer: GDF9 and BMP15 are detectable in female serum and their levels positively correlate with the number of oocytes retrieved during IVF.

What is known already: Oocyte quantity and quality are rate limiting to reproductive success. Current serum biomarkers of reproductive function, such as anti-Müllerian hormone (AMH), represent the follicular environment and hence are surrogate markers of oocyte biology. There are currently no serum oocyte biomarkers available for clinical use. The oocyte-secreted growth factors, growth differentiation factor-9 (GDF9) and bone morphogenetic protein-15 (BMP15), are requisite regulators of folliculogenesis, oocyte quality and fertility, and are essentially only produced by the gametes. These potential biomarkers of oocyte function have not previously been demonstrated as detectable in blood in association with reproductive potential.

Study design, size, duration: Novel immunoassays for the reliable detection of GDF9 and BMP15 were developed in-house, validated, and applied to excess serum samples from women having unstimulated or antagonist (FSH-stimulated) superovulated cycles. Bloods, matched to clinical data, were collected from women 27–45 years over six months and stored at -80° C prior to analysis. Serum levels of GDF9 and BMP15 were correlated with clinical, endocrine, and embryology data.

Participants/materials, setting, methods: For analysis of intra- and inter-cycle variation, GDF9 and BMP15 were measured in multiple bloods from unstimulated women (51 bloods from 9 women), from day 2 of the cycle prior to FSH stimulation, on multiple days during treatment, and/or across multiple cycles (167 bloods from 27 women). These data were analysed separately and combined with those of an additional 210 women for analysis in relation to clinical data.

Main results and the role of chance: The GDF9 and BMP15 immunoassays were validated for sensitivity, specificity and reproducibility using a serum pool as the reference preparation as non-parallelism was observed between recombinant protein preparations and biological samples. Stability studies using various collection methods and sample treatments further demonstrated their reliability. Assays for GDF9 and BMP15 detected proteins in female serum with notably similar profiles between the two assays. Serum GDF9 and BMP15 levels varied markedly between individual women (ranging from undetectable to 300-fold above baseline) but within subjects were unchanged throughout the ovarian cycle. Furthermore, serum levels were unchanged by antagonist stimulation treatment, regardless of FSH dose, and across different treatment cycles.

Serum levels of GDF9 positively correlated with the number of oocytes retrieved from non-PCO/PCOS women after superovulation ($r = 0.439$, $p < 0.05$, $n = 27$). However, this correlation was not observed in PCO/PCOS patients, indicative of oocyte-secreted factors being involved in this pathology. Furthermore, the GDF9:AMH ratio was on average 8.5 fold lower in PCO(S) compared to non-PCO(S) patients ($p < 0.0001$).

Serum levels of GDF9 and BMP15 did not correlate with age or baseline (day 2) endocrine measures of LH, oestradiol or progesterone. However, GDF9 positively correlated with baseline FSH ($r = 0.512$, $p < 0.05$, $n = 17$) and with AMH ($r = 0.478$, $p < 0.01$, $n = 36$).

Limitations, reasons for caution: The association of serum concentrations of GDF9 and BMP15 with oocyte yield during IVF supports the hypothesis that the proteins detected originate from the oocyte. However, the high concordance between serum levels of GDF9 and BMP15 warrants investigation into the exact form of the growth factors detected by these assays.

Wider implications of the findings: This is the first study to systematically demonstrate the diagnostic and predictive potential of oocyte-secreted GDF9 and BMP15 as serum biomarkers for use in reproductive medicine. Serum GDF9 and BMP15 may prove clinically valuable for diagnosing reproductive dysfunction and as predictors of fertility potential.

Trial registration number: Not applicable.

O-231 Effects and mechanisms of a novel mutation in basonuclin 1 gene on premature ovarian insufficiency

Y. Liu[1], J. Li[2], Y. Huang[2], Y. Wu[3], J. Liu[3], D. Zhang[3]

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Study question: What is the effects and mechanisms of Basonuclin1(BNC1) gene truncation mutation in premature ovarian insufficiency(POI)?

Summary answer: BNC1 dysfunction would lead to premature exhaust of follicles and POI. KLF17, CITED1, MPO, NPC2 may interact with BNC1 in maintaining normal ovarian aging.

What is known already: POI is one of the leading causes of female infertility owing to the absence, non-functionality or early depletion of ovarian reserve. We previously identified for the first time in the world a novel mutation——BNC1 truncation mutation in a large Chinese POI pedigree using whole-exome sequencing. Yet the effect and underlying molecular mechanism of BNC1 mutation on POI remains unknown.

Study design, size, duration: Transgene mouse model study. Generate BNC1 truncation mutation mice. Investigate ovarian aging of wild-type (+/+), heterozygous (+/tr), and homozygous (tr/tr) BNC1 truncation mutation mice. Assess transcriptomics study of wild-type and homozygous BNC1 truncation mutation mice ovaries. Explore the underlying mechanisms of BNC1 on ovarian aging.

Participants/materials, setting, methods: We generated BNC1 truncation mutation mice using homologous recombination. Genotypes were identified by PCR of tail DNA. Ovary size, fertility testing, serum hormone of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol were detected. Histological analysis of ovary tissues was performed and follicle numbers were counted. Transcriptomics study was carried out. Key differentiated genes were confirmed by RNA extraction and real-time RT-PCR.

Main results and the role of chance: BNC1 truncation mutation female mouse model exhibited infertility or subfertility, significantly increased serum FSH and LH levels, lowered estradiol level, decreased ovary size and reduced follicle numbers at every follicle developing stage. Taken together, the data from the mouse model indicated BNC1 truncation mice had impaired folliculogenesis and premature ovarian insufficiency. Go-analysis, pathway-analysis, Gene-act-network, co-expression network of differentiated expression genes were analyzed, and KLF17, CITED1, MPO, NPC2 were considered to be the possible genes involved in the mechanisms of BNC1 truncation mutation leading to premature ovarian aging.

Limitations, reasons for caution: The mechanism study between KLF17, CITED1, MPO, NPC2 and BNC1 regulation was limited, and need to be further investigated in our future research.

Wider implications of the findings: Our study describe the first time the effects and mechanisms of BNC1 truncation mutation in ovarian aging, which will contribute to improving female reproductive health with both scientific value and translational medical significance.

Trial registration number: No.

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O-232 Gonadotropin receptor variants are linked to pregnancy outcome in women undergoing in vitro fertilization

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[5]*Rigshospitalet, Laboratory of Reproductive Biology, Copenhagen, Denmark*

Study question: Can the gonadotropin receptor variants previously linked to pregnancy in the first *in vitro* fertilization (IVF) attempt also predict pregnancy in subsequent IVF cycles?

Summary answer: Women homozygous serine (S) in the follicle-stimulating hormone receptor (*FSHR*, N680S), and the luteinizing hormone receptor *(LHR*, N312S), had highest pregnancy rates in all IVF-cycles.

What is known already: Recently it was reported that women homozygous for S in aminoacids 680 and 312 of the FSHR and LHR, respectively, had four-fold increased pregnancy chance after their first IVF-cycle compared to women homozygous N. However, S-carriers required higher total dose of exogenous FSH before IVF compared to women with other genotypes, indicating decreased hormone sensibility for these women.

Study design, size, duration: Prospective, clinical study, in which n = 617 women were consecutively enrolled during the period 2007–2015, at Reproductive Medicine Center, Malmö, Sweden. Medical data were collected from records in 2016. The main outcome measures were age, stimulation regimens prior to IVF and pregnancy outcome after IVF.

Participants/materials, setting, methods: Inclusion criteria were: <40 years of age, body mass index <30 kg/$m^{2,}$ non-smoking, regular menstruation cycle of 21–35 days and bilateral ovaries. Leucocyte DNA was extracted and *FSHR* and *LHR* polymorphisms genotyped by allele specific PCR and direct sequencing of selected samples in order to verify PCR results.

Main results and the role of chance: Of the 617 originally included women, 370 (60%) completed a second IVF cycle and 217 (35%) continued to a third. Of these, 78 (13%) sustained 4–7 cycles. A majority of all participants (77%) were treated with follitropin alpha for ovarian hyperstimulation.

The allele frequencies were similar to previously reported, 30% homozygous N, 53% heterozygous N/S and 17% homozygous S in the FSHR gene. For the LHR, 16% were homozygous N/N, 46% were N/S, and 38% homozygous S/S.

Women homozygous S had significantly higher pregnancy rate compared to women with other variants also in subsequent IVF-cycles, being stastistically significant in the second (pregnancy incidence; 69% for S/S and 40% for N/N, p = 0,044) as well as in the second and third cycle combined, (47% for S/S and 35% for N/N carriers, p = 0.040). Significance was lost due to decreasing number of subjects in cycles 4–7.

Limitations, reasons for caution: Although the cohort used in this study was large, the vast majority of women were Caucasian. Other ethnic groups may show a different picture. Most women were treated with follitropin alpha and another result may be reached using other gonadotropin preparations and therapy modalities in relation to receptor genotype.

Wider implications of the findings: Genotyping of gonadotropin receptor variants are promising candidates for prediction of pregnancy and could in the future be used in an individualized stimulation strategy for women undergoing IVF.

Trial registration number: not applicable.

O-233 Prokineticin 1 (PROK1) is a new actor in human folliculogenesis

S. Brouillet[1,2], A. Gueniffey[1], C. Dunan-faure[1], L. Villaret[1], L. Azar[1], C. Douest[1], C. Lebayle[1], P. Martin[1], C. Perrot[1], P. Zavaroni[1], J. Bessonnat[1], N. Quenard[1], N. Alfaidy[2], P. Hoffmann[1,2], S. Hennebicq[1,3]

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Study question: To characterize the regulation of PROK1 secretion in follicular fluid (FF) throughout human folliculogenesis in *in vitro* fertilization (IVF).

Summary answer: In IVF, PROK1 secretion is significantly: 1) increased throughout late folliculogenesis, 2) up-regulated by gonadotropins (hCG and FSH), 3) down-regulated by smoking.

What is known already: Follicular fluid (FF) is an important component of the oocyte microenvironment influencing the acquisition of oocyte competence and the subsequent embryo implantation ability. Recent data from our laboratory reported that FF PROK1 measurement could be a new predictive biomarker of oocyte competence and embryo implantation potential in *in vitro* fertilization (IVF). On the basis of these observations, it was intriguing to investigate the regulation of its secretion in FF.

Study design, size, duration: A prospective study was performed between November 2014 and May 2016 in the Assisted Reproduction Unit, Department of Obstetrics and Gynecology, University of Grenoble, France. A total of 981 FF from 95 infertile couples undergoing IVF +/- ICSI were collected and analyzed for PROK1 concentration, follicular size, FSH starting dose, and smoking (*in vivo* study). Regulation of PROK1 secretion by FSH and hCG was investigated in primary follicular cells cultures (*in vitro* study).

Participants/materials, setting, methods: Samples were collected through CRB Germetheque (procedure approved by the institutional ethics review board, CPP Sud-Ouest). Signed informed consent was obtained from all patients who participated in the study. Follicular size (length and width) was measured by 2D-sonography during individual follicular puncture. Individual FFs were collected immediately after oocyte retrieval. Regulation of PROK1 secretion by gonadotropins was characterized in cumulus cell (CC) primary cultures. PROK1 concentration in FF was measured by enzyme-linked immunosorbent assay (ELISA).

Main results and the role of chance: PROK1 was detected in all individual FF samples with concentration ranging from 51 to 6,241 pg/ml (mean concentration of 2,186 ± 49 pg/ml). Follicles were divided into six groups according to their size (from 10 mm to 23 mm, mean size = 17.78 ± 0.11 mm). A significant increase of PROK1 concentration was observed with the growing size of follicles (n = 981, ANOVA, p = 10^{-14}). Moreover, individual FF PROK1 secretion was significantly increased in women with high FSH starting dose (>250 UI per day, n = 95, Mann-Whitney, p < 0.01). In CC primary cultures, FSH and hCG significantly increased PROK1 secretion (n = 15, Mann-Whitney, FSH, p < 0.001 and hCG, p < 0.001, respectively), probably *via* the AMPc pathway (n = 5, Mann-Whitney, Forskolin, p < 0.001). FF PROK1 secretion was significantly decreased in smokers (n= 95, Mann-Whitney, p < 0.01).

Limitations, reasons for caution: Extensive bleeding or concomitant aspiration of follicles could distort individual FF PROK1 concentrations. However, bloodstained FF and FF with unexpected volumes according to their size were discarded. Furthermore, PROK1 is up regulated in ovarian hyperstimulation syndrome (OHSS) and recurrent miscarriage. These conditions constitute two non-inclusion/exclusion criteria in our study.

Wider implications of the findings: PROK1 quantification in individual FF by ELISA is a quick and non-expensive test, which displays an appropriate sensitivity/specificity for clinical application. The long-term objective is to correlate individual FF PROK1 quantification with embryo implantation potential in order to evaluate its input in embryo transfer strategy.

Trial registration number: none.

O-234 Gonadotropin endocytosis as a biomarker of optimal FSH dosage – a randomised clinical trial

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Study question: Can standard practice of ovarian stimulation be optimised by choosing a starting FSH dose based on gonadotropin endocytosis *in vitro*?

Summary answer: Clinical results show that FSH dosage adjusted by gonadotropin endocytosis *in vitro* allows a more optimal outcome of ovarian stimulation compared to standard practice

What is known already: A key challenge in IVF is estimating the optimal FSH starting dose to obtain the desired ovarian response and prevent treatment

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complications like ovarian hyperstimulation syndrome. Peripheral blood monocytes are professional phagocytes that patrol and probe their environment by constitutive endocytosis. Gonadotropins are also internalised by monocytes and other phagocytes, consequently reducing the quantity that can reach target receptors in ovarian cells. We have invented a cell-based assay to measure FSH internalization by monocytes *in vitro*, and a nomogram to determine whether patients are in need of increased or reduced FSH doses

Study design, size, duration: This is an ongoing randomised controlled single-centre 2-year trial including 150 eligible patients undergoing IVF/ICSI allocated to FSH dosage by standard practice (control) or FSH dosage adjusted for gonadotropin endocytosis (intervention). The primary end point of the study is to assess standard deviation from optimal ovarian stimulation, *a priori* defined as 10 oocytes collected during treatment

Participants/materials, setting, methods: We recruited infertile women with tubal factor, male factor, or unexplained infertility, aged 18 – 38 years, BMI 18.5 – 33 kg/m^2, serum AMH 3 – 35 pmol/l. The patients' peripheral blood monocytes were isolated and the level of gonadotropin endocytosis *in vitro* was quantified by flow cytometry. Suggested adjustment of FSH dosage was calculated by a nomogram

Main results and the role of chance: Interim analysis was conducted when 81 patients have completed treatment, herein 35 randomised to intervention and 46 to control groups. Additional 49 patients have been excluded and 14 are dropouts. The geometric mean number of oocytes collected was 9.4 in the intervention and 6.8 in the control group, and 37% of women in the intervention group obtained 9 – 11 oocytes during stimulation, compared to 24% in the control group. Moreover, women in the intervention group achieved more consistent stimulation and less deviation from the a priori defined optimal stimulation (log-scaled $SD_{int} = 0.17$, $SD_{cont} = 0.33$, $F = 4.00$, $P = 0.00006$)

Limitations, reasons for caution: We excluded women with expected low or high response to stimulation, women with polycystic ovarian syndrome, endometriosis, autoimmune diseases, or other co-morbidities that may affect endocytosis by monocytes *in vitro*

Wider implications of the findings: Gonadotropin endocytosis by monocytes *in vitro* may reflect internalization of exogenous FSH by phagocytes, which may modulate the bioavailability of the drug during ovarian stimulation. Beyond predictors of ovarian reserve, like antral follicle count or serum AMH concentrations, gonadotropin endocytosis *in vitro* may help individualizing ovarian stimulation regimes for IVF

Trial registration number: ClinicalTrials.gov identifier: NCT02915900

SELECTED ORAL COMMUNICATIONS

SESSION 63: MODIFYING FACTORS IN RECEPTIVITY

Wednesday 5 July 2017 Room A 10:00–11:45

O-235 Live birth rate after oocyte donation is influenced by donor HLA-C: one step beyond conventional markers of success

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Study question: Do maternal KIR have an impact on pregnancy, miscarriage and live birth rates (LBR)/cycle in donor oocytes –ART by paternal and oocyte donor HLA-C?

Summary answer: The maternal KIR and parental donor HLA-C combination could predict which couple can benefit for donor selection by HLA-C in order to increase LBR.

What is known already: Increased risk of recurrent miscarriage (RM), preeclampsia, fetal growth restriction has been described in KIR AA mothers when the fetus has more HLA-C2 genes than the mother. Pregnancy disorders are predicted to reduce the frequency of KIR A/HLA-C2, and this selection is thought to have originated during human evolution. In ART oocyte donor cycles, oocyte HLA-C behaves as the paternal HLA-C and KIR-HLA-C combination is not presently taken into account during donors' selection. KIRAA women have lower live birth rates (LBR) after double embryo transfer (DET) in egg-donation ART cycles.

Study design, size, duration: Between April 2014 and September 2016, we performed a prospective study that included 201 women whose recurrent reproductive failure was of unknown etiology: recurrent implantation failure (RIF) (N = 113) and RM (N = 89), who had 138 oocyte donor-assisted reproductive (ART) transfers and 63 own oocyte transfers.

Participants/materials, setting, methods: All the patients were selected from IVI Clinics, and had normal karyotype, trombophilic, and immunological results. They had 201 embryo transfers (ET)(1 transfer/patient), of which 138 were with oocyte donation (57 DET, 81 SET). We performed genetic typing for maternal KIR and HLA-C, and for the HLA-C of their partners, oocyte and sperm donors, babies and product of conception after miscarriage. Pregnancy, miscarriage and LBR/transfer were studied by the maternal KIR haplotype and embryo HLA-C.

Main results and the role of chance: The median age of our patients was 40 years, and 25 years for oocyte donors.

In our cohort, 36.3% of women had KIR AA, 41.3% KIR AB and 22.4% KIR had the BB genotype.

Higher miscarriage rate/transfer after DET-oocyte donation was observed in KIRAA women (47.6%) compared with KIR AB (4.5%) and KIR BB (7.7%) ($p < 0.01$). Lower LBR/transfer was observed after DET-oocyte donation in KIR AA women (4.8%) compared with AB (22.7%) or BB (46.2%) ($p < 0.03$).

The study of LBR/transfer by maternal KIR and HLA-C and their embryos HLA-C revealed that LBR significantly lowered from 100% after transferring embryo HLA-C1 (N = 2) to 0% after transferring DET and HLA-C2 embryos (N = 11) in KIR AA women ($p < 0.000$). This trend was not observed in the KIR AB or BB patients.

LBR lowered in KIR AA patients as differences between embryo and mother HLA-C2 increased. When comparing both groups, i.e., Embryo HLA-C2 £ Mother HLA-C2 (group 1) and Embryo HLA-C2 >Mother HLA-C2 (group 2), a significantly higher LBR per transfer was noted in group 1 (57.1%) *vs.* group 2 (25%) ($p>0.01$) in the KIR AA women.

Limitations, reasons for caution: Our sample was small and this is the first report to observe differences in LBR by oocyte donor/embryo HLA-C in KIR AA mothers. However, apart from statistical significance, the association strength was noticeably high, which confers the findings more confidence.

Wider implications of the findings: We speculate that completing normal pregnancy is possible only for those KIR AA mothers who carry a baby with a least one non-self HLA-C1. Therefore, selecting HLA-C1 amongst oocyte and/or sperm donors for KIR AA patients who undergo egg donation could be more efficient and safer.

Trial registration number: not applicable since no intervention was made.

O-236 Uterine natural killer (uNK) cell number in the peri-implantation endometrium of normal fertile women and women with recurrent reproductive failure

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Study question: What is the difference in uNK cell number between fertile women and women with recurrent miscarriage (RM) and women with recurrent implantation failure (RIF)?

Summary answer: The number of uNK cells in women with RM and women with RIF was significantly higher than it in fertile controls.

What is known already: Some studies have demonstrated an increase in number of uNK cells from women with RM and/or women with RIF, while other studies found no difference between these groups. The controversy may arise from the relatively small sample size of the fertile control cohort and the lack of standardized methodology for uNK cell counting in the earlier studies.

Study design, size, duration: A total of 189 women from 3 university centres participated in the study, including 68 fertile women, 89 women with RM and 32 women with RIF. All endometrial biopsies were collected precisely on day LH+7 of the cycle

Participants/materials, setting, methods: Endometrial sections were immunostained for CD56 to identify uNK cells. Image capture and cell counting were performed by a standardised protocol agreed and published by the participating centres. Results were expressed as percentage of positive uNK cell/total stromal cells.

Main results and the role of chance: The number of uNK cells in women with RM and women with RIF was significantly higher than the fertile controls (RM: median 3.99%, range 0.52-8.58%; RIF: median 3.61%, range 0.68-8.27%; control: median 2.64%, range 0.73-5.32%). Using 5th and 95th percentile to define the lower and upper limits of uNK cell numbers in fertile controls, the reference range was from 1.12% to 4.55%. In samples from women with RM, 24% (21/89) of them were above the range while 13% (12/89) below the range. In women with RIF, 11 out of the 32 women (34%) had uNK cells count above the range and 4/32 (13%) below the range.

Limitations, reasons for caution: The prognostic value of uNK cell count measurement has yet to be confirmed.

Wider implications of the findings: Up to date, there is limited data on whether "low" uNK cell number correlate with adverse pregnancy outcomes. An established reference range for uNK cell count by standardised method should enable progress in this field.

Trial registration number: N/A.

O-237 Comparison of ectopic pregnancy risk among transfers of embryos vitrified on day 3, day 5 and day 6

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Study question: What's the relationship between the vitrification day of the embryos transferred and the subsequent risk of ectopic pregnancy (EP) in frozen-thawed embryo transfer (FET) cycles?

Summary answer: Transfers of day 6 vitrified embryos had a significantly lower risk of EP both than transfers of day 3 and day 5 vitrified embryos.

What is known already: EP is a leading cause of maternal mortality among the first trimester worldwide with unelucidated pathogenesis. Theoretically, blastocyst transfers could lower the likelihood of EP compared with cleavage stage embryo transfers, but results from reported clinical data varied a lot, and few studies compared EP rate between day 6 and day 5 or day 6 and day 3 vitrified embryo transfers.

Study design, size, duration: This was a retrospective cohort study involving a total of 11,204 pregnancies following FET cycles of in vitro fertilization/intracytoplasmic sperm injection from March 2003 to May 2015.

Participants/materials, setting, methods: EP rate was compared by patient and treatment characteristics, including maternal age, full-term birth history, EP history, Fallopian tubal surgery history, tubal infertility, male infertility, endometrial preparation, endometrial thickness on embryo transfer day, year of treatment, number and vitrification day of embryos transferred. Thereafter, we used goodness of fit to specify the best fitted generalized estimated equation regression models to calculate unadjusted and adjusted odds ratios for the association between EP and selected characteristics.

Main results and the role of chance: The overall rate of EP was 2.8%. In the univariate analysis, patients with EP history, Fallopian tubal surgery history and tubal factor infertility were associated with a significantly elevated proportion of EP compared with those without. In contrast, patients with male factor infertility had a significantly decreased rate of EP than those without. Additionally, EPs were less common among women who had a >11mm endometrium on the day of embryo transfer compared with those having a thinner endometrium. As to vitrification day of embryos transferred, EPs were more likely when day 3 and day 5 vitrified embryos were transferred than transferring day 6 vitrified embryos (3.1% vs. 1.9% vs. 0.6%, P<0.001). In the adjusted analysis, EP history (adjusted odds ratio [AOR] 1.62, 95% confidence interval [CI] 1.18-2.23), Fallopian tubal surgery history (AOR 1.35, 95% CI 1.04-1.76) and tubal factor infertility (AOR 2.52, 95% CI 1.67-3.81) were significantly associated with increased risk for EP. Besides, day 6 vitrified embryo transfers had a significantly lower risk for EP both than day 3 (AOR 5.29, 95% CI 2.35-11.91) and day 5 (AOR 2.88, 95% CI 1.05-7.87) vitrified embryo transfers.

Limitations, reasons for caution: The data were not derived from multiple centers. Moreover, female smoking habits were not available in the database, and the amount of day 5 vitrified embryo transfers was still limited.

Wider implications of the findings: The reduced EP risk in day 6 vitrified embryo transfers both than in day 3 and day 5 vitrified embryo transfers indicates embryo factors may play important roles in the pathogenesis of EP, and more individualized timing of embryo transfer in FET cycles may be needed to decrease EP risk.

Trial registration number: Not applicable.

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O-238 The impact of granulocyte colony stimulating factor on thin endometrium of an animal model with rats

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Study question: Is it possible to improve endometrial thickness of thin endometrium by influencing proliferative, angiogenic and apoptotic factors with granulocyte colony stimulating factor (G-CSF) injection?

Summary answer: G-CSF significantly improved endometrial thickness along with angiogenic, proliferative and apoptotic factors that crucial for implantation.

What is known already: Thin endometrium (≤ 7 mm) unresponsive to ovarian hyperstimulation is associated with decreased pregnancy rates after IVF. In an experimental animal model, G-CSF improved endometrial thickness and morphology by immunohistochemistry and western-blot with cytokeratin and vimentin. In clinical small case series of thin endometrium G-CSF administrations between the days of ovulation trigger and embryo transfer was found to be effective on pregnancy rates regardless significantly improvement of endometrial thickness

Study design, size, duration: An experimental rat model was conducted to evaluate the effect of G-CSF injection on endometrial thickness and expression of endometrial angiogenic, proliferative, and apoptotic markers. For this purpose, a total number of 24 female adult rats with either thin (12) or normal (12) endometrium were used. Each group was further divided into G-CSF or saline injection groups with 6 rats

Participants/materials, setting, methods: 2 estrous cycles after forming of thin endometrium by uterine injection of 0.2 ml 96% ethyl alcohol to 200-250 g weighing Sprague Rawley® rat models, 5 days of subcutaneous injections of 40 μg/kg G-CSF (Nuepogen) or saline were given. Endometrial thickness,

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immunohistochemically expression of vascular endothelial growth factor receptor-2 (VEGF-R2), proliferative cell nuclear antigen (PCNA) and fibronectin; apoptosis with TUNEL method were compared in specimens of hysterectomies among 4 groups of post-model rats.

Main results and the role of chance: Endometrial thickness was significantly improved with G-CSF in thin endometrium but not in normal endometrium group when compared to saline injection (239 ± 158.4 vs 151 ± 167, p = 0.025, 283.6 ± 189.1 vs 282.6 ± 94.9, p = 0.309, respectively). In thin endometrium groups, expression of PCNA (10.56 ± 4.25 vs 2.31 ± 2.01, p < 0.001) in stromal and glandular epithelium and pericapillary VEGF-R2 (3.11% vs 1.84%, p = 0.037) was significantly increased and apoptosis was significantly decreased with G-CSF when compared to saline injection (16.97 ± 4.42 vs 28.50 ± 7.92, p < 0.001). Fibronectin was also increased between G-CSF and saline in thin endometrium, but the difference was not statistically significant (0.79% vs 0.71%, p = 0.071), However, in normal endometrium, any maker was not improved by G-CSF when compared to saline injection.

Limitations, reasons for caution: The main limitation of this study is being an experimental rat model and the need of further clinical studies for confirmation of the results.

Wider implications of the findings: G-CSF improves endometrial thickness, proliferation, angiogenesis and DNA fragmentation in thin endometrium and in the light of these finding it may also have a role in recurrent implantation failure

Trial registration number: not applicable.

O-239 Live birth rate according to different treatment strategies in TPOAb-positive women with recurrent pregnancy loss

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Study question: Do positive thyroid peroxidase antibodies (TPOAbs) affect pregnancy outcome in women with recurrent pregnancy loss (RPL) and, if so, is this ameliorated by treatment with immunotherapy or thyroxine replacement therapy?

Summary answer: TPOAbs were not associated with pregnancy outcome. However, thyroxine replacement caused a trend towards a significant increase of live birth rate in TPOAb-positive women.

What is known already: Thyroid autoimmunity may be associated with pregnancy loss. A possible mechanism behind this is overactivation of the maternal immune system, which causes rejection of the fetal allograft. Studies of TPOAb-positivity in women with RPL are few and of limited sample size.

Study design, size, duration: Prospective cohort study of 900 women with RPL followed at the RPL Unit, Copenhagen University Hospital, from 2011-2016.

Participants/materials, setting, methods: Recurrent pregnancy loss was defined as ≥3 losses. Upon referral, women were screened for TSH (Roche Modular E170 electrochemiluminescense immunoassays) and TPOAbs (automated Kryptor immunoflourescent assay). TPOAb-positivity>60 kU/L. We performed test for trends by chi-square or independent t-tests as appropriate, and subsequently adjusted regression analyses including as covariates: maternal age at referral, TSH (logtransformed), total number of losses, immunotherapy, and thyroxine replacement therapy. The National Data Protection Agency approved the project (2017-41-5005).

Main results and the role of chance: We included 5219 pregnancies (76.6% spontaneous) in 900 women. 119 (13.2%) women were TPOAb-positive. TPOAb-positivity was neither associated with number of losses (p = 0.87, aOR 0.004(-0.3-0.3), p = 0.98), nor with number of pregnancies (p = 0.62, aOR 1.02(0.92-1-11) p = 0.76). Among TPOAb-positive women, TPOAb-concentration was not associated with number of losses (B=0.75 (-0.12-0.27), p = 0.45).

In women with a registered first pregnancy after referral, 72 of 557 (12.9%) were TPOAb-positive. Excluding women with extrauterine pregnancies and provoked abortions (n = 65), TPOAb-positivity was not associated with live birth rate (54.4% vs 60.6%, p = 0.35, aOR 0.98(0.5-1.9) p = 0.94). Total number of losses was the only predictor of live birth rate (aOR 0.54(0.45-0.66) p = 0.000). TSH-levels were positively associated with TPOAb-positivity (p = 0.00), but not with any investigated outcome. A total of 199 (40.4%) women received immunotherapy and 30 (6.6%) women received thyroxine replacement therapy. Live birth rate was not significantly affected by treatment with immunotherapy (59.3% vs 60.1%, p = 0.93, aOR 1.17(0.77-1.78), p = 0.45) or thyroxine replacement therapy (63.3% vs 59.5%, p = 0.85, aOR 1.69(0.67-4.30), p = 0.27). However, in TPOAb-positive women, live birth rate after treatment with thyroxine replacement therapy was 68.4% as compared to 49.0% in non-treated women (p = 0.18, aOR 3.2(0.89-11.55) p = 0.08). Immunotherapy did not alter live birth rate (53.6% vs 55.0%, p = 1.0, aOR 0.92(0.31-2.71) p = 0.89).

Limitations, reasons for caution: Although this study to our knowledge is the largest to date of women with RPL, there may be a lack of power in calculations of association between live birth rate and treatment strategies.

Wider implications of the findings: Treatment of TPOAb-positive women with thyroxine replacement (but not immunotherapy) caused a trend towards a significant increase of live birth rate. Results from multicenter intervention studies are awaited. If RPL is caused by an immunological reaction, thyroid autoimmunity seems to be neither the cause nor a sensitive marker hereof.

Trial registration number: Not applicable.

O-240 Is there a role for immunotherapy in IVF and recurrent miscarriage: a systematic review and meta-analysis

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Study question: What is the current evidence on the role of immunotherapy in IVF and in the management of recurrent miscarriage (RM)?

Summary answer: Current literature does not support the use of the immunotherapy for IVF outcomes or in the prevention of the RM.

What is known already: Imbalance within the immune system has been postulated to be one of the causes for adverse IVF outcomes and RM. Within this context, immunotherapy is presumed as the next logical step with an aim to improve pregnancy outcomes in these women. Various interventions to improve uterine receptivity, enhance implantation and prevent early miscarriages have been suggested. However, the benefit of using immunomodulators is still unclear. The objective of this systematic review is to summarize objectively the efficacy of immunotherapy in IVF and RM.

Study design, size, duration: We conducted a systematic review to assess the efficacy of commonly used immunomodulators such as: intravenous use of (1) immunoglobulin (IVIG), (2) lymphocyte immunotherapy (LIT) and (3) intralipid; intrauterine infusion of (4) granulocyte-colony stimulating factor (G-CSF) and (5) peripheral blood mononuclear cells (PBMC); subcutaneous administration of (6) TNF-alpha (TNF-α) inhibitors, (7) leukaemia inhibitory factor (LIF); and oral administration of (8) glucocorticoids. A literature search was performed using MEDLINE, PUBMED and EMBASE until December 2016

Participants/materials, setting, methods: Only randomized controlled trials (RCTs) were included and a meta-analysis was carried out where appropriate. Case-control or cohort studies were considered but not included in the meta-analysis. Participants included women undergoing IVF treatment with or without a previous history of recurrent implantation failure (RIF) and women with RM. The primary outcome was live birth rate (LBR); secondary outcomes were clinical pregnancy rate (CPR) and miscarriage rate (MR).

Main results and the role of chance: Meta-analysis of two RCTs that evaluated IVIG as the sole intervention in women with RIF undergoing IVF treatment showed no significant difference in the CPR and LBR between the IVIG and

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placebo control group (RR 0.92; 95% CI 0.26, 3.19; p = 0.89 and RR 1.79; 95% CI 0.67, 4.79; p = 0.25 respectively). Four RCTs evaluated the role of steroids in women undergoing IVF (heterogeneous population) showed no difference rate in CPR (RR 1.3; CI 0.90, 1.86; p = 0.16) or MR (RR 1,2; CI 0.25, 5.85, p= 0.83) versus the control group.

A total of 11 RCTs were analysed for the use of immunomodulators in women with RM. The study evaluating the role of G-CSF showed a significant improvement in the LBR compared to the control group (RR 5.14; CI 1.69, 15.63; p =0.004). Two studies evaluated LIT in women with RM, showing no significant difference in the LBR (RR 3.48; CI 0.19, 65.26; p = 0.4). Eight RCT's assessed IVIG treatment in RM showing no difference in LBR (RR 1.35; CI 0.92, 1.98; p = 0.13), CPR (RR 0.96; CI 0.54, 1.71; p = 0.90) or MR (RR 0.79; CI 0.5, 1.25, p = 0.31) versus the control group.

Limitations, reasons for caution: Limited number of RCTs with limited sample size are present in literature. Both RCTs and case-controlled studies are heterogeneous. Preparations, procedures, sample populations and outcomes vary across studies.

Wider implications of the findings: This study did not show a role for immunotherapy in the prevention of RM or in women undergoing IVF treatment. Immunotherapy should only be used in the context of research and should not be used in routine clinical practice to improve reproductive outcomes.

Trial registration number: Not required.

O-241 HLA-DR and –DQ alleles in Danish women with unexplained recurrent pregnancy loss and controls

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Study question: Are particular HLA-DRB1 alleles and especially HLA-DRB1*03 associated with recurrent pregnancy loss (RPL) in a Caucasian population?

Summary answer: HLA-DRB1*03 was borderline associated with RPL in this study. Combining three Danish case-control studies, HLA-DRB1*03 was significantly increased in RPL (p = 0.002).

What is known already: In two previous smaller studies, we found that the HLA-DRB1*03 allele is more frequent in Caucasian women with unexplained RPL than in controls. This HLA allele is associated with several autoimmune diseases. However, other studies have not confirmed this association.

Study design, size, duration: The study was a case-control study including 1078 RPL patients consecutively referred between January 2003 and May 2016 and 230 consecutive normal women referred for routine scan in pregnancy.

Participants/materials, setting, methods: HLA-DRB1 typing and in subsets of patients also HLA-DQB1 typing by DNA technology were done in Caucasian women with unexplained RPL (3 or more consecutive early pregnancy losses without uterine abnormalities, parental chromosomal aberrations or oligomenorrhea). The patients had been referred to the RPL clinic in Copenhagen. Similar HLA typing was done in Caucasian control women with a least one normal birth and maximum one pregnancy loss referred for routine ultrasound scan in mid-gestation.

Main results and the role of chance: The HLA-DRB1*03 allele was found borderline significantly increased in RPL patients compared with controls (Odds Ratio [OR] 1.39; 95% CI 0.99-1.93), p = 0.053. HLA-DRB1*07 was found with increased frequency in RPL compared with controls (OR = 1.56; 1.09-2.22), p = 0.015. Most HLA-DRB1*07 alleles are found on the HLA-DRB1*07-DQB1*02 haplotype and in order to investigate whether the DQB1*02 allele is the common susceptibility allele for RPL, investigation of HLA-DQB1 was undertaken in HLA-DRB1*07 positive women. This did not reveal any significant difference between the two groups.

The frequency of the HLA-DRB1*04 was significantly decreased in patients compared with controls (OR = 0.70; 0.55-0.89), p = 0.004. When comparing the frequency of HLA-DRB1*04 homozygous patients and controls, the difference became even stronger (OR = 0.45; 0.24-0.83); p < 0.01).

The frequencies of no other HLA-DRB1 allele was different between the two groups. There was no clear correlation between primary/secondary RPL status or number of pregnancy losses and presence of specific HLA alleles except for HLA-DRB1*03, which were more frequent in patients with ≥4 pregnancy losses.

In a meta-analysis of results from three Danish case-control studies, the combined OR of HLA-DRB1*03 in RPL was 1.42 (1.14-1.77), p = 0.002.

Limitations, reasons for caution: The number of RPL patients was the largest in any similar study so far; however, the number of controls was limited. Whereas the association of HLA-DRB1*03 to RPL was hypothesized before the study, the findings regarding HLA-DRB1*07 and HLA-DRB1*04 were new and must be confirmed in further studies

Wider implications of the findings: The clear association between HLA-DRB1*03 and RPL (and maybe other HLA-DRB1 alleles) strengthens suggestions that immunologic disturbances play a significant role in RPL since autoimmune diseases are known to be strongly associated with particular HLA-DRB1 alleles.

Trial registration number: None

SELECTED ORAL COMMUNICATIONS
SESSION 64: PATTERNS OF GENETIC DIVERSITY

Wednesday 5 July 2017 Room B 10:00–11:45

O-242 Profiling of human spermatozoal non-coding RNA to determine the male contribution to embryonic implantation potential

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Study question: To identify vital sperm-specific noncoding RNA (ncRNA) in men with unexplained infertility and normal semen parameters that can contribute to the non-implantation of good-quality embryos.

Summary answer: Underexpression of regulatory sperm-specific methylation ncRNAs may contribute to dysfunction in de-novo embryo methylation at the time of blastulation leading to decreased implantation potential.

What is known already: NcRNAs serve as functional and regulatory RNA molecules, which are transcribed from DNA, but do not undergo translation into proteins. Recent evidence suggests that sperm-specific ncRNAs may contribute to the reproductive potential of the male gamete by regulating post-fertilization embryonic development. One mechanism through which this is achieved is via increased genomic methylation at the blastocyst stage. It is posited that some men may not be able to achieve a pregnancy even in the presence of a normal semen analysis due to dysregulation of vital regulatory ncRNAs involved in embryonic development.

Study design, size, duration: Thirty-one consenting men undergoing infertility screening donated their ejaculated samples. RNA-sequencing (RNA-seq) was performed on these specimens, with special emphasis on identifying ncRNAs of interest i.e., small noncoding RNA (sncRNA) and long noncoding RNA (lncRNA). Following RNA-Seq, the expression of sncRNAs and lncRNAs was compared between infertile men who did not achieve a pregnancy with ART and a control group of 3 fertile men who were able to conceive naturally with their female partners.

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Participants/materials, setting, methods: RNA was isolated from 25x10^6 spermatozoa using a commercial spin column kit (RNeasy Mini Kit, QIAGEN, Germany). The spermatozoal nucleic acid quality and concentration were measured. After creation of paired-end libraries from RNA samples, 76 bp RNA-Sequencing (RNA-Seq) using an Illumina platform (NextSeq 500) was carried out and expanded to 60 M reads. Expression values were calculated in fragments per kilobase of transcript per million mapped reads (FPKM).

Main results and the role of chance: The 31 consenting men with a mean age of 39.6 ± 5 years had the following semen parameters: 46.3 ± 19 × 10^6/mL (concentration), 44.8 ± 14% (motility), and normal morphology. Five (68.9%) men, with female partners aged 34.8 ± 1 years, underwent ART that resulted in a fertilization rate of 71.4% and generation of 2.44 ± 1 good-quality embryos on day 3. However, no implantation of embryos occurred. There was no difference in the age demographics of men or women in the ART or control groups. RNA-seq of approximately 23,260 genes was performed, of which 28 ncRNAs showed decreased expression in the ART group. These 28 ncRNAs were classified into 7 (25%) sncRNAs and 21 (75%) lncRNAs. Interestingly, the expression of 4 ncRNAs (*SNORD68*, *SNORD104*, *AVP*, *ALOX15P1*) involved in methylation was completely absent in the ART group ($P < 0.001$). The expression of 5 other ncRNAs (*MIR181C*, *MIR636*, *MIR3687*, *MIR3689B*) involved in the stabilization of messenger RNA was completely decreased in the ART group compared to controls ($P = 0.08$). Of note, the majority of ncRNAs (81.3%) were products of genes located on autosomes, while the remaining ncRNAs were coded by genes located on the sex chromosomes.

Limitations, reasons for caution: The under or absent expression of major sncRNAs and lncRNAs in men with normal semen parameters but unexplained infertility needs to be confirmed prospectively in a larger cohort. Also, the molecular mechanisms by which dysregulation of sperm-specific ncRNAs involved in methylation impact the implantation potential of embryos needs further elucidation.

Wider implications of the findings: RNA-Seq of vital sperm-specific ncRNAs can provide crucial information about the epigenetic etiology of unexplained infertility in men with normal semen parameters, and may also reflect the overall contribution of the human spermatozooan to embryonic development and establishing a viable pregnancy.

Trial registration number: Not applicable.

O-243 Identification of extracellular vesicles in human oviductal fluid and small non-coding RNAs profiling

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Study question: Are there extracellular vesicles/exosomes in the human oviductal fluid and which is its composition?

Summary answer: Extracellular vesicles were identified in human oviductal fluid and small non-coding RNAs profile was analyzed in individual samples of human oviductal fluid.

What is known already: Extracellular vesicles (EV) in oviductal fluid were firstly reported in mouse. They carry and deliver to sperm the plasma membrane Ca^{2+}-ATPase4, which participate in the Ca^{2+} efflux pump and play an essential role in sperm fertilizing ability (Al-Dossary, et al 2013). Later, other studies reported the presence of oviductosomes in different species, showing that, in cattle, the addition of OVS from the isthmic region to the in vitro culture system without serum affects gene expression (with upregulation of factors such as AQP3, DNMT3A and, SNRPN), and improves the development and quality of the produced embryos (Lopera-Vasquez et al., 2017).

Study design, size, duration: 8 oviductal human fluid samples were collected individually from premenopausal women with benign uterine pathology submitted to abdominal hysterectomy in University Hospital Virgen Arrixaca, by direct aspiration of the tubal content with an automatic 200 µl pipette. Two of them were analyzed by western-blot, 2 were analyzed by nanoparticle tracking analysis (Nanosight) and 4 pairs oviductal fluid-serum were analyzed by small nc-RNA-seq (50 bp single end Illumina HiSeq NGS).

Participants/materials, setting, methods: After centrifugation of the samples, cellular debris was discarded and extracellular vesicles were isolated from the supernatant using the Exoquick exosome precipitation kit (SBI System Biosciences, Inc.) according to manufacturer´s instruction. Similarly, EVs were isolated from serum samples for the pairs oviductal fluid-serum samples.

Main results and the role of chance: Western blot analysis revealed the presence of CD9 and CD63 in the isolated EV from oviductal fluid. Nanoparticle tracking analysis showed the existence of vesicles with a size between 150-177 nm, displaying a Brownian motion with a concentration between 6.25x10^{11} to 1.48 × 10^{12} particles/ml. Concentration of small RNA isolated from individual was between 131-290 (ng/µl), with an average size of 29 nucleotides in the miRNA fraction. After RNA-sequencing the number of alignments were higher than 11,5 millions for all samples, with a percentage of reads aligned between 87.85 to 93.80 %. From RNA-seq data 22 categories of small non-coding RNAs were identified, including miRNAs (micro RNAs) and piRNAs (piwi-interacting RNAs). From miRNAs, some elements such as miR-126, miR-148, miR-152, miR-185, miR-21 and miR-29, with previously described functions on epigenome by targeting DNA methyltransferases DNMT1 and/or DNMT3A/3B were identified. Relevant differences for the level of expression were detected for some factors between oviductal fluid and serum.

Limitations, reasons for caution: A limited number of samples were analyzed and individual variation between samples could mask some additional differences between serum and oviductal fluid.

Wider implications of the findings: Results showed that extracellular vesicles are present in human oviductal fluid, and those vesicles contain at least 22 types of small non coding RNAs, showing a specific profile compared with serum from the same patients.

Trial registration number: Not applicable.

O-244 Investigation of possible incompatibilities between the mitochondrial and nuclear genomes that affect preimplantation embryo development

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Study question: Do particular haplotype combinations between mitochondrial and nuclear encoded genes involved in mitochondrial function lead to a mismatch affecting human embryo development?

Summary answer: Comparison of nuclear genes associated with the ETC identified SNPs in the *COQ9* gene in embryos with poor preimplantation development in three different families.

What is known already: Maternal factors that may lead to abnormal preimplantation development include mitochondria. Proteins required for mitochondrial function are encoded by both mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA) necessitating the coordination between the two genomes. Mitochondrial-nuclear mismatch has been proposed as a cause of embryonic cell death. Changes in ATP synthesis and metabolic reactions in the mitochondrial electron transport chain (ETC) have been linked to female infertility. This study explores the effect of different combinations of mtDNA haplotypes with 53 nuclear encoded genes that have mitochondrial function, by assessing preimplantation embryo development.

Study design, size, duration: This was a cohort study including two groups. The first group was made up of 11 fertile couples undergoing PGD for single gene disorders and their corresponding embryos (n = 57). The embryos were collected on day 6-7 post fertilization. The other group was composed of 12 couples with repeated miscarriage (RM) for unknown reasons.

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Participants/materials, setting, methods: Maternal and paternal mtDNA was analysed from 11 fertile and 12 couples suffering repeated miscarriage (RM) by Long range PCR and NGS. Each sample was assigned a mitochondrial haplogroup using HaploGrep and confirmed by EMMA software. The selected 53 nuclear genes were sequenced from parental genomic DNA and from embryos following whole genome amplification by MDA using SureSelect QXT from Agilent. Nuclear DNA genotypes that only occurred in the embryos with poor morphology were identified.

Main results and the role of chance: Several mitochondrial haplogroups were identified. Overall more couples from the repeated miscarriage group (4/12) had identical mtDNA haplotypes between partners, compared to the fertile group (1/11). Within the fertile group the presence of the T haplogroup in one of the partners was associated with poor blastocyst formation. Analysis of sequences in embryos from the selected nuclear genes identified 3 SNPs in the *COQ9* gene in embryos with poor preimplantation development in three different families. In these families, mitochondrial haplogroup T, was identified in the male partner. Although mitochondria from the male partner are not present in the embryo, we considered the male mitochondrial haplogroup as a surrogate marker for possible combinations of nuclear genes that could be transmitted to the embryo leading to a mitochondrial nuclear mismatch. This analysis suggests that the nuclear background (specifically SNPs on *COQ9*) where one partner has mitochondrial haplogroup T may contribute to poor embryo development. Other studies have suggested that haplogroup T is associated with poor sperm motility and fertilisation. Whilst most of the mitochondrial haplogroups T were present in the male partner in our study, all fertilization was achieved following ICSI, therefore sperm motility was not a factor in our observation.

Limitations, reasons for caution: The main limitation is the small number of samples due to the difficulty in obtaining human embryos. Also, many SNPs where filtered to avoid the possibility of ADO or because they were non-infomative. Another limitation is the difficulty in performing functional analysis on the same embryo.

Wider implications of the findings: COQ9 is necessary for the synthesis of Ubiquinone; a lipid found in most biological membranes and a co-factor in many redox processes including mitochondrial ETC. Changes in the sequence of *COQ9* may affect ubiquinone synthesis disturbing mitochondrial ATP production, increasing oxidative stress and apoptosis affecting many processes including embryogenesis

Trial registration number: not applicable.

O-245 Effect of FSHR gene polymorphism on the rate of chromosomal abnormalities in blastocysts

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Study question: Is the rate of chromosomal abnormalities in human blastocysts influenced by different alleles of the c.2039 G>A (p.Ser680Asn) polymorphism in the FSH receptor gene (FSHR)?

Summary answer: We have found that patients who are not carriers of the risk allele (Asn) have the highest rate of normal embryos.

What is known already: The risk alele (Asn) of studied FSHR gene polymorphism is linked to the ovarian response to stimulation by FSH and the risk of ovarian hyperstimulation syndrome (OHSS). In vitro studies show, that high levels of FSH during oocyte maturation increase aneuploidy in resulting oocytes and it has also been shown, that higher intrafollicular FSH is associated with oocyte aneuploidy. But it is not known if individual differences in ovarian response and the occurence of OHSS are linked to the aneuploidy rate in clinical IVF cases possibly through different sensitivity of the oocytes to the FSH stimulation depending on the polymorphism.

Study design, size, duration: This is a retrospective study from the years 2012-2016. We have analyzed data from 284 cycles with PGS performed in 224 female patients. There were 42 cycles with 109 blastocysts in Ser/Ser homozygotes, 159 cycles with 449 blastocysts in Ser/Asn heterozygotes and 83 cycles with 233 blastocysts in Asn/Asn homozygotes.

Participants/materials, setting, methods: We have compared the results of preimplantation genetic screening of aneuploidy (PGS) using microarray comparative genomic hybridization (aCGH) on trophectoderm samples in patients divided according to the results of molecular genetic examination of the FSHR gene Ser680Asn polymorphism using High Resolution Melting (HRM).

Main results and the role of chance: The groups were not significantly different with regards to the age (Ser/Ser: average age 36 years, Ser/Asn: 36.8 years, Asn/Asn: 36.2 years, Kruskal-Wallis test, $p = 0.3$).The rates of normal embryos were in the Ser/Ser group: 57 % (95% confidence interval: 47-66 %), in Ser/Asn: 44 % (39-48 %) and in Asn/Asn 48 % (42-54 %). The differences in the proportions are significant (Fisher's Exact Test, $p = 0.04$). Patients with Ser/Ser genotype had the highest proportion of normal blastocysts. Our results support the hypothesis, that variants in the FSHR gene might influence the risk of aneuploidy.

Limitations, reasons for caution: We have not found any further decrease in the rate of normal embryos in Asn/Asn homozygotes compared to Ser/Asn heterozygotes.

Wider implications of the findings: Elucidating the role of individual genetic variants in the response to the controlled hyperstimulationovarian stimulation including the rate of chromosomal abnormalites in oocytes and embryos might lead to better and more personalized treatments.

Trial registration number: not applicable.

O-246 Luteinizing hormone beta gene polymorphism affects clinical pregnancy rate after in-vitro fertilization and embryo transfer in GnRH antagonist cycles

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Study question: Does luteinizing hormone (LH) beta gene polymorphism affect the outcome of in-vitro fertilization (IVF) and embryo transfer (ET)?

Summary answer: Clinical pregnancy rate after IVF-ET is lower in patients with LH beta gene variant in GnRH antagonist cycles.

What is known already: Trp8Arg polymorphism of LH beta gene is common and shows decreased bioactivity in vivo. Previous studies showed association between LH beta variant protein and hypo-sensitivity to exogenous FSH in controlled ovarian hyperstimulation (COH) for IVF-ET. In another study, carrier frequency of LH beta gene polymorphism was 11% (4 out of 36 patients) in poor ovarian response group, while carrier frequency in normal response group was 17% (17 out of 98 patients). Therefore, further studies are needed to evaluate the effect of LH beta gene polymorphism on the outcome of IVF-ET.

Study design, size, duration: In this retrospective cross sectional study, 591 patients who had undergone IVF-ET at Maria Fertility Hospital during 2003-2016 were recruited. Among them, 289 patients had undergone COH with GnRH antagonist protocol and 302 patients had long GnRH agonist cycles.

Participants/materials, setting, methods: Trp8Arg polymorphism of LH beta gene was analyzed in subjects using realtime TaqMan assay. Patients with wild type allele of LH beta gene were regarded as noncarrier group and those with variant allele(s) as carrier group. Age, basal FSH, AMH, number of retrieved oocytes, total dose of gonadotropin, duration of COH, serum estradiol on hCG day, pregnancy rate and clinical pregnancy rate were compared in both groups.

Main results and the role of chance: Variant LH genotype was found in 70 (11.8%) patients out of 591 subjects: 67 hetrozygous and 3 homozygous. In GnRH antagonist cycles, basal characteristics including age, basal FSH and AMH were comparable between carrier and noncarrier groups. Numbers of retrieved oocytes were similar in carrier and noncarrier groups (11.4 ± 7.9 vs. 10.4 ± 6.3, respectively, $p = .377$). Clinical pregnancy rate per embryo transfer was significantly lower in carrier group than that in noncarrier group (18.9% vs.

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37.1%, respectively, p = .030). Other parameters including total dose of gonadotropin, duration of COH and serum estradiol on hCG day were comparable in both groups. In long GnRH agonist cycles, basal characteristics including age, basal FSH and AMH were comparable between carrier and noncarrier groups. Numbers of retrieved oocytes were similar in carrier and noncarrier groups (11.8 ± 7.0 vs. 11.8 ± 5.9, respectively, p = 0.996). Clinical pregnancy rate per embryo transfer (45.2% vs. 38.4%, respectively, p = 0.467) were similar in carrier and noncarrier groups. Other parameters including total dose of gonadotropin, duration of COH and serum estradiol on hCG day were comparable in both groups.

Limitations, reasons for caution: Because of the retrospective nature of this study, further study is necessary to apply this result to larger population. In our study, COH was done with exogenous FSH or hMG. Homogenous exogenous gonadotropin use might clarify the effect of LH beta gene polymorphism on IVF-ET outcome.

Wider implications of the findings: Common LH beta gene variant was associated with lower clinical pregnancy rate in GnRH antagonist cycles but not in long GnRH agonist cycles. Evaluating single nucleotide polymorphism might be applied to individualize COH protocol to each patient in the future.

Trial registration number: Not applicable.

O-247 A Deep Dive into the Human Blastocyst: Correlating the Embryo Transcriptome with Preimplantation Genetic Screening Results and Embryo Morphology

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Study question: Can gene sets predict human embryo sex and morphology from RNA expression data and, subsequently, can the embryo's transcriptome be analyzed through single cell technology?

Summary answer: Embryo sex can be identified reliably from the RNA transcripts of whole embryos and trophectoderm biopsies. Identified gene sets correlate with embryo quality.

What is known already: Genomic analysis is being increasingly utilized as a clinical test for In Vitro Fertilization with aims of increasing implantation rates per embryo transfer. This tool has not yet enabled the sole selection of competent human embryos, prompting investigation of other techniques. Single cell RNA sequencing has been studied with marked improvement in techniques requiring very low input of nucleic acids, enabling application to analysis of human embryos. Deep sequencing analysis of the embryo transcriptome has the potential to unearth details about coding and non-coding RNA (Ribonucleic Acid) transcripts, both of which have likely functional relevance on the embryo's developmental potential.

Study design, size, duration: This is a cohort study in which donated embryos for research were thawed under an IRB approved protocol over a 12-month duration. Slow frozen blastocyst stage embryos were thawed and cultured overnight in a standard incubator using a single step media. Embryos were biopsied at the blastocyst stage. 30 embryos were included on the analysis over the duration of the experiment.

Participants/materials, setting, methods: Thawed slow frozen embryos meeting established morphologic criteria were selected for inclusion. Blastocyst stage embryos were double biopsied and RNA Sequencing was performed from the trophectoderm (TE) biopsy and the remaining whole embryo, while the other TE biopsy was sent for Preimplantation Genetic Screening. Some embryos were processed whole after dissolution of the zona pellucida. Using single cell RNA sequencing, a gene expression profile for each embryo was quantified and differential analysis was performed.

Main results and the role of chance: Comparative analysis of genetically screened male and female embryos identified a set of genes that are characteristically expressed in male versus female embryos at the late blastocyst stage. To validate our gene set, we used it to blindly assign the sex of individual cells and embryos of a separate recently published study (Petropoulos, 2016), and found that our sex assignments agreed with the independent assessment made in that study. Furthermore, using embryos that underwent PGS, we identified a gene expression profile differentiating high quality (Grade 4AA or 4BB by Gardner criteria) euploid blastocysts from poorly developed blastocysts with C grade TE and inner cell mass. Intuitively, aneuploid embryos are far more likely to exhibit poor morphology than their euploid counterparts. Analysis of expression differences between poorly developed embryos and high quality embryos revealed a set of genes activated in the poor quality embryos. By GO (Gene Ontology) analysis, these genes are involved in negative regulation of cellular metabolism and signaling. These results suggest that common pathways are activated in embryos with poor morphology, regardless of the specific abnormality or mutation.

Reference: Petropoulos et al. Single-Cell RNA-Seq Reveals Lineage and X Chromosome Dynamics in Human Preimplantation Embryos. Cell, 2016. 165, 1-15

Limitations, reasons for caution: This is a small data set using samples processed in a single laboratory. All embryos were frozen-thawed embryos that had been frozen for varying durations of time; transcriptome results are thus not applicable to fresh embryos and the impact of prolonged embryo storage to the results is unknown.

Wider implications of the findings: We are able to establish embryo sex through gene expression analysis with good correlation between whole embryos and TE biopsies. We have also identified a gene set that correlates to embryo quality. Further aspects of this research examine the embryo transcriptome through developmental stages and correlate it with morphokinetic milestones.

Trial registration number: N/A.

O-248 Sex differences of histone modifications in mouse PGCs after erasure of DNA methylation

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Study question: The mechanism by which the sex-specific gene expressions of PGCs are regulated by epigenetic modifications, especially histone modifications, remains to be elucidated

Summary answer: The histone modifications are major regulators for the sex-specific gene expressions in the male and female PGCs after DNA methylation erasure.

What is known already: In a mammalian germline, dynamic reprogramming of sex-specific epigenome modifications resulted in the formation of oocytes and sperms, which each have unique epigenome modifications. In mice, primordial germ cells (PGCs) are first appear at E7.5. By E13.5, after proliferation and migration into the genital ridge theses PGCs are specified into male and female cells. DNA methylation, a major epigenome modification, is erased in mouse PGCs by E13.5. Nevertheless, the PGCs exhibit sex-specific gene expression profiles at that stage. These findings indicate that the PGCs have already specified into female and male germlines, although DNA methylation marks are erased.

Study design, size, duration: Here, to obtain better understanding of the regulation of gene expression in mouse PGCs by epigenetic modifications, we conducted a genome-wide analysis of six histone modifications, H3K4me1, H3K4me3, H3K27ac, H3K9me2, H3K9me3 and H3K27me3. The histone modifications were evaluated by ChIP-seq analysis followed by next generation sequencing. The ChIP-seq data sets were then compared to the gene expression profiles.

Participants/materials, setting, methods: PGCs were collected using an FACS cell sorter to detect GFP-positive cells from E13.5 embryos of Pou5f1-⊿PE-GFP mice. A total of 10^4 cells were digested by MNase, and the chromatin was applied for immunoprecipitation. Indexed sequencing libraries were constructed using purified DNA from the immunoprecipitation followed by amplification of 12-14 PCR cycles. Sequencing was performed on an Illumina NextSeq sequencer; the generated 76–77-base pair tags were aligned to the reference (mm10), using bowtie2-2.2.3.

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Main results and the role of chance: Comparison analysis showed that a positive correlation with RNA-seq data sets (Sakashita et al., 2016) was found in H3K4me3 and H3K27Ac, which were enriched to the transcription start site. In the inhibitory markers, H3K9me2 and H3K9me3 selectively accumulated in the repressed genes, which would change the chromatin structure to the repression status, and H3K27me3 was specifically enriched to the low expressed genes. In PGCs, approximately 30% of H3K4me3 modifications were found to be concentrated in CGIs, whereas 13.79% was enriched in embryonic stem cells. This indicates that the H3K4me3 modification is specifically enriched in CpG islands, which are targets of DNA methylation and regulate gene expression. The histone modification profiles, especially in H3K4me3, H3K4m1 and H3K27ac, which are active markers, were well associated with sex specific gene expression profiles of male and female PGCs. This study was supported by Grants-in-Aid for Scientific Research from AMED-CREST and JST.

Limitations, reasons for caution: Shown only one species, mice, was studied.

Wider implications of the findings: Our data could provide information for future studies to elucidate the role of epigenetic modifications in the development, function and sex differences of germline and stem cells.

Trial registration number: The data were obtained in biological duplicates at least.

SELECTED ORAL COMMUNICATIONS

SESSION 65: MARKERS AND MECHANISMS OF ENDOMETRIOSIS

Wednesday 5 July 2017 Room W+X 10:00–11:45

O-249 Impact of peritoneal fluid ceramides on endometriosis associated infertility (EAI)

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Study question: In endometriosis, do specific ceramides within the peritoneal environment lead to poor oocyte quality, impede intrinsic maturation potential and reduce a patient's ability to conceive?

Summary answer: Subfertility in endometriosis is influenced by a defective peritoneal environment rift with increased very-long-chain ceramides. These ceramides may alter oocyte maturation potential.

What is known already: Endometriosis is associated with marked subfertility, altered oocyte quality and accumulated levels of potentially oocytotoxic compounds in peritoneal fluids (PF).

Due to the juxtapositioning of ovaries and released oocytes to the PF, an altered PF may have detrimental effects on the maturation potential of oocytes thereby impairing fertilization. Ceramides have been found to be toxic to oocytes and embryos both *in vivo* and *in vitro*. Little is known, however, how PF ceramides contribute to oocyte quality and plausibly EAI.

Study design, size, duration: A case-control study of 39 women undergoing laparoscopic procedures for various indications were recruited into the study in the KK Women's and Children's Hospital, Singapore. To assess the effect of ceramides on oocyte maturation potential, 150 denuded GV oocytes from BALB/c mice were matured in PF of subjects and also in the presence of ceramides at increasing concentrations for 14 hour.

Participants/materials, setting, methods: Women between 22-40 years were recruited, presence of endometriosis recorded and staged, and fertility characteristics collected. Subjects were excluded if FSH > 10 IU/L or had male infertility contributions. PF were aspirated during laparoscopy and had their lipids profiled via liquid chromatography-mass spectrometry. Linear regression was conducted to identify PF ceramides associated with EAI. Assessment of GV to MII oocyte maturation potential was made via polar body extrusion and mitochondrial reactive oxidative species.

Main results and the role of chance: Comparing infertile women with endometriosis and non-endometriotic women, three very-long chain ceramides, C22:0 ceramide ($p = 0.006$), C24:0 ceramide ($p = 0.006$) and C24:1 ceramide ($p = 0.026$) were significantly associated with a combined Area Under Curve (95% CI) of 0.95 (0.83-1.00). With non-endometriosis subjects as reference, Odds Ratios indicated that C24:1 ceramide increases risk (OR=1.017), whereas Cer C22:0 ceramide (OR=0.927) and C24:0 ceramide (OR=0.989) mitigate the risk.

PF from women with and without endometriosis compromised the maturation potential of murine oocytes. The maturation potential of murine oocytes varied with exposure to the very long chained ceramides. By 14 hours, C24:1 ceramide arrested oocytes mainly at GV and MI stage, obtunding maturation to MII. Mitochondrial reactive oxidative species was significantly increased in C24:1 ceramide-treated oocytes. Conversely, C24:0 ceramide significantly improved the maturation potential of GVs to MIIs in a dose-dependent manner relative to vehicle control.

Collectively, the results suggest that any underlying pathology in the peritoneal fluids of women with endometriosis may contribute to compromised oocyte maturation potential and potentially affect fertility in various stages of reproduction, whether by natural conception or IVF, in the ampulla contiguous to the peritoneal cavity where fertilisation typically takes place.

Limitations, reasons for caution: Our results may be confounded by a small sample size; a larger scale study in this specific subgroup of women with non-male EAI is needed to verify our findings. In addition, murine oocytes may have varied response to ceramides compared to human oocytes

Wider implications of the findings: The identification of functionally important oocytotoxic ceramides provide a direct functional readout of pathophysiology in a defective peritoneal environment in endometriotic women. In addition, information derived from the peritoneal fluid may provide a mechanistic insight into its oocytotoxicity.

Sequencing experiments focused understanding transcriptomic changes to ceramide-exposed oocytes are ongoing.

Trial registration number: Not applicable.

O-250 The comparison of effects of ranibizumab, zoledronic acid, danazol, buserelin acetate and dienogest on experimental model of endometriosis in rats

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Study question: Comperative effects of known and promising drugs in the treatment of endometriosis in rat endometriosis model.

Summary answer: Zoledronic acid and ranibizumab treatments may have a potential clinical utility in the treatment of endometriosis.

What is known already: There is no study about zoledronic acid treatment in endometriosis.

Study design, size, duration: This is an experimentally induced endometriosis study on female rats. After the treatment, volumes and histopathological properties of the implants were evaluated by scoring system and immunochemistry. Comparisons were made accordingly.

Participants/materials, setting, methods: Experimental endometriosis was induced in 52 adult female rats. The rats were divided into 6 groups; zoledronic acid, ranibizumab, dienogest, danazol, buserelin acetate and as control % 0,9 NaCl groups. After four weeks of treatment, the volumes were measured and the lesions were excised for histopathologically scoring and immunohistochemistry (CD- 31, NF-κB, BcL- 2).

Main results and the role of chance: Among the groups, the histological score was significantly lower in zoledronic acid and ranibizumab groups in when

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compared with controls($p < 0.001$). There was a statistically significant difference in terms of cell numbers according to the degree of BcL-2, NF-κB and CD31 staining ($p < 0.001$). For BcL-2 staining, the staining rate of the group treated with zoledronic acid was statistically significantly lower compared with dienogest and danazol groups($p < 0.05$). CD31 and NF-κB staining were significantly lower in zoledronic acid and ranibizumab groups in when compared with controls ($p < 0.05$). There were no significant differences in terms of ellipsoidal volume levels between groups($p>0.05$). There was no statistically significant difference in BcL-2, CD31 and NF-κB staining in the binary comparisons between the other groups($p>0.05$).

Limitations, reasons for caution: There was no limitation.

Wider implications of the findings: Zoledronic acid -used in the treatment of osteoporosis- has antiangiogenic properties, and has fewer side effects, by doing so, there may be more acceptable drug option in the treatment of endometriosis. Ranibizumab is anti-VEGF drug, used in ophthalmology. Ranibizumab may serve as another logical option in the treatment of endometriosis.

Trial registration number: TUBITAK Project Number: 213S002.

O-251 ER stress-induced apoptotic cell death in human endometrial cell cycle

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Study question: Whether is endoplasmic reticulum (ER) stress regulated by ovarian steroid hormones (estrogen and progesterone) in human endometrial stromal cells (HESCs), and is it associated with apoptosis?

Summary answer: Estrogen inhibits ER stress in HESCs. This inhibition is reversed by progesterone during the secretory phase, which is directly involved in apoptosis induction.

What is known already: ER stress is a common cellular stress response, which is known to activate apoptosis signaling to trigger cell death. Recent studies have shown that apoptotic cell death is reduced by estrogen through inhibition of ER stress in some cell types including osteoblasts and pancreatic β-cells, which suggests that ER stress-induced apoptosis can be regulated by ovarian steroid hormones. Therefore, endometrial cell apoptosis may be also controlled by ovarian steroid hormones via ER stress during human endometrial cell cycle.

Study design, size, duration: HESCs were cultured with tunicamycin to induce ER stress. To evaluate the effects of estrogen and progesterone on ER stress and apoptosis, tunicamycin-treated HESCs were cultured with estrogen and/or progesterone. And, mifepristone (progesterone receptor modulator) or salubrinal (ER stress inhibitor) were also added in culture medium to block progesterone effects and ER stress, respectively. In addition, to evaluate the cycle-dependent induction of ER stress and apoptosis, endometrial cells were collected according to menstrual cycle.

Participants/materials, setting, methods: The expression level of glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) was measured by Western blot to evaluate ER stress induction. In addition, apoptosis was evaluated by measuring the expression of apoptosis associated protein (BAX, BCL2 and cleaved caspase-12) and by performing annexin-V and PI staining.

Main results and the role of chance: The expression levels of CRP78 and CHOP protein were markedly increased in HESCs treated with tunicamycin compared with control. However, treatment with estrogen decreased tunicamycin-induced CRP78 and CHOP expression levels by dose-dependent pattern, which suggests that estrogen suppresses ER stress in HESCs. In contrast, progesterone treatment significantly increased CRP78, CHOP, cleaved caspase-12 expression, BAX/BCL2 ratio and the proportion of apoptotic cells in estrogen-treated HESCs. And, progesterone-induced CRP78 and CHOP expression was suppressed by the addition of either mifepristone or salubrinal. This suppression was accompanied by decreased apoptosis induction. These results suggest that progesterone reverses estrogen-induced inhibition of ER stress, which is directly involved in apoptosis induction in HESCs. In addition, these data were coincided with the *in-vivo* findings that ER stress and apoptosis increased significantly at secretory phase compared to proliferative phase.

Limitations, reasons for caution: Further study regarding ER stress-related downstream pathways such as PERK, IREα1 and ATF6 pathways is needed to determine the more detailed mechanism of ER stress-induced apoptosis.

Wider implications of the findings: These results give us new insights into the hormonal regulation mechanism for endometrial cell apoptosis during menstrual cycle.

Trial registration number: This study is basic researchis basic research.

O-252 Expression patterns of mRNAs and miRNAs in adenomyosis and their potential uses as diagnostic biomarkers

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Study question: The aim of this study was to improve molecular characterization of adenomyosis and identify biomarkers. For this purpose, transcriptomes and miRNomes of endometrium patients with and without adenomyosis were investigated.

Summary answer: We identified specific mRNAs and miRNAsin endometrium from patients with adenomyosis compared with endometrium from healthy patients used as control.

What is known already: Adenomyosis is a gynaecological disease which is poorly molecularly characterized. In addition, to date, there are very few studies using large scale profiling methods (OMICS) to investigate molecular signatures of this enigmatic pathology.

Study design, size, duration: Endometrial biopsies were obtained from hysterectomy specimens of patients with ($n = 6$) pure and without ($n = 6$) adenomyosis. Presence or absence of adenomyosis was validated by anatomopathological examination and all endometrial specimens were during the proliferative phase. RNAs were extracted and gene and miRNA expression profiles were investigated using the Affymetrix HG-U133 plus 2.0 microarrays and *Affymetrix miRNA 4.1 Arrays*. The differential gene and miRNA expression profiles between pathological and control groups were evaluated with bioinformatics system.

Participants/materials, setting, methods: The differential gene and miRNAexpression profiles between endometrial samples were investigated in patients with adenomyosis (fold-change >2, p-value <5%) without adenomyosis. The lists of significant genes were cross-intersected to identify of list of genes exclusive to the pathological group. Potential endometrial biomarkers of adenomyosis were investigated by RT-qPCR.

Main results and the role of chance: Using transcriptomic analysis, we identified a list of 542 genes exclusive to adenomyosis group with 280 and 262 genes up- and down-regulated in endometrium respectively. The top up- and down-regulated genes exclusive to the endometrium from the adenomyosis patients were ATP8A2 (x35), SH2D3A (x32), KLHL31 (x15), ADAMTS16 (x14), FOXP2 (x-31), F2RL2 (x-29), DGKB (-14), LEFTY2 (x-12) and play roles in several functions including membrane trafficking, migration and extracellular remodeling. In the other hand, miRNome analysis revealed that 3 miRNAs (miR-490-3p, miR-6738-5p, miR-4763) were differentially expressed in endometrium in patients with adenomyosis compared with control patients. Supervised clustering using these 3 miRNAs revealed a great segregation between adenomyosis and control groups, opening news perspectives in the diagnosis of adenomyosis. Interestingly, one miRNA targets 16 mRNAs identified in our transcriptomic analysis that were reached to several biological functions including the BMP signaling pathway and the estrogen receptor signaling playing crucial roles in the physiopathology of adenomyosis.

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Limitations, reasons for caution: Although our pre-screening of mRNAS and miRNAs have been performed in a small number of patients, the relevance of certain biomarkers of adenomyosis in endometrium are being validated in independent cohort of patients.

Wider implications of the findings: These results could contribute for improving our understanding of the physiopathology of adenomyosis and should open new perspectives in the diagnosis of this pathology.

Trial registration number: Not applicable.

O-253 Suppression of endometrial stromal cells autophagy regulates differentiation and function of CD3- CD56+ CD16- NK cells via Hematopoietic Cellular Kinase in patients with endometriosis

Y. Wang

Shanghai Jiaotong University, reproductive, Shanghai, China

Study question: Is differentiation of CD3-CD56+CD16- NK cells affected by degree of Endometrial stromal cells(ESCs) autophagy and what mechanism is involved in it?

Summary answer: The down-regulation of Hck(Hematopoietic Cellular Kinase)mediated by suppression of ESCs autophagy regulates CD3- CD56+ CD16- NK cells differentiation and function.

What is known already: mTOR(mammalian target of rapamycin), the major negative regulator of autophagy, is abnormally increased in endometriotic lesions and is involved in the direct regulation of ESCs apoptosis. Uterine natural killer cells (uNK) constitute a major lymphocyte population during early gestation in the uterus. The uterine natural killer cells are recognized owing to their CD3-CD56+CD16- phenotype.

Study design, size, duration: Autophagy was measured by transmission electron microscopy and immunofluorescence, and in vitro analysis was used to measure HCK-mediated CD3- CD56+CD16- NK cells.

Participants/materials, setting, methods: A total of 30 controls and 30 women with histologically confirmed endometriosis were included. We transfected ESCs with plasmid GV230-Hck or Hck-RNAi, and determined the effect of plasmid transfection on Hck protein expression in ESCs by Western Blot analysis, then they were co-cultured with isolated NK cells for 48 h. And NK cells were analyzed for the expression of CD16, KIR2DL1, KIR3DL1, NKp30, NKp44, NKp46 and IFN-γ, perforin and granzyme B by flow cytometry.

Main results and the role of chance: ESCs were treated with rapamycin (autophagy inducer) or 3-methyladenine (autophagosome inhibitor, 3-MA) for 4 h, and then co-cultured with CD3-CD56+ NK cells for 48 h. The ratio of CD16-NK to CD16+NK was significantly increased after treated with 3-MA ($P < 0.05$). Surface tolerance molecules KIR2DL1、KIR3DL1 were up-regulated($P < 0.05$) and surface molecules NKp30,NKp44 were down-regulated ($P < 0.05$) in CD3-CD56+NK cells in 3-MA treated group. While expression of Intracellular cytokine IFN-γ、Perforin and Granzyme B were decreased in that group($P < 0.05$). Lysate or supernatant from control (secretory phase, $n = 10$), and corresponding 3-MA-treated ESCs ($n = 10$) were characterized by Proteomic microarray. According to Kegg database, we predicted the target of NK cells in proteomics' differential proteins. ESCs control and ESCs treated with rapamycin or 3-MA were then assessed for the differential expression of Hck by Western blot analysis, which showed that Hck is decreased along with the decreased autophagy in endometriosis-derived ESCs($P < 0.05$).We transfected ESCs with plasmid GV230-Hck or Hck-RNAi, and determined the effect of plasmid transfection on Hck protein expression in ESCs by Western Blot analysis. KIR2DL1、KIR3DL1 were up- regulated and NKp30, NKp44, IFN-γ、Perforin and Granzyme B were down-regulated in the GV230-Hck treated ESCs co-cultured group, while in the Hck-RNAi treated ESCs co-cultred group showed opposite results($P < 0.05$).

Limitations, reasons for caution: Further studies are needed to examine the mechanism of ESCs autophagy on CD3-CD16-CD56+ NK cells differentiation and function.

Wider implications of the findings: Measures to increase numbers of CD3-CD16-CD56+ NK cells might be a valid, novel approach to facilitate the implantation of embryos in patients with endometriosis.

Trial registration number: None.

O-254 Pelvic Hypoxic Microenvironment Promotes Endometrial Stromal Cells Ectopic Adhesion Ability via TGF-β1/Smad2 Signaling Pathway in Endometriosis

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Study question: Do hypoxic microenvironment modulate endometrial stromal cell ectopic adhesion ability through mechanisms involving TGF-β1/Smad2 signaling pathway in endometriosis.

Summary answer: Hypoxic microenvironment enhanced endometrial stromal cell ectopic adhesion ability through TGF-β1/Smad2 mediated signaling pathway in endometriosis.

What is known already: Hypoxic microenvironment developed a closed relationship with endometriosis and upregulated HIF-1α played vital roles in the establishment of ectopic lesions. An important step in the aetiology of endometriosis and establish of ectopic lesion is the integrins mediated aberrant adhesion between endometrial fragments and surrounding tissues.

Study design, size, duration: This is a laboratory-based observational study that sampled primary endometrial stromal cells (ESCs) from 13 healthy volunteers to explore their response to hypoxia in vitro. We analyzed ectopic lesions from 5 patients of reproductive age with endometriosis and 5 normal endometrial samples from women without endometriosis. A total of 20 BALB/c nude mice and 22 C57BL6 mice received implantation of proliferative endometrial fragments from 21 individuals without endometriosis.

Participants/materials, setting, methods: The effects of hypoxia on ESCs adhesion, migration and invasion were evaluated by cell counting kit-8, scratch and transwell assays. The effects of hypoxia, TGF-β1 and TGF-β1 receptor inhibitoron on integrins' expression were determined by quantitative PCR and Westerns. Contribution of hypoxia on endometriosis were analyzed using a xenograft model in immunodeficient nude mice. The underlining mechanism of hypoxia influenced adhesion were explored by immunofluorescence assay, lentivirus interference test and enzyme-linked immuno sorbent assay.

Main results and the role of chance: Our results demonstrated that hypoxia culture significantly promoted ESCs adhesion, migration and invasion compared with normoxia culture. Expression levels of integrins in ESCs were dramatically elevated after hypoxia treatment. Of special note was that ESCs secreted higher levels of TGF-β1 in hypoxia culture compared with normoxia culture. Furthermore, both hypoxia culture and TGF-β1 co-culture significantly increased expression of integrin-α5 and integrin-β5, which was reversed by TGF-β1 receptor inhibitoron (SB-431542). Mechanistic researches indicated that TGF-β1/Smad2 signaling pathways in ESCs were activated by HIF-1α. Animal experiments and immunological histological chemistry of huamn endometriotic tissues showed that HIF-1α, TGF-β1 and integrins all remarkedly increased in endometriotic cysts, which enhanced the functions of HIF-1α,TGF-β1 and integrins in the establishment of ectopic lesions.

Limitations, reasons for caution: This is the first time to identify hypoxia in aberrant adhesion of ESCs in endometriosis. However, endometrial epithelial cells also contained abnormal expression of HIF1-α and TGF-β1, and maybe involved in the pathogenesis of endometriosis. Therefore, further studies are required to elucidate the exactly effects of hypoxia in endometriosis.

Wider implications of the findings: Hypoxia may be involved in the development of endometriosis through activating the classical TGF-β1/Smad2 signaling pathway and inducing high expressions of integrins, which lead to the formation of ectopic lesions.

Trial registration number: No clinical trails, none trial registration number.

O-255 TGFβ1 promotes ectopic epithelial cells activation through cadherin-11 to aggravate invasion joint inflammation in endometriosis

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Study question: Previous studies showed that TGFβ1 are involved in endometriosis and we hypothesized that TGFβ1 might induce cadherin-11 (CDH11) expression and invasion in endometriosis.

Summary answer: The expression of CDH11 was enhanced by TGFβ1 and was predominantly overexpressed in endometriotic epithelial cells compared to eutopic endometrial epithelial cells.

What is known already: Previous studies showed that TGFβ1 has pivotal role in the pathogenesis of endometriosis, in terms of fibrosis, inflammatory reaction and epithelial mesenchymal transition (EMT). CDH11 is a downstream effector of TGFβ1, and a cell-to-cell adhesion molecule associated with tissue remodeling, EMT and migration. The expression and function of CDH11 in endometriosis has not been studied. According to our RNA seq data, CDH11 was significantly over-expressed in endometriotic epithelial cells compared to eutopic endometrial epithelial cells.

Study design, size, duration: The sections of endometrioma (N = 13) and endometrium (N = 3) were obtained from patients, who underwent excision of endometrioma or hysterectomy for benign gynecologic disease. All women experienced regular menstrual cycles and did not receive hormonal treatment for at least 3 months before surgery. For in vitro experiments, we used immortalized endometriotic epithelial cells.

Participants/materials, setting, methods: The sections of endometriotic tissues and eutopic endometria were obtained from patients, who underwent excision of endometrioma or hysterectomy for benign gynecologic disease with written informed consent. The expression of CDH11 in endometrioma or eutopic endometrium was examined by immunostaining. The invasion of immortalized endometriotic epithelial cells was evaluated after the induction of TGFβ1 or the stimulation of rCDH11 by transwell invasion assay and CDH11 or interlukin-6 (IL-6) was detected by qPCR and Western blot.

Main results and the role of chance: First, immunostaining showed that CDH11 was highly expressed on ectopic epithelial cells of endometrioma comparing to eutopic endometria. Second, TGF-β1 upregulated the expression of CDH11 ($P < 0.05$) and increased the expression of IL-6 in a time-dependent manner ($P< 0.05$). Third, transwell invasion assay showed that rCDH11 increase the number of infiltrated epithelial cells. Accordingly, we provided several lines of evidence to show that TGFβ potentiates cadherin-11 expression in ectopic endometrial epithelial cells, thereby contributing to epithelial cell invasion and aggravating inflammation.

Limitations, reasons for caution: In vitro experiments using immortalized cell lines.

Wider implications of the findings: We found CDH11 was predominantly overexpressed in endometriotic epithelial cells and enhanced invasive potency of endometriotic epithelial cells, which implies that CDH11 can be a new target for endometriosis treatment.

Trial registration number: Not availiable.

SELECTED ORAL COMMUNICATIONS
SESSION 66: PSYCHOLOGY AND COUNSELLING

Wednesday 5 July 2017 Room C 10:00–11:45

O-256 Psychological distress of women with primary ovarian insufficiency: effect of counseling training for clinician

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Study question: Does specific counselling training for clinicians in Reproductive Medicine & Infertility sub-specialty in disclosing primary ovarian insufficiency (POI) to infertile couple reduce the anxiety and alleviate distress.

Summary answer: Structured and specific training of clinician to diagnose, disclose and counsel women with primary ovarian insufficiency helps them positively to cope better with the condition.

What is known already: Infertility and its treatment have considerable impact on mental health of the couple, primary ovarian insufficiency to them becomes more challenging for the clinician because of its profound effect on quality of life and bleak chances of women begetting her genetic child. Over 75% women with POI feel unprepared to hear the news and most of them underscore the need of more considerate communication. Clinicians need to develop the skills to deliver troubling news, communicate the spectrum of problems associated with it and accurate information about the treatment modalities available to be able to reduce the emotional suffering experienced.

Study design, size, duration: A prospective study conducted in an IVF centre. Women who came for infertility workup, when diagnosed with primary ovarian insufficiency after investigations between March 2015 and October 2016 (N = 62) were included. Of which 29 women were counselled before (Group A) and 33 women after the clinicians attended specific training for information based supportive counselling (Group B). Data were represented as percentage; differences in these measures between the groups were assessed by means of chi-square analysis.

Participants/materials, setting, methods: A questionnaire consisting of 28 questions regarding the condition and counselling, was sent to all patients 2 weeks after the consultation. This questionnaire included specific questions to assess the feelings after the consultation, the adequacy of information delivered to them, whether or not all queries were answered in the consultation, how considerate the clinician was in dealing with them and whether a second consultation was taken elsewhere for this.

Main results and the role of chance: The mean age of the patients was 35 years. The women counselled by trained clinicians were significantly more satisfied as compared to women who consulted before training of clinicians (Group B-81.8% and Group A- 44.8% $p = 0.002$). Eighty eight percent women in group B felt they were given adequate time during counselling compared to 58.6% in group A ($p = 0.02$). Revisit rate was significantly higher in group B 66.7% compared to group A 31.03% ($p= 0.01$).

Limitations, reasons for caution: Although it is prospective study, done before and after training, it has limitation of small sample size.

Wider implications of the findings: Clinicians who convey diagnosis of POI to women present them with life-altering information. Our findings emphasise the need of specific counselling training of clinicians to deal with women with POI to significantly improve satisfaction and treatment continuity.

Trial registration number: Not Applicable.

O-257 Does ART treatment increase the risk for divorce? A registered-based study 1994-2010

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Study question: Are couples who initiate ART treatment at higher risk for marital dissolution compared to other couples?

Summary answer: There is no effect of ART treatments on risk of future marital dissolution over a period of 16 years when adjusting for all confounders.

What is known already: Findings regarding marital stability and fertility treatments have been sparse and controversial. While there is data showing higher divorce rates among women who go through fertility treatments, there is also some evidence of this experience bringing couples closer by forcing them to communicate more and to deal with the surrounding stigma. Using a population-based study and couple-level data, we investigated the extent to which ART treatment increases the risk for divorce/ partnership dissolution during up to 16 years of follow-up.

Study design, size, duration: Register-based national cohort study including all women registered with ART between January 1st 1994 and September 30th 2009 (n = 42845). Marital/cohabiting status was confirmed by matching these women to partners with whom they were married or shared an address. To account for having a significant relationship at baseline (two years), marital/cohabiting status was confirmed by accessing this variable before the establishment of the cohort back to January 1st 1992.

Participants/materials, setting, methods: A comparison group from the background population including five controls per ART-treated woman and

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matched to female age at baseline was prospectively sampled, and participants could change status during follow-up if they entered ART. Final sample had 148873 couples, followed until marital dissolution, death of women/spouse, migration, or December 31st 2010. We used Cox regression models adjusting for female and male age, education, marriage, common child at baseline and live-born child during follow-up.

Main results and the role of chance: At baseline, the majority of couples were married (69%). More non-ART couples opted for marriage (70% vs. 64%; P<.0001) and already had common children at study entry (43% vs. 9%; P<.0001). During the 16 years of follow-up the majority of couples had children with their baseline partners (56% non-ART vs 65% ART), and 22% ended up separated or divorced (20% ART vs 22% non-ART). Findings revealed a lower risk of break-up among ART couples (crude HR 0.84, 95% CI 0.82-0.86), even after adjusting for both partners' age, education and partnership status (adj HR 0.83, 95% CI 0.80-0.86). However, when subsequent common children (time-dependent) was added to the model, no difference in the risk of dissolution was found (adj HR 1.00, 95% CI 0.99-1.01). A significant interaction between ART status and common children showed that the risk of break-up is mainly influenced by the childlessness regardless of having gone through ART treatment. While no difference in the risk of break-up was found among childless couples, there was a slightly higher risk of break-up among ART couples compared to non-ART couples with common children (HR 1.02, 95% CI 1.00-1.04).

Limitations, reasons for caution: There are important predictors of divorce that were not considered. We were unable to adjust for religion, existence of previous marital relationships, income, employment, health status of parents and child(ren), and quality of relationship.

Wider implications of the findings: While the finding that going through ART does not increase the risk of break up per se is reassuring for couples who underwent ART and have children or are contemplating to start ART, findings on the protective nature of parenthood can be particularly helpful for increasing patient adherence to treatments.

Trial registration number: N/A.

O-258 Successful IVF is not associated with maternal perinatal depressive symptoms

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Study question: Is conception by in-vitro fertilization (IVF) associated with maternal depressive symptoms during pregnancy and postpartum?

Summary answer: Successful IVF is not associated with maternal depressive symptoms during pregnancy or postpartum, even when controlling for several potential confounders.

What is known already: Subfertility and its treatment are a known psychological burden. On the other hand, maternal perinatal depressive symptoms are common and contribute to maternal morbidity and mortality. However, few and heterogeneous controlled studies have so far examined the association between successful IVF and maternal depressive symptoms.

Study design, size, duration: Longitudinal observational study of 3283 women with a singleton pregnancy receiving antenatal care and delivering in Uppsala, Sweden. These women were recruited between 2010 and 2015 into the BASIC project (Biology, Affect, Stress, Imaging, Cognition), a population-based study on psychological well-being during pregnancy and postpartum.

Participants/materials, setting, methods: Pregnant women who had conceived via IVF (n 167; 5%) or spontaneously (n 3116; 95%) were delivered a web-based questionnaire including sociodemographic, clinical and pregnancy-related items, as well as the Edinburgh Postnatal Depression Scale (EPDS), at 17 and 32 gestational weeks, as well as at six weeks and six months postpartum. The main outcome variables were the prevalence of significant depressive symptoms (defined by EPDS≥12) and EPDS scores, and were compared between the groups.

Main results and the role of chance: Women older than 34 ($p < 0.001$), primiparae ($p < 0.001$), and caesarean deliveries (p 0.006) were all significantly more frequent among the IVF mothers. Other demographic and clinical characteristics were similar between the groups. Significant depressive symptoms, defined by EPDS≥12, were reported by 12.8%, 12.4%, 13.8% and 11.9% of women at 17 and 32 gestational weeks, and at 6 weeks and 6 months postpartum, respectively. No significant differences in prevalence of depressive symptoms (at chi-square test) and EPDS scores (at Mann-Whitney U test) were observed between women who were pregnant spontaneously or through IVF. No statistically significant association between mode of conception and maternal depressive symptoms was found at any time-point, even when adjusting for several possible confounders (age, BMI, parity, education, depression history and stressful life events) at multiple logistic regression.

Limitations, reasons for caution: IVF mothers often have different characteristics than those spontaneously pregnant. Our study included only singleton pregnancies, and multiple confounders were considered. The gap between women conceiving spontaneously or via IVF in Sweden may be narrower because of factors such as universal healthcare coverage, single-embryo-transfer policy, and unmedicalized obstetric care.

Wider implications of the findings: Our findings are reassuring, considering subfertility's psychological burden. IVF mothers may be less likely to suffer from depressive symptoms than the general subfertile population. It would be interesting to verify whether health policies, such as single-embryo-transfer or unmedicalized obstetric care, reduce the risk of perinatal depression among subfertile women.

Trial registration number: not applicable.

O-259 Quality of life, physical and psychosocial wellbeing among 1023 women during their first ART treatment: Secondary outcome to RCT comparing GnRH-antagonist and GnRH-agonist protocol

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[2]*University of Copenhagen, Department of Public Health, Copenhagen, Denmark*

Study question: Is self-reported quality of life, psychosocial and physical well-being similar in short GnRH-antagonist and long GnRH-agonist protocol among women in their first ART treatment?

Summary answer: Women in short antagonist protocol rated psychosocial- and physical wellbeing during their first ART treatment superior to women in long GnRH-agonist protocol.

What is known already: It is stressful and straining to undergo ART treatment and the psychological burden for the couple can be a major reason for discontinuing treatment. However, the literature on quality of life, psychosocial- and physical wellbeing during different ART treatment protocols is scarce. GnRH-agonists have been associated with negative mood symptoms as depression, fatigue, anhedonia and anxiety due to the medically induced hypogonadism. The agonist protocol is also associated with a higher risk of severe ovarian hyperstimulation syndrome (OHSS). On the other hand, GnRH-antagonist protocol has been associated with more pronounced mood variations during the stimulation phase compared to GnRH-agonist protocol.

Study design, size, duration: Quality of life, psychosocial- and physical well-being was secondary outcomes in a phase IV, dual-centre, open-label, RCT including 1023 women, who started gonadotrophin stimulation in their first ART cycle. Women were randomized to either short GnRH-antagonist or long-GnRH agonist protocol using a web-based concealed randomization code in a 1:1 ratio and enrolled over a five-year period. The aim was to compare self-reported quality of life, physical- and psychosocial wellbeing in the two treatment groups.

Participants/materials, setting, methods: Women referred for first ART cycle <40 years of age were included. All started standardized ART protocols with age-adjusted rFSH doses. A questionnaire on self-reported physical well-being was completed 4 times during treatment and self-reported quality of life and psychosocial wellbeing was completed at the end of treatment. Quality of life and psychosocial wellbeing were analysed in a regression model controlling for; treatment protocol, age, male-factor, previous delivery, pregnancy, severe OHSS and years of infertility.

Main results and the role of chance: Baseline characteristics were similar and the overall response rate for all required questionnaires were 79.4% (419/528)

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in the GnRH-antagonist group compared to 74.3% (368/495) in the GnRH-agonist group (P = 0.06). For the single item self-evaluated quality of life during ART treatment no difference was found between the two treatment groups (P = 0.25), the only covariate predictive of reduced quality of life was the development of severe OHSS (AOR=3.89; 95%CI: 2.34-6.46; P < 0.01). However, women in the short GnRH-antagonist group had a lower adjusted risk of being emotional during treatment (AOR=0.74; 95%CI: 0.56 to 0.97; P = 0.03), they had a lower adjusted risk of experiencing unexpected crying (AOR=0.69; 95% CI: 0.52 to 0.91; P = 0.01) and had a lower adjusted risk of worse quality of sleep (AOR=0.65; 95%CI: 0.48 to 0.88; P < 0.01) compared to women in GnRH-agonist treatment.

Three days after embryo transfer more women in the GnRH-agonist group felt worse physically (P < 0.01), felt bloated (P < 0.01) and had shortness of breath (P = 0.03). At the day of the pregnancy test more women in the GnRH-agonist group had stomach pain (P < 0.01). Finally, significantly more women in the long agonist group experienced side-effects to the prescribed medication during ART treatment.

Limitations, reasons for caution: Overall response rates were satisfying, however, response rates were significantly lower for women without embryo transfer. The randomization procedure makes the risk of bias less likely. Finally, neither physicians nor patients were blinded to GnRH-treatment group.

Wider implications of the findings: ART protocols have improved over the years, but less focus has been on the physical- and psychosocial wellbeing among women treated with different protocols. We found that women in short GnRH-antagonist protocol have better physical and psychosocial wellbeing during their first ART treatment compared to women in long GnRH-agonist protocol.

Trial registration number: EudraCT #: 2008-005452-24; ClinicalTrial.gov: NCT00756028.

O-260 Getting Through the Two Week Wait – A Self-Help Intervention for Women Anxiously Waiting for Their in Vitro Fertilization Result

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Study question: How effective the I-BMS self-help intervention in reducing the anxiety among women during the two week wait?

Summary answer: The anxiety level during the 2-week-wait of the women in intervention groups increased less than that of control group.Their need for parenthood decreased after intervention.

What is known already: IVF treatment is always considered as a stressful experience to women. Among all the treatment stages, it has been proven that women's anxiety level peaks at the two week wait,i.e. the waiting period between embryo transfer and pregnancy test.The situation is especially difficult for Chinese women who bear the obligation to extend the family tree.

The psychosocial self-help model adopted Traditional Chinese Medicine (TCM) as the framework, which aims at maintaining a harmonious balance in holistic wellbeing. It has been proven effective in alleviating anxiety, improving marital satisfaction and achieving holistic well-being of the Chinese women in face of IVF treatment.

Study design, size, duration: 536 women who were going to undergo IVF treatment were recruited in infertility clinics in Hong Kong. 412 who agreed to study participation were randomly assigned to three groups after the two-hour briefing session: Spiritual-behavioral (SB) group (n = 143) received a self-help exercise book on bodily exercises and spiritual reflections for home practicing; Spiritual (S) group (n = 107) received only spiritual stories; Control group (n = 103) received health information on infertility and ART treatments.

Participants/materials, setting, methods: All participants were invited to complete a set of self-administered questionnaires which comprised of the Chinese State-trait Anxiety Inventory (C-STAI) and Fertility Problem Inventory (FPI) on the day of embryo transfer (T1) and the day of pregnancy test (T2). The results were analyzed by repeated measures of ANOVA.

Main results and the role of chance: The mean age of the participants was 36.4 years old (SD:3.2), and the mean duration of marriage was 6.7 years (SD: 3.2). They had been diagnosed of subfertility for 3.8 years (SD: 2.4) in average, and 34.2% of them had gone through more than one treatment cycle. The reasons of subfertility were mostly due to male factors (37.4%), followed by female factors (29.2%), unexplained factors (18.1%) and mixed factors (15.3%).

Participants in the intervention groups (SB group: *T1=42.3+10.9, T2=49.8 +10.6, p < 0.05, Difference=+17.7%*; S group: *T1=42.2+11.8, T2= 48.6+14.0, p < 0.05, Difference=+15.2%*) showed lowered increase in state anxiety level than the control group (*T1=45.1+11.3, T2=55.5+8.8, p < 0.05, Difference= +23.1%)* as measured by C-STAI.

Apart from the anxiety level, participants in SB group showed significant reduction in their need for parenthood (*T1=36.4+7.7, T2=34.9+8.1, p < 0.05*) as measured by the sub-scale of FPI, while S group showed no significant changes (*T1=38.5+7.0, T2=39.3+7.6, p>0.05*). In contrast, the control group demonstrated the highest increase in their need for parenthood (*T1=37.4+6.7, T2=38.2+7.8, p < 0.05*).

Limitations, reasons for caution: The sample size was not standardized for the three groups due to the on-going recruitment of the study. Moreover, since it was a quantitative study, the women's treatment experience and their opinions towards to self-help intervention during the period were subject to further study.

Wider implications of the findings: The self-help intervention allows more autonomy, flexibility and privacy at lower cost to fulfill the women's special needs during the two week wait. It could prepare them psychologically and physically for the treatment outcomes. With limited existing psychosocial services specific for this period, it could provide another choice of support.

Trial registration number: N/A.

O-261 What do people know about fertility and infertility risk factors? A systematic review on fertility knowledge (FK) levels and associated factors

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Study question: What are the levels of fertility knowledge (FK) among study participants and how is FK associated with gender, age, educational level, reproductive status?

Summary answer: Study participants report low to moderate FK. Females, highly educated individuals and people who report difficulties in getting pregnant or previous pregnancies present higher FK.

What is known already: Recent evidence indicates that people in reproductive age groups have inadequate knowledge concerning fertility, infertility risk factors and the consequences of delaying childbearing. To date, no study tried to explore and summarize the results of these studies and analyze which factors are associated with FK; namely age, gender, educational level and reproductive status.

Study design, size, duration: A systematic review of literature was conducted to identify studies assessing FK. A literature search was performed from inception to October 2016 in EBSCO, Web of Science, Scielo and Scopus electronic databases. PRISMA guidelines were followed and the review protocol was registered at PROSPERO (registration number CRD42016050186; http://www.crd.york.ac.uk/PROSPERO).

Participants/materials, setting, methods: A search was conducted using combinations of keywords and MeSH term (e.g., ´knowledge´ AND´ fertility´; ´fertile period´; 'assisted reprod*´). Original studies published in English, French, Spanish and Portuguese were included. Eligible studies had to evaluate quantitatively FK and to describe the measure or have a detailed description of questions used. Assessment of eligibility and data extraction was performed by two researchers independently. A narrative synthesis approach was used to conduct the review.

Main results and the role of chance: Out of 7696 studies initially identified, 63 of them met eligibility criteria and were included in the review. Studies investigating FK with separate items revealed low to moderate knowledge regarding infertility definition and the fertile period. Knowledge regarding infertility risk

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factors, e.g., alcohol, smoking and sexually transmitted diseases were high. However, knowledge regarding causes of infertility was low. A significant proportion of studies showed inaccurate beliefs about infertility causes. Knowledge regarding female age as an infertility risk factor and knowledge regarding the most fertile ages was moderate to high. However, knowledge concerning age-related fertility decline was low, with study participants overestimating the fertility potential and success rates of fertility treatment. Knowledge on risks for the mother and the baby as consequences of delaying childbearing was moderate. Studies using a structured instrument for measuring FK found moderate FK. Evidence showed that being a female, having a higher educational level, difficulties in getting pregnant, and previous pregnancies were associated with higher FK. Being a parent or desiring parenthood was not associated to FK. Age seems inconsistently linked to FK, with some studies showing positive associations and others a negative or no association with FK.

Limitations, reasons for caution: Studies presented high heterogeneity in FK assessment and methodological limitations. A significant proportion of studies focused exclusively on women, undergraduate students and samples were predominantly from USA and Europe, increasing the likelihood of a risk bias on the findings. Conclusions need to be interpreted with cautions.

Wider implications of the findings: This is the first review addressing FK levels. The findings suggested that interventions to increase FK are welcomed, with particular benefits to men, low-educated groups and before preconception care takes place. Prospective high-quality studies using FK validated instruments and exploring the role of FK on reproductive decisions are needed.

Trial registration number: NA.

O-262 Single-mothers-by-choice: parent-child relationships, social support networks and the well-being of their children

M. Brewaeys, H. Bos

University of Amsterdam, College of Child Development and Education, Amsterdam, The Netherlands

Study question: Are there differences between single-mother-by-choice (SMC) and heterosexual two parent families, looking at parent-child relationship, social support of the parent and well-being of their children.

Summary answer: No differences were found between family types looking at parent-child relationships and child development. SMC had a larger social support network.

What is known already: Children growing up without a father in a single mother family have more developmental problems. This is mostly measured after an emotional trauma (like divorce of death). It is likely that secure attachment and parent-child relationships are negatively influenced by this trauma and not by the absence of a father. SMC knowingly choose to raise their child alone. This means that their children do not suffer from this kind of trauma. Little research on the subject has been done but it shows that children of SMC do not have any more developmental problems than children from two parent families.

Study design, size, duration: This cross sectional study, consists of 69 single-mothers-by-choice and 59 mothers from heterosexual two-parent families with a child between the ages of 1.5 and 6 (M_{age}=3.12, SD_{age}=1.50). Participants filled out a questionnaire consisting of standardized instruments. Parent-child relationships, social support network and children's well-being were compared between family types (single-mothers-by-choice versus heterosexual two-parent households). Then the relationship between parent-child relationship and social support, and children's well-being were analyzed within the SMC group.

Participants/materials, setting, methods: SMC were recruited through a fertility center in the Netherlands. Mothers from the comparison group were recruited through day care centers. Inclusion criteria were that the relationship status did not change since the child was born, and additionally for the single mothers, that they had knowingly chosen to raise their child alone. The mothers completed the Parenting and Child Rearing Support Questionnaire (PCRSQ), the Child-Rearing Practices Report (CRPR) and the Child Behavior Checklist $1^{1/2}$-6 (CBCL).

Main results and the role of chance:

(1) There were no significant differences in emotional involvement (p = .936) or parental stress (p = .474) between family types.
(2) SMC showed significant higher scores on received social support (p = .012), and interestingly also on wanting more social support (p = .030); there was no significant difference between the mothers in both famiy types on their satisfaction of support (p = .112).
(3) There were no significant differences on the offspring's internal (p = .769) and external (p = .985) problem behavior (well-being) between both family types.
(4) When looking at the offspring's problem behavior in the SMC families, a significant negative correlation between satisfaction with social support and internalizing problem behavior was found (r= -.386, p = .007). Likewise, a significant negative correlation between satisfaction with social support and externalizing problem behavior (R= -.319, p = .027) was found in the SMC families.

Limitations, reasons for caution: Because the study has a cross-sectional design nothing can be said about the cause and effect of the findings. Additionally, social support questionnaires about child rearing are originally made for couples. This means we had slightly alter one of the questionnaires. This did not influence the reliability of the instrument.

Wider implications of the findings: Children in both family types are doing well in terms of their well-being. SMC and their children benefit from a good social-support network. In the counseling of women who want to have and raise a child without a partner, one should emphasize the importance of having a good supportive network.

Trial registration number: not applicable.

INVITED SESSION

SESSION 67: FROM BEGINNING TO END: CHROMOSOMES MADE TO FAIL

Wednesday 5 July 2017 Plenary I 12:00–13:00

O-263 Gamete origins of early pregnancy failure

L. Gianaroli

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Abstract text

Although not unique to humans, aneuploidy is frequent in human embryos, due to malsegregation at meiosis and/or mitosis. Data from natural pregnancies report that the vast majority of aneuploidies are traced back to the maternal chromosomes in an age dependent manner. As miscarriages occurring during the first weeks of gestation may be undetected and untested, the incidence of aneuploid conceptuses can be higher than that reported by data from miscarriages or prenatal/postnatal diagnoses. This is confirmed by the data from preimplantation genetic testing for aneuploidy (PGT-A) that has provided comprehensive information on the incidence of chromosome errors in IVF oocytes and embryos.

There are several possible causes of oocyte predisposition to aneuploidy. Although meiotic errors can occur during foetal oogenesis, PGT-A of oocytes has revealed that the incidence of malsegregation at the second meiotic division is higher than that occurring at the first division. Possibly, the long time between the initiation and the completion of oocyte maturation contributes to the alteration/degradation of factors important for chromosome fidelity. Interestingly, the origin of autosomal aneuploidy in foetuses mostly resides in the first meiotic division suggesting that those happening after oocyte activation are hardly compatible with clinical pregnancies.

The formation and positioning of the meiotic spindle is controlled by the subcortical maternal complex (SCMC), a multiprotein complex essential for both the meiotic spindle migration and the progression beyond the first divisions. The genes encoding for the SCMC complex are expressed during oogenesis, and the transcripts are degraded after embryonic genome activation. SCMC proteins are found until the blastocyst stage, where they segregate to the outer cells being absent in the inner cell mass. These proteins are involved in mitochondria translocation at appropriate timing during embryogenesis. Clearly, the provision of ATP at crucial steps in oocytes and embryos is fundamental for the

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events associated with fertilization and developmental competence. It has been proposed that ageing may affect oocyte competence in targeting the cytoplasmic quality, and particularly mitochondria. The consequences include the generation of aneuploidy that is increased by oxidative stress, confirming the close relationship between mitochondria and oocyte quality. Interestingly, some mtDNA-haplogroups are protective against the decline of ovarian reserve.

Despite the preponderant role of the oocyte in relation to aneuploidy, sperm contribution to the resulting embryo cannot be disregarded. Following chromosomal analysis, aneuploidy is found in approximately 6% of sperm cells, but this figure increases significantly in severe male factor samples. Besides releasing into the oocyte its genome and the phospholipase C-zeta, the sperm also contributes its centrosome that, after duplicating and separating its two centrioles, pull the chromosomes apart during the first embryonic divisions. Certain forms of male infertility involve centriole abnormalities, and may induce the formation of mosaic embryos.

Results from sequential biopsies suggest the existence of aneuploidy corrective mechanisms. The potential mechanisms of aneuploidy rescue, either of meiotic or mitotic origin, include the formation of micronuclei encapsulating missegregated chromosome, multi-polar division, aneuploid blastomere exclusion or fragmentation. These corrective mechanisms existing in viable oocytes help restoring a condition compatible with the generation of euploid conceptuses.

In carriers of translocations, the risk of generating unbalanced gametes is higher in reciprocal than in Robertsonian translocations. In reciprocal translocations, this frequency varies depending upon the type of chromosomes engaged in the translocation, and the size and the position of the exchanged segments. PGT results have shown that the vast majority of embryos are unbalanced both in reciprocal and Robertsonian translocations. In case of male carriers, the analysis of sperm cells for the chromosomes involved in the translocation provides an estimation of unbalanced gametes, as well as of the type of segregation.

O-264 Genetic testing in early pregnancy failure

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Abstract text

The loss of a pregnancy during the first trimester occurs in about 10%-15% of all clinical pregnancies. Once a woman is 40 years of age, the percentage of first trimester losses increases up to 40% of all clinical pregnancies. We know that half of the first-trimester miscarriages are caused by fetal chromosome abnormalities. The prevalence of these foetal chromosome abnormalities after a single sporadic miscarriage has been estimated to be 45% (95% CI: 38-52; 13 studies, 7012 samples). The prevalence of chromosome abnormalities in women experiencing a subsequent miscarriage after preceding recurrent miscarriage appears to lie within the same range (39%, 95% CI: 29-50; 6 studies 1359 samples).

According to conventional karyotyping nine out of 10 abnormalities are due to numerical aberrations, other reasons are structural abnormalities and more rarely problems like mosaicism and translocations. Conventional karyotyping may be hampered by maternal contamination, culture failure or overgrowth and poor quality of the chromosomal preparations. No fetal karyotype result is available in about 20% of the samples tested. Another disadvantage is the limited resolution.Chromosomal Comparative Genomic Hybridization (CGH), array-Comparative genomic hybridization (array-CGH), fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and quantitative fluorescent polymerase chain reaction (QF-PCR) are alternative techniques that might deal with (a part of) the limitations of conventional karyotyping though each technique has its limitations. The expectations of the molecular techniques, in particular the array-CGH, are high. These techniques do not seem to detect more chromosomes but are able to detect chromosome abnormalities in case of failed cultures. Furthermore, FISH and MLPA can detect pre-selected submicroscopic abnormalities while array-CGH can detect chromosome abnormalities in the whole genome. Hence these techniques enable us to detect submicroscopic chromosome abnormalities which cannot be found with conventional karyotyping.

Knowledge on prevalence of cytogenetic abnormalities in miscarriage samples may provide important information for the woman or couple in question. Still, the relevance of the molecular techniques for daily clinical practice is a point of discussion. Opinions differ as to the usefulness of genetic testing of miscarriage samples for routine clinical practice.

INVITED SESSION

SESSION 68: INTRA-UTERINE INSEMINATION: WHY, WHEN AND HOW

Wednesday 5 July 2017 Plenary 2 12:00–13:00

O-265 Evidence based IUI: what we do and do not know

B. Cohlen[1], A. Bijkerk[1], W. Ombelet[2], S. Van der Poel[3]

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Abstract text

Background: Intrauterine insemination (IUI) has become a first-line treatment option for many infertile couples, worldwide. However, the practice of IUI with the use of ovarian stimulation (OS) varies across centers and countries; and, complication rates, such as multiple pregnancies rates, greatly differ. The World Health Organization (WHO) had initiated a multi-year project to establish best practice guidance within the field of infertility, and had therefore recognized the need to evaluate the evidence for use of IUI.

Objective and rationale: 14 prioritized questions covering IUI with and without OS were established through WHO processes. The best available evidence was collected and research gaps identified during development of draft global, evidence-based guidelines.

Methods: Specific search terms were used to find the available evidence in MEDLINE (1950 to May 2015) and The Cochrane Library (until May 2015). We also hand searched references of relevant reviews and included more recent studies up to January 2016. Articles found relevant were read and analyzed by two investigators (AB and BC) and critically appraised using the Cochrane Collaboration's tool for assessing risk of bias and AMSTAR in case of systematic reviews. The quality of the evidence was assessed using the WHO GRADE system.

Outcomes: An assessment and synthesis of the evidence will be presented, as well as with identification of evidence gaps. In general, quality of the evidence found was poor and in many cases, outdated. Recent studies showed IUI with OS still to be the most cost-effective treatment option in couples with moderate male or unexplained subfertility when prognosis is less than 30% of becoming pregnant without assistance in the forthcoming year. There is insufficient evidence to recommend for or against IUI (with or without OS) in male infertility because of the lack of high quality evidence. Evidence-based details on how to perform IUI will be presented. Patient-centred practice and the importance of patient groups in writing guidelines is highlighted, with a need that patients be provided options for what can be a preferred less invasive intervention of IUI over IVF//ICSI.

Clinical judgment completes the triad of evidence-based medicine. Preventing complications such as multiple pregnancies, especially in low resource settings, are critical to decrease maternal/newborn morbidity and mortality. Primary and secondary measures will be presented to keep multiple pregnancy rates to a minimum.

Wider implications: Because IUI is a widely applied treatment option, there is great need for clear practical best practice guidance that are cost-effective and better ensure preventable complication rates. Future research should focus on the gaps that were identified in the search for evidence that will help to support worldwide guidelines.

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O-266 Prognostic factors influencing IUI success

N. Dhont[1]

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Abstract text

Background: Intra uterine insemination aims to increase gamete density at the site of fertilization. It is a widely used treatment mainly for couples with unexplained infertility, cervical factor and mild male factor. Questions remain about the effectiveness of this method. There is a considerable amount of literature on several prognostic factors either related to patient characteristics or the IUI procedure itself, often with controversial results.

Objective and rationale: In this lecture we will review the prognostic factors influencing success rates of IUI.

Methods: For all the prognostic factors we searched the available evidence in MEDLINE (up to January 2017) and The Cochrane Library (until January 2017) using specific search terms. We also hand searched references of relevant reviews and included more recent studies up to January 2017.

Outcomes: Female age is the most important prognostic factor influencing the likelihood of pregnancy in IUI with a sharp decline after 40 years. For IUI with donor sperm acceptable pregnancy rates can be achieved up until 42 years. In general IUI is performed if one tube is patent; there is some evidence however that in case of unilateral distal tubal blockage IUI pregnancy rates decrease significantly. The effects of female weight, male and female smoking and male age are controversial. The most important male factor is sperm quality parameters. Based on a literature review the following cutoff parameters can be identified: TM (Total Motility) >30%, TMSC (Total motile count native sample) > 5-10 million and IMC (Inseminating Motile Count) > 1-2 million.

For unexplained infertility, minimal to mild endometriosis and mild male factor addition of ovarian stimulation improves pregnancy rates in IUI, gonadotrophins more than clomiphene citrate. This is not the case for moderate male factor and cervical factor. The increase in pregnancy rates with ovarian stimulation need to be balanced against the increase in multiple pregnancy rates. There is some evidence that luteal support with progesterone after IUI with gonadotrophin ovarian stimulation improves pregnancy rates.

IUI should be performed once 12-36 hours after HCG injection. There is no sperm preparation technique (wash and centrifugation, swim up, density gradient centrifugation) proven to be superior. 15 minutes of bed rest after IUI has been generally advocated, based on 2 RCTs showing beneficial effect. However this has recently been challenged by a new RCT showing no improvement after bed rest.

Wider implications: IUI remains a relatively simple, noninvasive, cheap treatment option for a well selected population with patent tubes and a sufficient number of motile sperm. If performed with proper timing and with ovarian stimulation when indicated, IUI result in cumulative pregnancy rates up to 50 % after 6 cycles and can prevent couples moving on to IVF.

INVITED SESSION

SESSION 69: ENDOGENOUS RETROVIRUSES AND PREGNANCY

Wednesday 5 July 2017 Room A 12:00–13:00

O-267 Implication of human endogenous retrovirus envelope proteins in placental functions

B. Barbeau[1], **C. Toudic**[1], **A.G. Lokossou**[2], **S. Bourgault**[3], **X. Elisseeff**[1], **J. Lafond**[1], **E. Rassart**[4], **P.T. Nguyen**[3], **L. Leduc**[5]

[1]Université du Québec à Montréal, Sciences biologiques, Montréal, Canada
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[3]Université du Québec à Montréal, Chimie, Montréal, Canada
[4]Université du Québec à Montréal, Sciences biologiques, Montréal, Canada
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Abstract text

Human Endogenous Retroviruses (hERV) are derived from ancestral retroviruses, which have been co-opted in our genome for over several million years. These sequences harbour viral-like genes, some of which have retained their encoding capacity. Interestingly, two hERV sequences encode former envelope proteins, known as Syncytin-1 and Syncytin-2, which are implicated in the formation of the syncytiotrophoblast, a multinucleated cell layer localized on the periphery of placental villi. This structure plays a pivotal role in the process of nutrient and gas exchange but also contributes in producing important hormones. Most importantly, this layer is very dynamic and its maintenance is ensured by constant fusion events with underlying villous cytotrophoblasts. Our team has been focussing on the role of Syncytin-2 in placental function through its association to the membrane of cytotrophoblasts and have shown that this protein, via its fusogenic properties, is greatly responsible in mediating cytotrophoblast fusion. In addition, we have accumulated other evidence showing that Syncytin-2 contributes to placental formation and function in a similar manner to envelope proteins at the surface of retroviruses. We have in fact recently demonstrated that Syncytin-2 is present at the surface of placental exosomes and that this latter protein can provide a form of tropism to these extracellular vesicles towards target cells through receptor-mediated binding and fusion. Herein, two additional examples of such retrovirus-related functions of Syncytin-2 are provided. Galectin-1 is an extracellular lectin, which acts as a dimer and binds specific glycans. This soluble protein has been associated to apoptosis and can increase infection by various viruses, such as HIV-1. Interestingly, this factor is highly expressed by cytotrophoblasts and has been shown to increase fusion between trophoblasts. We thus hypothesized that, like for other viruses, galectin-1 might favour the interaction between Syncytin-2 and its receptor. Using HIV-1 retroviral particles pseudotyped with Syncytin-2 and expressing the luciferase reporter gene, we demonstrate that galectin-1 importantly augmented the rate of infection of Syncytin-2-positive viruses. This increment in infectivity was more pronounced on 293 T cells overexpressing the Syncytin-2 receptor, MFSD2a. No such positive impact was noted when galectin-3 was added and, in addition, non-pseudotyped or VSV envelope-pseudotyped viruses were not responsive toward galectin-1 treatment. Incubation of galectin-1 with pseudotyped viruses during contact with cell target was shown to be essential for its effect on viral infection and suggested no role for galectin-1 on the fusion process. As a second approach, we assessed the immunosuppressive function of the Syncytin-2 protein through the analysis of its immunosuppressive domain, a domain which is shared with envelope proteins from various retroviruses. When a dimerized form of the synthesized peptide corresponding to the amino acid sequence of the immunosuppressive domain was tested, cytokine production of T cells lines or activated PBMCs was greatly hampered. Using the same assay, the inhibitory potential of exosomes isolated from cultured primary cytotrophoblasts was confirmed over activated PBMCs. Of interest, when the presence of Syncytin-2 was strongly reduced at the surface of exosome by siRNA-mediated silencing of its expression in cultured villous cytotrophoblasts, the immunosuppressive potential of resulting exosomes was severely downmodulated, thereby suggesting that Syncytin-2 plays a very important role in the immunosuppressive feature of placental exosomes. Overall, our results have demonstrated that Syncytin-2 plays multiple roles in the placenta and that its functions are reminiscent of those associated to envelope proteins of exogenous retroviruses. Through its association with placental exosomes, Syncytin-2 might also contribute to intercellular communication during pregnancy. Furthermore, this association has a potential diagnostic value to obstetric disorders in which Syncytin-2 presents important variation in its expression, such as preeclampsia.

O-268 Retroviruses facilitate the rapid evolution of the mammalian placenta

E. Chuong

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Abstract text

Endogenous retroviruses (ERVs) are remnants of ancient retroviruses that invaded host germline DNA, and constitute 6-14% of vertebrate genomes.

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Multiple genome defense mechanisms have evolved to repress ERV activity, which has long been associated with diseases including cancer and autoimmunity. Nonetheless, ERVs contain sequences capable of modulating transcription, and the propagation of these "pre-built" regulatory sequences throughout the genome has long been proposed to fuel the evolution of gene regulatory networks.

We are investigating a role for ERVs in shaping the evolution of transcriptional networks underlying various processes involved in reproduction and immunity. Our previous work identified ERVs as a substantial source of species-specific enhancers in the developing placenta. More recently, we identified ERV-derived enhancer elements that are activated during the interferon (IFN) response, a major branch of innate immunity. Through an analysis of STAT1 and IRF1 ChIP-seq data from IFN-treated human cell lines, we identified over 7,000 IFN-inducible binding sites that derived from ERV elements. We focused on an ERV family named MER41 that invaded primate genomes 50 million years ago and deposited hundreds of IFN-inducible enhancers by "copy and pasting" multiple STAT1 binding motifs. CRISPR-Cas9 deletion of individual MER41 elements in the human genome impaired expression of adjacent IFN-induced genes and revealed their involvement in the regulation of essential immune functions, including activation of the AIM2 inflammasome in response to viral infection. In mouse, we identify a largely distinct repertoire of rodent-specific ERVs that may have independently rewired the mouse IFN regulatory network. While these regulatory sequences likely arose in ancient viruses, they now constitute a dynamic reservoir of regulatory variation that may be co-opted for host biological functions, catalyzing the evolution of gene regulatory networks.

SELECTED ORAL COMMUNICATIONS

SESSION 70: CLINICAL IMPACT OF LABORATORY TECHNIQUES

Wednesday 5 July 2017 Plenary 1 14:00–15:15

O-269 Prospective evaluation of a cumulus-corona gene expression based scoring, combined with morphological scoring, reveals improved clinical pregnancy rates

T. Adriaenssens[1], I. Van Vaerenbergh[1], W. Couck[2], I. Segers[3], G. Verheye[3], M. De Vos[3], J. Smitz[1]

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[3]*Universitair Ziekenhuis Brussel UZ Brussel, Centre for Reproductive Medicine, Jette Brussels, Belgium*

Study question: Can quantitative cumulus cell gene expression analysis in addition to embryo morphology scoring improve the clinical pregnancy rate in patients undergoing single embryo transfer (SET)?

Summary answer: The Corona-test significantly improves the clinical pregnancy rates of day 3 SET compared to day 3 and 5 SET with morphological selection only.

What is known already: Because clinical pregnancy rates from IVF/ICSI cycles with SET stagnate between 30% and 40%, multiple embryo transfer is still common practice to increase the chance of live birth per treatment cycle. Cumulus and corona cells (CC) communicate bidirectionally with the oocyte, can be easily collected and are dispensable. CC mRNA analysis with quantitative PCR (qPCR) can provide an objective approach to oocyte competence evaluation.

Study design, size, duration: A prospective case-matched control study, performed from 2013 until 2016, comprised one experimental arm where the Corona-test complemented the morphological scoring with transfer on day 3 (n = 62) and two case-matched control arms where elective SET (eSET) was done on day 3 (n = 62) or day 5 (n = 44) based on morphological scoring only.

Participants/materials, setting, methods: All patients were stimulated with HP-hMG in a GnRH antagonist protocol and scheduled for ICSI and eSET. Case-control pairs were matched for age, number of good quality embryos (GQE) on day 3, and to be nearest in treatment time. In the experimental arm, the oocytes were denuded individually and CC were used to calculate a Corona-test score based on the normalized gene expression of specific genes. The GQE with the highest Corona-test score was transferred.

Main results and the role of chance: eSET decided on the basis of the Corona-test score yielded a significantly higher clinical pregnancy rate of 63%, compared to the clinical pregnancy rate of 27% ($p < 0.001$) and 43% ($p < 0.05$, Chi-square test) in the controls with eSET based on only morphological scoring and transfer on day 3 and day 5, respectively. Further analysis of the data indicated that the gain in clinical pregnancy rates using the Corona-test is independent of patients' age or number of available GQE. More importantly, the time-to-pregnancy was significantly shorter with three frozen/thawed embryo transfer cycles that could be avoided by performing a Corona-test analysis in the fresh cycle ($p < 0.05$, Kaplan-Meier analysis).

Limitations, reasons for caution: The Corona-test was evaluated in patients who underwent HP-hMG stimulation before ICSI treatment and with eSET on day 3. Wider generalization would necessitate validation studies in diverse stimulation protocols, other ethnicity and in patients undergoing IVF without ICSI.

Wider implications of the findings: The Corona-test reduces the time-to-pregnancy. This will have a great impact on the reduction of patient burden and the workload in the ART clinic and health care expenses for ART. The Corona-test further decreases the need to transfer multiple embryos and its associated risk of neonatal and maternal complications.

Trial registration number: not applicable.

O-270 Intracytoplasmic sperm injection versus conventional in vitro fertilization for non-male factor infertility and decreased ovarian reserve: a systematic review and meta-analysis

C. Wu[1], R. Bassil[2], J. Haas[2,3], E. Barzilay[3], D. Nayot[2], R. Casper[2,4]

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[2]*TRIO Fertility Partners- University of Toronto, Department of Obstetrics and Gynecology- Division of Reproductive Sciences, Toronto, Canada*
[3]*Sheba Medical Center- Tel-Hashomer- Sackler School of Medicine- Tel Aviv University, Department of Obstetrics and Gynecology, Tel Aviv, Israel*
[4]*Lunenfeld-Tanenbaum Research Institute, Division of Reproductive Sciences, Toronto, Canada*

Study question: Does intracytoplasmic sperm injection (ICSI) yield improved fertilization and clinical pregnancy rates compared to conventional in vitro fertilization (IVF) for non-male factor infertility with and without decreased ovarian reserve (DOR)?

Summary answer: ICSI yields better fertilization but similar clinical pregnancy rates when compared to conventional IVF in couples with non-male factor infertility.

What is known already: ICSI has surpassed conventional IVF as the preferred method of insemination in recent years. Threshold for using ICSI has been consistently lowered, and ICSI is now widely used for non-male-factor infertility and DOR. Previous studies examining fertility outcomes following IVF versus ICSI for these indications have yielded conflicting results.

Study design, size, duration: We systematically searched the Cochrane Library, EMBASE and MEDLINE databases from inception to November 2016. Keywords used included ICSI, IVF, non-male factor infertility, female infertility, and pregnancy. Studies were included if they examined couples with normal semen parameters, had insemination performed with either or both conventional IVF and ICSI depending on study design, and reported at least one of the outcomes of interest (fertilization, implantation, pregnancy, or miscarriage rates).

Participants/materials, setting, methods: Two reviewers independently extracted data from included studies. Fertilization and clinical pregnancy data were pooled across trials using a random-effects model.

Main results and the role of chance: Eighteen randomized controlled trials (RCTs) and 7 observational studies investigating fertility outcomes in couples with non-male-factor infertility were included in our systematic review. Six of

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the included studies examined couples with DOR and were included as part of a subgroup analysis. The systemic review included 1,884 IVF cycles and 2,038 ICSI cycles performed on a total of 3,583 patients. When data were pooled across RCTs, ICSI yielded better fertilization rates when compared to conventional IVF (odds ratio [OR] = 1.43; 95% CI = 1.05, 1.94) but similar clinical pregnancy rates (OR = 0.93; 95% CI = 0.69, 1.27) in non-male-factor-infertile couples. In the subgroup analysis of patients with DOR, IVF was superior to ICSI in clinical pregnancy rates (OR = 0.48; 95% CI = 0.32, 0.72), but comparable in fertilization rates (OR = 1.19; 95%; CI = 0.63, 2.24).

Limitations, reasons for caution: Studies comparing IVF and ICSI are heterogenous in methodology, patient selection, and outcome measures, making direct comparisons and data aggregation challenging.

Wider implications of the findings: Our meta-analysis provides an updated overview of currently published literature on fertility outcomes following IVF and ICSI cycles. It does not yield demonstrable evidence in favor of the routine use of ICSI in patients with non-male-factor infertility or DOR.

Trial registration number: Not applicable.

O-271 The reproductive outcome of donor ICSI cycles and embryo morphokinetic parameters are not compromised by high levels of sperm DNA fragmentation

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Study question: Is there an association between the level of sperm DNA damage and the reproductive outcome of ICSI cycles with donor oocytes?

Summary answer: An increased level of sperm DNA damage does not have a negative impact on the reproductive outcome of ICSI cycles with donor oocytes.

What is known already: The degree of sperm DNA fragmentation reflects the integrity of the genetic material and chromatin integrity seems essential for sperm to fully express their fertilizing capacity. Transmission of damaged sperm DNA, particularly at levels that exceed the DNA repair capacity of the oocyte, could have serious consequences on embryo quality (morphokinetic parameters, morphology), fertilization and live birth rates in couples with high sperm DNA fragmentation index (DFI). Whether the extent of DNA damage is a prognostic factor of a man's fertility potential and if it is linked to embryo quality and miscarriage rates is still a matter of debate.

Study design, size, duration: For this prospective study, semen samples were collected from 54 men undergoing ICSI cycles with donor oocytes in a private IVF center (October 2015-June 2016). Sperm was subjected to DNA fragmentation analysis before and after sample processing utilizing a previously defined cut-off value. Morphokinetic parameters (t5, s2, CC2) of 352 embryos were evaluated with time-lapse. Maximum 2 embryos were transferred and supernumerary embryos were vitrified. Cycles were followed up to record their reproductive outcome.

Participants/materials, setting, methods: Sperm samples from men undergoing ICSI cycles with donor oocytes were assessed using the Sperm Chromatin Dispersion test with a cut-off value 24.8% following ejaculation and after density-grade processing. The development of 352 embryos was monitored with time-lapse (Primo Vision). Student's *t*-test was used to assess statistical differences, with $p < 0.05$ considered significant. Means were compared with one-way ANOVA. The Spearman rank correlation was performed with raw scattered plots. The results are expressed as means ± SD.

Main results and the role of chance: Cycles were classified based on the DFI prior to sperm sample processing into: Group A with DFI<24.8% ($n = 29$) and Group B with DFI>24.8% ($n = 25$). Fifty-four ICSI cycles using donor oocytes resulted in 70.4% pregnancy rate, 66.6% clinical pregnancy rate, 59.3% ongoing pregnancy rate and 53.7% delivery rate per embryo transfer. There was no statistical difference between groups A and B with regards to the pregnancy rate ($p = 0.341$), clinical pregnancy rate ($p = 0.173$), ongoing pregnancy rate ($p = 0.4$), miscarriage rate ($p = 0.532$) and delivery rate ($p = 0.435$). Regarding the embryo morphokinetic parameters, no correlation was found between the DFI and t5, s2, CC2 ($p = 0.401$) nor with embryo quality on day 3. Scatter plot analysis showed a negative correlation of the DFI with sperm concentration. Furthermore, there was a significant decrease in the DFI of sperm samples (26.63 ± 13.57%) following density-grade centrifugation (15.02 ± 13.82%) ($p < 0.0001$).

Limitations, reasons for caution: The results need to be confirmed with a larger sample size. It is possible that current methods for determining the DFI cannot detect the DNA damage that is clinically important. New DFI cut-off values may need to be validated. The effect of SDF on blastocyst formation should also be evaluated.

Wider implications of the findings: A successful reproductive outcome depends both on the traits of sperm quality and function, as well as the influences of the oocyte. However, it seems plausible that the DNA repair mechanisms of high quality donor oocytes offset the negative impact of increased sperm DNA damage on the reproductive outcome.

Trial registration number: N/A.

O-272 Influence of commercial embryo culture media on preimplantation development and pregnancy outcome after IVF: a single-center RCT

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Study question: An RCT was conducted to compare preimplantation development and pregnancy outcome between three embryo culture media systems that are used widely in IVF programs.

Summary answer: Embryo culture media used in IVF affected preimplantation development, including blastocyst yield, but the overall pregnancy rates (PRs) after ET did not differ between media.

What is known already: Numerous commercial embryo culture media are now available for IVF, which raises the question as to whether any medium is superior. Significantly, the ability of a particular culture medium to yield a high embryo development percentage *in vitro* does not necessarily mean that these embryos are viable. For example, it was previously common to include blood serum in animal embryo culture media to stimulate blastocyst formation, but this resulted in impaired embryonic, fetal, and offspring health. Given the importance of culture media for treatment outcome, well-designed RCTs are needed, but the existing data are insufficient to select the best medium.

Study design, size, duration: This study included 525 healthy patents receiving their first IVF treatment cycle at our clinic between February and December 2016. They were randomized by computer-generated tables into three groups and underwent our standard oocyte retrieval and IVF/ICSI procedures, and the embryos were cultured in G1/G2 Plus (Vitrolife) (A), Global Total (LifeGlobal) (B), or Sequential Cleav/Blast (Origio) (C) media. Of the patients, 27 with no 2PN oocytes 18 h after insemination were excluded from the study.

Participants/materials, setting, methods: During embryo culture (1-5/50 μL), for cycles where the patients had only one good-quality (GQ) embryo by day 3 (D3), the embryos were vitrified on D2/3. When the patients had ≥2 GQ embryos by D3, ≤2 GQ embryos were vitrified on D2/3, the culture of the remaining embryos was extended, and all GQ blastocysts were vitrified on D5/6. The ET data for these vitrified embryos performed by the end of December 2016 were analyzed.

Main results and the role of chance: Patient age (years) was similar between groups A (35.8 ± 0.3, n = 165), B (36.2 ± 0.4, n = 170), and C (36.5 ± 0.3, n = 163). A total of 1098, 1170, and 1145 2PN oocytes were cultured in groups A, B, and C, respectively. The percentages of vitrified D2/3 embryos per 2PN oocytes were similar between groups A (19.3%), B (19.6%), and C (19.0%). The percentages of vitrified D5/6 blastocysts per 2PN oocytes were 25.7%, 38.4%, and 32.5% in groups A, B, and C, respectively (A vs. B, $P < 0.0001$; A vs. C, $P = 0.0004$; B vs. C, $P = 0.0031$). Groups A, B, and C underwent a total of 186 (D2/3 ET=56, D5/6 ET=115, 2-step ET (combining a D2/3 ET with a D5/6 ET)=15), 192 (D2/3 ET=55, D5/6 ET=120, 2-step

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ET=17), and 163 (D2/3 ET=55, D5/6 ET=96, 2-step ET=12) ET cycles with these vitrified embryos, respectively. Most embryos survived warming (A=220/220, B=222/225, C=191/192), and only one ET cycle was cancelled, which was in group C. The mean number of embryos transferred (A=1.16 ± 0.03, B=1.17 ± 0.03, C=1.18 ± 0.03), implantation rates (A=44.0%, B=40.6%, C=36.6%), clinical PRs per ET (A=48.9%, B=45.3%, C=40.7%), and ongoing PRs per ET (A=37.6%, B=35.9%, C=30.9%) did not differ between the groups.

Limitations, reasons for caution: Our single-center prospective RCT will help clarify whether commercial embryo culture media have an effect on IVF success rates. Further studies with more participants, including a follow-up study on the perinatal and long-term health of children born after embryo culture, are warranted.

Wider implications of the findings: In this RCT, the PR of a culture system yielding fewer blastocysts was comparable to or slightly better than those of other systems yielding more blastocysts. It would be important to differentiate between the ability of commercial culture media to support preimplantation development and its ability to yield viable embryos.

Trial registration number: UMIN000020910.

O-273 Cumulative live birth rates after fresh cleavage-stage versus blastocyst-stage transfer in combination with vitrification on day 5

I. De Croo, K. Tilleman, F. Vandekerckhove, E. Vanden Abbeel, P. De Sutter

Ghent University Hospital, Centre for Reproductive Medicine, Ghent, Belgium

Study question: What is the cumulative live birth rate per oocyte-collection-cycle (OCC) after cleavage-stage and blastocyst-stage transfer in combination with day 5 vitrification?

Summary answer: The cumulative live birth rate after fresh blastocyst-stage transfer is significantly higher as compared to fresh cleavage-stage transfer.

What is known already: A recent Cochrane analysis (2016) demonstrated a benefit favouring blastocyst transfer in fresh cycles, however it remains unclear whether the day of transfer impacts on cumulative live birth rates. In day 3 transfer cycles, freezing supernumerary embryos at the cleavage stage results in high cumulative live birth rates as compared to freezing at the blastocyst stage in day 5 transfer cycles. Recent published data however, reveal that vitrification of blastocysts results in equal or even better cumulative live birth rates in day 5 transfers cycles.

Study design, size, duration: In patients (n =1216) treated between January 2012 and December 2015 and having between 5 and 9 zygotes available on day 1, we retrospectively analysed cumulative live birth rate in patients having fresh embryo transfer on day 3 (n = 629) (group 1) or on day 5 (n = 587) (group 2). Cumulative live birth rate was defined as the outcome of the fresh treatment cycle in combination with warming cycles within 1 year following the OCC.

Participants/materials, setting, methods: In both groups, supernumerary blastocysts with at least an expansion status 1, inner cell mass score A, B or C and trophectoderm score A, B or C were vitrified. Continuous variables were compared using Mann-Whitney U test and categorical variables were compared using Fisher's exact test. Cox's hazard regression was used to estimate the hazard ratio (HR) ($p < 0.05$).

Main results and the role of chance: In group 1 and 2, mean number of oocytes retrieved (11.4 ± 4.1 vs 11.6 ± 4.3; NS) and mean number of embryos vitrified (2.5 ± 1.4 vs 2.5 ± 1.4; NS) were similar. The transfer cancellation rate was significantly lower in group 1 as compared to group 2 (2.7% (17/629) versus 8.0% (47/587) ($p < 0.0001$). The cumulative live birth rate per OCC in group 1 was significantly lower (36.4% (229/629) as compared to group 2 (42.1% (247/587) ($p = 0.046$). Cox regression analysis showed that for fresh transfer, group 2 had significantly higher hazards for live birth rates compared to group 1 (HR=1.54, 95% CI=1.24-1.92, $P < 0.001$). For the first, second and more warming cycles, the hazard rate did not differ significantly ($p = 0.17$ and $p = 0.556$, respectively).

Limitations, reasons for caution: This study is limited by its retrospective design and patients in group 1 were treated from January 2012-December 2013 and patients in group 2 from January 2014-December 2015. For both groups, not all vitrified blastocysts have been warmed yet.

Wider implications of the findings: In patients having between 5 and 9 zygotes on day 1 of the oocyte-collection-cycle, the fresh transfer policy should be blastocyst transfer.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 71: FEMALE (IN)FERTILITY - BASIC RESEARCH

Wednesday 5 July 2017 Plenary 2 14:00–15:15

O-274 Investigation of the protective effect of erythropoietin against cisplatin-induced ovarian damage: a rat model

C. Dayangan Sayan[2], O. Banu Tulmac[1], G. Karaca[4], Z.S. Ozkan[1], S. Yalçin[5], T. Devrim[3], N. Dindar Badem[6]

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Study question: Could erythropoietin (EPO) reduce the cisplatin induced ovarian damage in rats?

Summary answer: Concomitant EPO treatment with cisplatin chemotherapy preserves rat ovarian reserve.

What is known already: Cisplatin is a widely used chemotherapeutic agent for treatment of epithelial carcinomas. Cisplatin kills the dividing cells. It's toxic effects on central nervous system, kidney, testis, ovary have been notified widely. Ovarian failure due to cisplatin has been announced with an odd ratio of 1.77. Ovarian damage of cisplatin brings on menstrual irregularity, premature menopause, infertility, decreased life quality and raised medical costs. EPO, hematopoietic growth factor originated from kidney, has antiapoptotic and antioxidant effects. Recent studies have also shown that EPO can reduce the damage of cisplatin on neural system, kidney and heart.

Study design, size, duration: Thirty, female, 10-12 weeks old, 250–280 g, Wistar-Albino rats were used in the study. Rats were randomly divided into three groups: Control group (N = 10): Intraperitoneal salin infusion. Cisplatin group (N = 10): Intraperitoneal 7 mg/kg cisplatin (Cisplatin®, Koçak Farma, Turkey) twice a week. Cisplatin+EPO group (N = 10): Intraperitoneal 7 mg/kg cisplatin (Cisplatin®, Koçak Farma, Turkey) twice a week and subcutaneous 200 IU/kg/day EPO (Eprex®; Janssen-Cilag AG, Switzerland) for 7 days.

Participants/materials, setting, methods: After a midline laparatomy, the right ovaries were removed and blood samples were obtained from heart. Serum AMH concentrations were measured by ELISA method. Primordial, preantral, small antral and large antral follicles were counted after hematoxylin and eosin staining. Ovarian damage; including follicular cell degeneration, vascular congestion, hemorrhage and inflammation (neutrophil infiltration), was scored histologically using a graduated scale (0 = none; 1 = mild; 2 = moderate; and 3 = severe).

Main results and the role of chance: In cisplatin group, there was a significant decreament in posttreatment AMH level compared to pretreatment AMH level (17.69 ± 5.6 vs 14.93 ± 5.1; $p < 0.01$). But, there was no significant difference between pre- and post-treatment serum AMH levels in control and Cisplatin + EPO groups. Total ovarian damage score of cisplatin group was significantly higher than scores of control and cisplatin+EPO groups. The mean primordial follicle counts of control (7.8 vs 3.8, $p>0.01$) and cisplatin+EPO (8.4 vs 3.8, $p < 0.01$) groups were significantly higher than that of cisplatin group. The mean preantral follicle count of control group was significantly higher than that counts of cisplatin (8.4 vs 3.1) and cisplatin+EPO (8.4 vs 3.4) groups. We observed positive correlation between preantral follicle counts and posttreatment AMH levels (R=0.45, $p = 0.012$). After categorization of cisplatin and cisplatin+EPO groups according to having total ovarian damage score and post-treatment AMH level higher or lower than mean value, binary logistic regression

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analysis was performed. Total ovarian damage score showed no influence on posttreatment AMH level.

Limitations, reasons for caution: We could not investigate ovarian tissue AMH level, so we could not be able to compare serum AMH levels with tissue AMH levels. This is an experimental study and our results can not generalize in humans.

Wider implications of the findings: EPO administration to cisplatin chemotheraphy could ameliorate the ovarian damage, serum AMH levels and get better ovarian reserve in female rats. After additional studies in animals and humans, EPO administration to cisplatin therapy might be suggested against ovarian reserve decreament.

Trial registration number: none.

O-275 Metaphase II oocytes obtained after luteal phase stimulation have the same intrinsic quality as eggs from the follicular phase stimulation.Results from a multicentre prospective study

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Study question: Are metaphase II (MII) oocytes obtained after the follicular phase stimulation (FPS) and luteal phase stimulation (LPS) equivalent in terms of developmental and reproductive competence?

Summary answer: MII oocytes from LPS showed a similar intrinsic quality as eggs from FPS for fertilization,blastulation,euploidy rates and ongoing pregnancy after euploid single embryo transfer (eSET)

What is known already: Follicular development is a dynamic process. Evidences suggest that 'waves"of antral follicles develop cyclically 2-3 times during the same menstrual cycle, overtaking the classic theory that in women only one cohort of antral follicles grows during the follicular phase (FP) of a menstrual cycle. Indeed, ovarian stimulation could be initiated at any time of the cycle, also in the luteal phase (LP). These observations led to the introduction of two new protocols for ovarian stimulation: double stimulation in the same menstrual cycle (Duostim) for poor prognosis patients and random-start for fertility preservation.Preliminary promising clinical data stimulated a deeper investigation of these strategies

Study design, size, duration: Multicenter prospective study performed between January2015-October2016. 208 patients(mean female age=39.7 ± 3.2 range 30.0-44.0)with reduced ovarian reserve(AMH≤1,5 ng/ml,antral follicular count ≤6,and/or ≤ 5oocytes retrieved in a previous cycle)underwent oocyte retrieval after FPS and LPS.The primary outcomes were MII oocytes' intrinsic quality defined as fertilization,blastulation,euploidy rates and ongoing implantation after eSET of blastocyst obtained in the LPS versus FPS from the same patients.Secondary outcomes were the number of MII oocytes,blastocysts, euploid blastocysts and ongoing pregnancies obtained in LPS versus FPS.

Participants/materials, setting, methods: Both FPS and LPS were performed with rFSH in an antagonist protocol. After the first oocyte retrieval from FPS we waited five days before starting LPS. All embryos obtained were cultured to blastocyst, underwent trophectoderm biopsy and vitrification. A single chromosomal analysis was performed through qPCR on all the biopsies obtained after both FPS and LPS. eSETs were performed in a subsequent natural or artificial cycle

Main results and the role of chance: The number of MII oocytes after FPS and LPS were 763 (3.6 ± 2.3;1-16) and 960 (4.5 ± 2.9;1-15), respectively ($p < 0.001$). The fertilized oocytes were 509 (2.4 ± 2.0;0-15) and 707 (3.3 ± 2.0;0-13), ($p < 0.001$). The fertilization rate was 66.7% versus 73.6% (NS). The blastocyst obtained after FPS and LPS were 234 (1.1 ± 1.0;0-4) and 364 (1.7 ± 1.6;0-9) ($p < 0.001$). The blastulation rate was 46.0% versus 51.5% (NS). The euploid blastocysts obtained after FPS and LPS were 99 (0.5 ± 0.7;0-3) and 154 (0.7 ± 1.0,0-6), respectively ($p = 0.005$). The euploidy rate was 42.3% (n = 99/234;95%CI:35.9%-48.9%) versus 42.3% (n = 154/364;95% CI:37.1%-47.5%) in FPS and LPS and 12.9% (n = 99/763; 95%CI=10.7%-15.6%) and 16.0% (n = 154/960; 95%CI=13.8%-18.5%) in FPS and LPS, per biopsied blastocyst and MII oocytes, respectively (NS). Overall, the rate of cycles with at least one euploid blastocyst increased from 34.4% (n = 74/215; 95%CI=28.1%-41.1%) in the FPS-only to 57.7% (n = 124/215; 95%CI=48.4%-61.7%) after Duostim ($p < 0.001$). Thirty-four eSETs of blastocysts from the FPS and 37 from the LPS were performed up to date and no difference was shown in terms of ongoing pregnancy rate: 41.2% (n = 14/34; 95%CI=24.7%-59.3%) versus 45.9% (n = 17/37; 95%CI=29.5%-63.1%) (NS).

Limitations, reasons for caution: A more thorough assessment of oocyte quality after LPS versus FPS in terms of gene expression, metabolism and dynamic developmental parameters is still eagerly needed. Moreover, the neonatal outcomes and the cost-effectiveness especially of Duostim need yet to be evaluated.

Wider implications of the findings: The evidence of multiple follicular waves during a single menstrual cycle in women opened important implications for the treatment of infertility. Here, we report that LPS allows higher yields without affecting the developmental and reproductive potential of the oocytes obtained.Thus, Duostim and random-start ovarian stimulation may be considered clinically-valuable strategies.

Trial registration number: none.

O-276 In Vitro Maturation (IVM) culture conditions: the effect of oxygen tension and medium volume

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Study question: Does modulation of oxygen tension and culture medium volume during human IVM influence maturation efficiency and embryo development?

Summary answer: Reduction in oxygen tension (5%) and culture medium volume (1 Cumulus Oocyte Complex (COC)/20 µl) in a 30 h grouped culture IVM system significantly impaired blastocyst development.

What is known already: Low (5%) oxygen tension improves embryo development in vitro (*Nastri et al., 2016*). However, the optimal oxygen tension during IVM of human oocytes has not been established. Differential oxygen tension during IVM in mice induced differences in developmental potential (*Banwell et al., 2007*) and cumulus cell gene expression (*Kind et al., 2015*). Oocytes and cumulus cells in antral follicles have different oxygen requirements, with oocytes dependent on oxidative phosphorylation (*Cinco et al. 2016*). Dissolved oxygen available to the oocyte depends on both oxygen tension and IVM medium volume. Grouped IVM culture may have a beneficial effect on IVM outcome.

Study design, size, duration: A RCT on sibling oocytes was performed in 29 consecutive PCOS patients who had at least 11 immature oocytes retrieved after non-hCG triggered IVM. COC were randomly divided into either groups of 8-10 COC/500 µl in IVM medium at atmospheric oxygen tension (20% O_2; 'High O_2-IVM') or groups of 3-5 COC in 60-100 µl medium at 5% O_2 ('Low O_2-IVM' with a strict ratio of 1 COC/20 µl).

Participants/materials, setting, methods: IVM was performed in Origio IVM System: COC were washed in LAG medium and incubated for 30 h in IVM medium supplemented with 75 mIU/ml HP-hMG, 100 mIU/ml hCG and

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10 mg/ml HSA in four-well dishes with 500 µl or macrodroplets of 60-100 µl under oil. IVM was performed in incubators containing 6% CO_2 at 37°C under either 20% O_2 or 5% O_2. After IVM and ICSI, embryos were cultured individually in 25 µl Cleav™ and Blast™ medium (Origio).

Main results and the role of chance: In total, 169 COC were randomly allocated to 'High O_2-IVM', 137 COC to 'Low O_2-IVM'. Using mixed-effects regression analysis (to account for the fact that multiple oocytes derived from the same patient), comparable maturation rates (49% vs 48%; p = 0.36), fertilization rates (73% vs 73%; p = 0.92) and embryo quality scores on day 3 (top quality: 40% vs 39%; good quality: 29% vs 29%; p = 0.9) were obtained in 'High O_2-IVM' vs 'Low O_2-IVM' respectively. For 14 patients, ≥4 good-quality embryos were available on day 3 and cultured to day 5/6. On day 5/6 a utilization rate (embryos of sufficient quality for transfer or cryopreservation per fertilized oocyte) of 44% was obtained in 'High O_2-IVM', 18% in 'Low O_2-IVM' (p = 0.002).

The combined reduction of oxygen tension and COC/volume ratio during the IVM phase only specifically affected blastocyst formation, with fewer top quality blastocysts and more arrested embryos after extended culture. This finding was unexpected considering good blastocyst development by others when culturing individual COC in 5% O2 during IVM (Walls et al., 2015). Although fewer good quality blastocysts were produced in our 'Low O2-IVM' system, clinical pregnancies were obtained in both 'Low O2-IVM' and 'High O2-IVM' culture conditions.

Limitations, reasons for caution: Since oxygen tension conditions and COC/volume ratio during IVM were investigated in parallel, we could not identify whether blastocyst development was impaired by oxygen tension, COC/volume ratio or both. The results are based on a small number of IVM cycles.

Wider implications of the findings: Every specific in vitro culture system, including human IVM, requires fine-tuning of physico-chemical parameters. Seemingly small adjustments in culture conditions can have profound, unexpected effects on embryonic development. Extended culture to day 5 provides important additional information on IVM-derived embryo development under differential IVM culture conditions.

Trial registration number: not applicable.

O-277 Liver X Receptors in granulosa cells regulate human oocyte meiosis resumption

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Study question: Do Liver X Receptors (LXRs) pathway in granulosa cells regulate the human oocyte meiosis resumption?

Summary answer: Our results show an increase of LXR activity in the activation of human oocyte meiosis resumption.

What is known already: Previous studies in animal model showed clearly that lipid homeostasis influences the oocyte meiosis. However, the signaling pathway is not clearly identified. Oxysterols are molecules derived from the cholesterol synthesis pathway. They are the ligands of the liver X receptors (LXRs). In mice deficient of LXR genes, the oocytes are unable to resume meiosis. In human, The LXR signaling pathway has never been investigated in this process.

Study design, size, duration: From 73 oocytes of 15 women (35 ± 3 years) with tubal infertility, Granulosa cells surrounding oocytes were isolated during in vitro fertilization (IVF) process and analyzed. The oocyte meiosis resumption was considered as positive (GC+) when two pronuclei and two polar bodies were observed at zygote stage and negative (GC-) when the oocyte remained blocked at metaphase II stage (at day 1). The embryos derived from CG+ oocytes were all top quality.

Participants/materials, setting, methods: The lipid accumulation in granulosa cells was assessed after oil red O staining. The expression levels of key enzymes of oxysterol synthesis, LXRs and their downstream genes were measured by qPCR.

Main results and the role of chance: Lipid accumulation, measured by oil red O staining, was higher in GC- (66 ± 4.9%) compared with CG+ (43 ± 17.8%; p = 0.02). We showed that the accumulations of HMGCoS, SQLE, LSS and Cyp51 (genes involved in oxysterols synthesis) were upregulated in GC+ versus GC- respectively at 102 ± 14.1, 60 ± 9.9, 56 ± 17 % and 48 ± 10.9% (p < 0.05). Although LXRs genes expression was not affected, we observed an increased level of some direct target genes such as LDLr (58 ± 16.2 %) involved in cholesterol uptake (p < 0,05).

Limitations, reasons for caution: These results should be confirmed by measurements of oxysterols levels in granulosa cells. In addition, LXRs protein levels and its target genes should be quantified by western blot and immunofluorescent experiments.

Wider implications of the findings: The analysis of LXR pathway could be used to detect in reliable way the oocytes with high potential of resumption meiosis in a IVF program. Moreover, these novel findings may help to better understand the physiopathology of oocyte meiosis failures.

Trial registration number: None.

O-278 The motility and number of mitochondria in the advanced age embryos

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Study question: What is difference of mitochondria between advanced age embryo and young embryo?

Summary answer: The mitochondria movement dramatically decreases in the advanced age embryo.

What is known already: Mitochondrial functionality is important for oogenesis and embryo development. However, the motility of mitochondria has not yet been clearly identified for embryo development. Mitochondria are unique cellular organelles that have their own genome, the mitochondrial DNA (mtDNA). The mtDNA encodes proteins involved in oxidative phosphorylation and ATP synthesis. Therefore, it is important for maintaining life via modulating metabolic pathways. The specific mitochondrial localization and movement are important for their function and activity. Mitochondrial activity affects the quality of oocytes and embryos directly. Low-quality oocytes show mitochondrial dysfunction, such as altered mitochondrial morphology and decreased number of mitochondria.

Study design, size, duration: set-up of functional activation of mitochondria for advanced age female infertility model study. Sixty-head female mouse (30 weeks old) during1years study.

Participants/materials, setting, methods: All procedures for animal breeding and care were approved by the IACUC of Sahmyook University, Seoul, Republic of Korea. Superovulation was induced in 30-weeks-old female mice and in a 2~4weeks female mice as controls. We then performed the mitochondria analysis by PCR and image analysis in embryos with the mitotracker-RFP stained /tubulin-GFP injected embryo. Then we measured the mitochondria's moving speed in young and old mouse embryos by the ZESS 2016 program.

Main results and the role of chance: Young age embryos showed hyperactive motility of the mitochondria from one-cell to a blastocyst. However, advanced age embryos had no mitochondrial motility from all pre-implantation development. Specifically, young embryos presented one nm per one second of the speed ratio base on live imaging analysis. Mitochondria motility is important to profile for the functionality and the biogenesis support to cell behavior. The process for embryo development requires amount of energy from mitochondria at a specific location like the nuclear and the endoplasmic reticulum region. However, the loss in movement of the mitochondria means that it cannot support well-conducted biogenesis for specific regions for cellular processing. Therefore, it needs to improve mitochondrial functional activity by enhancing movement to specific localization. We need to further study for the enhancement of mitochondrial motility and the functional activity for the advanced age embryo.

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Limitations, reasons for caution: This study is limited animal study for pre-clinical application.

Wider implications of the findings: The oocyte and embryo needs the functional activity of the mitochondria for high quality embryo. Our findings will probably contribute to finding ways to restore the function of mitochondria in advanced age embryos. Furthermore, it shows the possibility of overcoming infertility due to aging in humans.

Trial registration number: This is not a critical trial

SELECTED ORAL COMMUNICATIONS

SESSION 72: NEW ENDOCRINE INTERVENTIONS

Wednesday 5 July 2017 Room A 14:00–15:15

O-279 Transvaginal hydrolaparoscopy versus hysterosalpingography in the work-up of subfertile women, a randomised clinical trial

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Study question: What is the diagnostic accuracy and acceptability of transvaginal hydrolaparoscopy (THL) compared to hysterosalpingography (HSG) as a primary tool to assess tubal patency?

Summary answer: THL and HSG are safe and well-tolerated. The number of women diagnosed with tubal pathology is comparable. THL gives more information about adhesions and endometriosis.

What is known already: Tubal pathology is found in 10-20% of infertile women. To assess tubal function in these women, various diagnostic tests are available, among which HSG has been around for over a century. THL is a relatively new technique, that allows direct visualisation of the pelvic cavity and tubes in an outpatient setting. Here, we compare THL and HSG as a first line tubal patency test in a randomised clinical trial (RCT).

Study design, size, duration: We performed a multicenter RCT (NTR3462) in 4 teaching hospitals in the Netherlands. We included subfertile women. The primary outcome of the trial was cumulative live birth rate at 24 months. In this abstract we present findings at THL and HSG, as well as complication rates and VAS scores for pain and acceptability. To exclude a difference in live birth rate larger than 6%, we needed to randomize 1,330 women (alpha .05, beta .80).

Participants/materials, setting, methods: Subfertile women scheduled for tubal patency testing were eligible. Women with a positive Chlamydia PCR, prior tubal testing or tubal surgery, retroverted uterus, masses/or cysts in the pouch of Douglas, or allergies to iodine or methylene blue were excluded. After written informed consent, women were randomized to THL or HSG. Pain and acceptability were measured on a visual analogue scale (VAS). Here, we compare the findings at THL and HSG.

Main results and the role of chance: Between May 2013 and October 2016, we allocated 152 women to THL and 151 to HSG, of whom 17 and 12 women conceived naturally before the scheduled procedure, respectively. In seven women allocated to THL (5%) the procedure was not possible, either due to preperitoneal placement of the trocar or due to pain, while in one women allocated to HSG the procedure was stopped due to pain (RR 6.9, 95% CI 0.87 to 56).

In women undergoing THL we found no abnormalities in 86%, endometriosis in 8%, adhesions in 4%, unilateral tubal occlusion in 6%, and bilateral tubal occlusion in 1% of women. In women undergoing HSG, we found no abnormalities in 90%, unilateral tubal occlusion in 8% and bilateral tubal occlusion in 2%. The risk of detecting an abnormality was 13.2% versus 10.1% (RR 1.3 95% CI 0.69 to 1.5).

In the THL group one woman had a bowel perforation which was handled conservatively, while two women had a bleeding of the posterior vaginal wall that needed stitching. In the HSG group one woman had a bleeding after the procedure. Pain scores (4.9 versus 5.4, $p = 0.17$) and acceptability scores (1.9 versus 2.3, $p = 0.30$) did not differ significantly.

Limitations, reasons for caution: Our original sample size was set at 1,330 women. Since attempts to fund the study were not successful, the original sample size could not be reached and the study was halted after 3 years and randomization of 303 women.

Wider implications of the findings: Both THL and HSG are in terms of safety and pain acceptable methods to test tubal patency. THL gives more information about endometriosis and adhesions than HSG, but the clinical implication of these findings is unclear.

Trial registration number: NTR3462.

O-280 Multivariate Meta-analysis is required for partially reported multiple endpoints and demonstrates the benefit of rh-LH supplementation on live birth for Poor Ovarian responders

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Study question: Compared with clinical or ongoing pregnancy, Live Birth is much less reported in trials. How to correct important biases and inconsistencies observed on the separate results of these endpoints?

Summary answer: Compared with separate meta-analysis of each endpoint, the multivariate meta-analysis model provides consistent estimates, better precision, and minimizes biases and paradoxical results.

What is known already: In ART, almost all the meta-analyses report results of multiple endpoints, although these endpoints were partially reported. When analyzing separately and independently each endpoint, results cannot be compared as there are based on different studies and number of studies. Necessitating a longer follow-up, live birth (LB) is the less reported endpoint, other measurements such as biochemical (BP), clinical (CP), On-going pregnancies (OP) being more often reported. Reporting independent analyses of these endpoints generates inconsistent results essentially caused by power difference, and because the correlation between these endpoints is not accounted for. No appropriate technique was used as yet.

Study design, size, duration: We illustrate this problem on the widely debated question of the benefit of LH-supplementation in ART cycles. We conducted a meta-analysis based on the largest possible selection of randomized controlled trials having compared LH supplementation added to FSH with FSH Alone. 54 RCTs (n = 7702, 36 and 18 studies on Poor(POR) and Normal (NOR) Ovarian responders). Pregnancy was partially reported by 26, 47, 16 and 8 studies for BP, CP, OP and LB endpoints, respectively.

Participants/materials, setting, methods: We compared the results obtained by separate and independent analyses with those found with a random multivariate model based on BP, CP, OP and LB, their sequential occurrence modeled following an ARMA hetero-scedastic autoregressive AR(1). Intent to treat analysis was only considered. The risk ratio RR (rh-LH+FSH/FSH) on POR compared with NOR constituted the main endpoint and was tested by using the binary moderator POR-NOR as fixed meta-regressor.

Main results and the role of chance: Strong correlations were found between the four endpoints and in particular between sequential endpoints confirming our autoregressive hypothesis. We compared the Risk Ratio RR (95%CI and Pvalue) between the univariate separate model and the multivariate model (table 1).

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Table 1 Comparison of the results of the univariate separate analyses with the multivariate result for Biochemical (BP), clinical (CP) and ongoing (OP) pregnancies and Live birth (LB).

	Univariate				Multivariate			
	RR	95%CI		P	RR	95%CI		P
BP (22)	1.36	.58	2.19	.29	1.19	.81	1.73	.23
CP (39)	1.29	1.02	1.64	.003	1.27	1.03	1.59	.04
OP(13)	1.22	.78	2.05	.18	1.36	1.02	1.88	.03
LB (8)	1.68	.64	3.96	.21	1.41	1.06	2.07	.01

The precision of univariate estimates was poor, heterogeneous, and inconsistent among sequential endpoints: Univariate model found a strong although non-significant RR=1.68 for LB poorly consistent with a significant RR=1.29 for CP. In contrast, multivariate estimates and related p-values were more consistent and stable with respect to sensitivity analyses: A non significant effect is identified for the first pregnancy measurement (BP) followed by a continuous increase of the observed benefit until final endpoint (LB) characterized by a significant effect.

Limitations, reasons for caution: This never used approach in ART considerably improves the consistency of the results, however, results remain dependent of some hypothesis underlying the correlations between pregnancy measurements. The multivariate model is also more complex which may limit its use in clinical applications.

Wider implications of the findings: Life Birth constitutes the ultimate and paradoxically the less reported endpoint in controlled trials and caused important biases in previous meta-analyses. Multivariate meta-analysis based on the between-endpoint correlation provides consistent, unique and comparable estimates during the luteal phase, providing evidence of a significant benefit of rh-LH supplementation on live birth.

Trial registration number: not applicable.

O-281 Follicle developmental inhibition at the primordial stage without increasing apoptosis, by the combination of everolimus and verapamil: a preclinical randomized study

I. Kosmas[1], M. Pargianas[2], C. Kitsou[3], A. Dalkalitsis[4], S. Markoula[5], E. Kolibianakis[6], B. Tarlatzis[6], I. Georgiou[7]

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Study question: The aim of this study was to test whether follicular stop and reactivation can be achieved and at what extend.

Summary answer: There is significant increase in the primordial follicles after the inhibitory effect of Everolimus plus verapamil.In parallel, atresia was reduced at the control levels.

What is known already: TSC/Mtor pathway manipulation has a profound effect in rapid follicular activation and Tsc1 and Tsc2 proteins play the major role in this over-activation (Adhikari et al 2009, 2010). Both Mtor complexes (Tsc1 /Raptor and Tsc2 /Rictor) will be inhibited alone or in combination, with Everolimus and Fisetin respectively. The combination of Everolimus plus Verapamil (an CYP3A inhibitor and everolimus enhancer) will be used, in order to lower the dose of everolimus and increase the effect. It is not known yet whether after follicular inhibition, activation could be achieved. Therefore, an effort to activate follicles afterwards, with FSH, will take place.

Study design, size, duration: 45 female immature Wistar rats were divided, after randomization, in five groups.All animals received the intervention from 9th week and for 8 weeks in total.Control group received 0.2 ml of intraperitoneal saline. Next group received everolimus (1,5mgr/Kg daily). The third group received Fisetin (10 mg/kg daily).The fourth group received Everolimus (1,5mgr/Kg daily) plus Fisetin (10 mg/kg daily) every other day. Fifth group received Everolimus (0,75mgr/kg daily) plus (Verapamil 2,5mgr/kg every other day).

Participants/materials, setting, methods: Following to, left ovary of each animal was removed and primordial, primary, secondary, antral, atretic follicles and Corpora Lutea measured.After three weeks, animals received 35 IU FSH for 4 days and 35 IU of hCG on the 5th day. After that, right ovary examined for the same parameters. AMH, estradiol and progesterone measurements took place at the end of the first and second intervention.

Main results and the role of chance: Significant difference has been observed at the primordial stage of development (P=0, 0159) (33, 3 ± 15,6) at the Everolimus plus Verapamil group compared with Everolimus alone (28 ± 13,7) and the control group (25,2 ± 16,4). This group, had the less number of atretic follicles (12, 16 ± 6, 26) (P=0, 0001) followed by the control group (15, 66 ± 6, 68).After ovarian stimulation, no significant difference has been observed between the five groups for the primordial (P=0,214), primary (P=0,435) follicles and corpus lutem (P=0,201). From the other side, significant difference has been observed in the pre-antral follicles (P=0,022), antral follicles (P=0,0001), Graafian (secondary) follicles (P=0,044) and atretic follicles (P=0,0001). Especially in the atretic follicles, control and everolimus plus verapamil group showed the lowest number (7, 7 ± 2,1 and 18,7 ± 7,8) respectively.

Limitations, reasons for caution: A randomized study that assigns pharmaceutical interventions to all animal groups. Both ovaries serve as internal control for each animal. Both pathologists, performed the histological analysis were blinded for the intervention examined on each slide.

Wider implications of the findings: Patients that wish to withhold their fertility for a long time or patients that must undergo cytotoxic therapies should have the option of keeping their ovarian follicles in a dormant state. Such pharmaceutical schemes may have position in POF and fertility preservation.

Trial registration number: None.

O-282 Assessment of the reliability of the Bologna criteria in predicting ovarian response and live birth rates in subsequent IVF attempts: An external validity study

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Study question: Are the Bologna criteria reliable in predicting ovarian response and live birth rate in subsequent IVF cycles?

Summary answer: The Bologna criteria based on age and oocyte yield in the first IVF cycle successfully predict ovarian response and clinical outcome in subsequent IVF attempts.

What is known already: The ESHRE Working Group on Poor Ovarian Response defined a set of variables to standardize the definition of poor responders. Several concerns have been raised regarding their prognostic significance and their external validity, which have not been vigorously tested.

Study design, size, duration: This retrospective study included 2047 women who had undergone two (n= 1370) and three (n = 667) consecutive IVF cycles between April 2010 and December 2013.

Participants/materials, setting, methods: We analyzed the ovarian response and live birth rates in subsequent IVF attempts of patients who were defined as poor responders according to the Bologna criteria based on a history of collection of ≤ 3 oocytes in two prior IVF cycles for women aged <40 years or; in a single cycle for women aged ≥40 years.

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Main results and the role of chance: Age and ovarian response in the first IVF cycle were closely related with the prospects in the subsequent stimulation cycles. The Bologna criteria defined poor responders were more likely to show recurrent poor ovarian response both in the second (72.4% vs. 17% respectively, p < 0.0001; OR(95%CI): 0.07 (0.047-0.13)) and the third IVF cycle (85.4% vs. 18.5% respectively, p < 0.0001; OR(95%CI): 0.03 (0.02-0.07)) compared to women who did not fulfil the criteria. Likewise, Bologna criteria defined poor responders had significantly lower live birth rates in their 2nd (6.9% vs. 29.9% respectively, p < 0.0001; OR(95%CI): 5.76 (2.63-12.4)) and 3rd IVF attempts (11.5% vs. 18.5% respectively, p < 0.001; OR(95%CI): 2.81 (1.51-5.3)). In the 3rd IVF cycle, younger Bologna criteria defined poor responders had significantly lower live birth rate compared to younger optimal responders (13.5% vs 28.6% respectively, p < 0.05; OR(95%CI): 0.38 (0.17-0.87)) whereas live birth rate of older patients who were defined as poor responder according to the Bologna criteria was not any different from those who do not fulfill the criteria (9.1% vs. 13.7% respectively, p = 0.56; OR(95%CI): 0.63 (0.20-2.1)).

Limitations, reasons for caution: The retrospective design and inability to evaluate all of the Bologna criteria due to unavailability of ovarian reserve tests are the major limitations of the study.

Wider implications of the findings: The Bologna criteria are the best available tool for the definition of poor responders and should be adopted universally into clinical practice. Given the heterogeneity of the population covered by the criteria, their descriptive or prognostic validity might be increased by detailed description of patient characteristics in future clinical trials.

Trial registration number: None.

O-283 Low serum Progesterone the day of embryo transfer is associated with diminished ongoing pregnancy rate in artificial endometrial preparation cycles. A prospective study

E. Labarta, G. Mariani, N. Holtmann, P. Celada, J. Remohí, E. Bosch

Instituto Universitario IVI Valencia, Human Reproduction, Valencia, Spain

Study question: Is there a relationship between serum Progesterone (P) and endometrial volume the day of transfer with the ongoing pregnancy rate in artificial endometrium preparation cycles?

Summary answer: Patients with serum P <9.2 ng/ml the day of embryo transfer had significantly lower ongoing pregnancy rate than the rest of patients.

What is known already: A window of optimal serum P levels during the embryo implantation period (e.g. after 7 days of P administration) has been described in artificial endometrium preparation cycles. This interval of P concentration has been defined to be between 70 and 99 nmol/L (22.0-31.1 ng/mL), while serum P levels < 50 nmol/L (15.7 ng/mL) and > 100 nmol/L (31.4 ng/mL) are related to significantly lower pregnancy rates. Also, it has been shown that an endometrial volume <2.5 ml is related to a poorer outcome, and that a volume < 1 ml is associated with a null pregnancy rate.

Study design, size, duration: Prospective cohort study including 244 patients undergoing embryo transfer after an artificial endometrial preparation cycle with estradiol valerianate and vaginal micronized progesterone (400 mg/12 hours). The study was performed between February 22nd, 2016 and October 25th, 2016 (8 months). Sample size was calculated to detect a 20% difference (35-55%) between 2 groups according to serum P levels, in a two sided test with a statistical power of 80% and a confidence level of 95%.

Participants/materials, setting, methods: Patients undergoing their 1st/2nd oocyte donation cycle, aged <50, BMI <30 Kg/m^2, with a normal uterine cavity in 3D ultrasound, a triple layer endometrium >6.5 mm, and being transferred 1-2 good quality blastocysts; in a private infertility centre. Serum P determination and 3D ultrasound of the uterine cavity were performed the day of embryo transfer. Endometrial volume measurements were done using VOCAL system. Primary endpoint was ongoing pregnancy rate beyond the 12th week of pregnancy.

Main results and the role of chance: Of 244 patients recruited, 211 fulfilled all the inclusion/exclusion criteria. In 27 patients a Müllerian abnormality was diagnosed with the 3D ultrasound (19 T-shaped, 6 septate partial/complete, 1 hemiuterus and 1 bicorporeal uterus). In 6 cases there was a protocol violation.

The mean age of the included women was 41.3 ± 4.4; BMI: 22.3 ± 2.6; Endometrial thickness: 8.9 ± 1.7 mm. Serum P the day of embryo transfer was 12.7 ± 5.4 ng/mL. (p25: 9.2; p50:11.8; p75:15.8).

The ongoing pregnancy rates according to serum P levels were: <p25: 32.7%; p25-p50: 49.1%; p50-p75: 58.5%; >p75: 50.9%. Women with serum P <p25 (<9.2 ng/mL) had a significantly lower ongoing pregnancy rate compared to the rest of patients: 32.7% vs. 52.8%; p = 0.016; RR (95% CI): 0.62 (0.41-0.94).

Endometrial volume was 3.2 ± 1.3 ml. Serum P the day of embryo transfer did not correlate with the endometrial volume. Only women with a very low endometrial volume (n = 10), (<p5 = 1.4 ml) were associated with a poor ongoing pregnancy rate (22.2% vs. 48.3%).

A logistic regression analysis adjusting for all potential confounders showed a statistically significant relationship between serum P the day of embryo transfer and the likelihood of ongoing pregnancy (OR: 1.10: 95%CI: 1.02-1.19), p = 0.01.

Limitations, reasons for caution: Only women with a normal uterine cavity, an appropriate endometrial thickness and good quality blastocysts transfer were included. Extrapolation to an unselected population needs to be validated. The role of endometrial volume could not be fully defined, as only a few patients presented a very low volume.

Wider implications of the findings: The present study suggests that there is a minimal threshold of serum P values the day of embryo transfer that needs to be reached in artificial endometrial preparation cycles to optimize the outcome. An upper threshold could not be defined.

Trial registration number: The www.clinicaltrials.gov registration number is NCT02696694

SELECTED ORAL COMMUNICATIONS

SESSION 73: NEWS AND VIEWS ON CLINICAL ANDROLOGY

Wednesday 5 July 2017 Room B 14:00–15:15

O-284 Prospective comparison of vitrification in two different devices and slow freezing techniques for cryopreservation of small numbers of human spermatozoa

S. Mnallah[1], C. Herbemont[1], M. Grynberg[2], C. Sifer[1]

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Study question: When cryopreservation of small numbers of human spermatozoa is needed, is vitrification more efficient than the slow freezing (SF) method? If yes, in which device?

Summary answer: Sperm vitrification in the Cell Sleeper as device enables higher recovery and survival rates, while requiring less time than vitrification in stripper tip or SF.

What is known already: Sperm cryoconservation is widely used in combination with ART (Assisted Reproductive Technology) techniques. Currently, the reference method remains the conventional SF protocol. However, despite many years of research, significant numbers of spermatozoa still do not survive cryopreservation or cannot be retrieved because of the large volumes commonly contained in straws (30-500 µL). Hence, the SF procedure is not suitable for single spermatozoa. Vitrification, using different devices, is a novel technique that has been widely used for embryo storage, but it has not been applied yet to routine sperm cryopreservation.

Study design, size, duration: Prospective study, conducted between June and December 2016, in a university hospital. Two sperm vitrification devices, the ready-to use Cell Sleeper (Nipro, Japan) (n = 12) (group 1) and the stripper tip (Origio, Denmark) (n = 11) (group 2) were tested, and compared to the SF method, using a classical straw (CBS, France) (n = 14) (group 3). Selected sperm from ejaculates were obtained from the same fertile man after written consent.

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Participants/materials, setting, methods: Ten motile spermatozoa per device were isolated with an ICSI pipette and frozen with Spermfreeze (Fertipro, Belgium). Vitrification devices were cooled in liquid nitrogen (LN2) vapor for 2 minutes, plunged and stored in LN2. For SF, a programmable freezer was used. Samples were warmed at room temperature for 1 minute. Frozen-thawed spermatozoa were searched within 20 minutes per device. Motility, recovery and survival rates, using the hypo-osmotic test if needed, and recovery time were compared.

Main results and the role of chance: Briefly, vitrification in Cell Sleeper (group 1) provided higher recovery rate, motility and survival rate (98.5%; 13,0%; 76.4%, respectively) than the SF procedure (group 3) (37.8% [$p < 0.0001$]; 2.3% [$p = 0.05$]; 28.7% [$p < 0.001$]). Only recovery rate was higher in group 1 than when using vitrification in stripper tip (group 2) (84.5% [$p = 0.03$]). Moreover, recovery time per spermatozoa was faster in group 1 (1.3 minute) than in both groups 2 and 3 (2.2 minutes and 6.6 minutes, respectively [$p < 0.01$]). Furthermore, statistical significance was also achieved when comparing the four above parameters between groups 2 and 3 concluding to the superiority of vitrification in stripper tip over SF method.

Limitations, reasons for caution: Sample size must be increased and further studies are required to assess if ART outcome could be improved using our vitrification procedures in case of severe male factor infertility.

Wider implications of the findings: We conclude that the vitrification of small numbers of human spermatozoa especially using Cell Sleeper device should be applied to patients with a limited sperm resource.

Trial registration number: Not applicable.

O-285 The outcomes of intracytoplasmic sperm injection (ICSI) using sperm of Japanese men with Y chromosome microdeletion

K. Yamaguchi[1], T. Ishikawa[2], T. Takeuchi[3], T. Habara[4], S. Kokeguchi[5], M. Ohashi[6], H. Hibi[7], T. Hara[8], K. Ichioka[9], T. Kawamura[10], T. Matsuyama[11], H. Yoshida[12], S. Okamoto[13], F. Tawara[14], H. Masuda[15]

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[14]*Tawara IVF Clinic, infertility, Shizuoka, Japan*
[15]*Aino Hospital, male infertility, Osaka, Japan*

Study question: To clarify the results of microdissection testicular sperm extraction (micro TESE) of Japanese with Y chromosome microdeletion and ICSI outcomes using sperm from those men

Summary answer: Sperm was retrieved from over 70% patients with azoospermia factor (AZF) c deletions, however, fertilization rate was fairly-low in ICSI using sperm from those men.

What is known already: Microdeletions of Y chromosome, especially in the AZF, are the most common known genetic cause involving spermatogenesis. Deletion frequencies are 2-10% reflecting the composition of study population. Although these deletions are associated with disruption of spermatogenesis, some previous reports revealed the tendency to retrieve spermatozoa by micro TESE from patients with AZFc deletion rather than azoospermic men without Y chromosome microdeletions. When compared with ICSI outcomes for oligozoospermic men without Y chromosome microdeletions or controls, some authors described that ICSI using sperm from men with those deletions resulted in significant low fertilization rates, and high percentage of aneuploidies of embryos.

Study design, size, duration: This study was retrospectively examined a total of 1934 azoospermic or severe oligozoospermic patinets who undergone genetic testing for AZF deletions in 18 Japanese medical centers from April 2007 to June 2016.

Participants/materials, setting, methods: A total of 1934 azoospermic or sever oligozoospermic patinets were examined genetic testing for AZF deletions by Promega Y Chromosome AZF Analysis System (version 2.0). We firstly investigated each type of AZF deletions frequencies, and sperm retrieval rate (SRR) by micro TESE of the azoospermic patients with AZF deletions. In addition, we analyzed the ICSI results using testicular or ejaculatory sperm of men with AZF deletions.

Main results and the role of chance: AZF microdeletions were found in 189 cases (9.8%): 20 AZFa, 15 AZFb, 72 AZFc, 1 AZF a+b, 58 AZFb+c, and 23 AZFa+b+c. Of men with isolated AZFc deletion, spermatozoa were retrieved in 71.4% (30/42) by micro TESE. We could not find spermatozoa from patients with other AZF deletions.

Twenty-nine couples underwent ICSI using testicular sperm retrieved by micro-TESE, and 3 couples underwent ICSI using ejaculatory sperm. In totally, 572 metaphase 2 oocytes were retrieved by 62 oocyte retrieval cycles. Fertilization rate was 46.1% (220/477). We performed 62 embryo transfer (ET) cycles, mean replaced embryos were 1.39 per ET. Implantation rate per total transfer embryos were 19.8% (17/86), and clinical pregnancy rate per ET cycles were 27.4% (17/62). Abortion rate was 23.5% (4/17). Although we could not detect significant differences, the results of ICSI using ejaculatory sperm had better propensity in fertilization rate, implantation rate, and clinical pregnancy rate compared with the results of ICSI using testicular sperm (72.7% vs 44.1%, 50.0% vs 17.5%, 75.0% vs 24.1%, respectively).

Limitations, reasons for caution: The cohort size is relatively low and the study is still ongoing at our institution.

Wider implications of the findings: This is the first relatively large Japanese study to clarify the outcomes of ICSI using sperm of patients with AZFc deletions. The results of this study provide useful actual clinical information for Japanese infertile couples with AZF deletions concerning the adoption of the treatment.

Trial registration number: none.

O-286 Is thyroid hormone evaluation of clinical value in the work-up of males of infertile couples?

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Study question: Is thyroid hormone (TH) evaluation of clinical value in the work-up of males of infertile couples?

Summary answer: Our results suggest that TH evaluation is not mandatory in the work-up of male infertility.

What is known already: A few previous studies performed on a limited series of subjects reported a negative impact of hyper- and hypo-thyroidism on semen volume, sperm density, progressive motility and normal morphology. No previous study has systematically evaluated associations between TH variation, semen parameters and ultrasound characteristics of the male genital tract.

Study design, size, duration: Cross-sectional analysis of a consecutive series of 172 subjects seeking medical care for couple infertility from September 2010 to November 2014.

Participants/materials, setting, methods: Of the entire cohort,163 men (age 38.7 ± 7.6 years) free of genetic abnormalities were studied. All subjects underwent a complete andrological and physical examination, biochemical and hormonal assessment, scrotal and transrectal colour-Doppler ultrasound (CDUS) and semen analysis (including seminal interleukin 8 levels, sIL-8) evaluation within the same day.

Main results and the role of chance: Among the patients studied, 145 (88.9%) showed euthyroidism, 6 (3.7%) subclinical hyper- and 12 (7.4%) subclinical hypo-thyroidism. No subjects showed overt hyper- or hypo-thyroidism. In a multivariate model, after adjusting for confounders such as age, BMI, smoking habit, sexual abstinence, calculated free testosterone, prolactin and sIL-8

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levels, we observed a positive association between fT3 levels, ejaculate volume and seminal fructose levels. When CDUS features were investigated, using the same multivariate model, we found positive associations between fT3 levels and seminal vesicles (SV) volume, both before and after ejaculation (adj. $r = 0.354$ and adj.$r = 0.318$, both $p < 0.0001$), as well as with SV emptying (Δ SV volume; adj.$r = 0.346$, $p < 0.0001$) and echo-texture inhomogeneity. In addition, negative associations between fT4 levels and epididymal body and tail diameters were found. No significant associations between TSH or TH levels and CDUS features of other organs of the male genital tract were found. Finally, when the features of subjects with euthyroidism, subclinical hypo- and hyperthyroidism were compared, no significant differences in seminal or hormonal parameters were found. Conversely, subjects with subclinical hyperthyroidism showed a higher difference between the SV longitudinal diameters measured before and after ejaculation as compared to that of subclinical hypothyroid men, even after adjusting for confounders ($p < 0.007$).

Limitations, reasons for caution: First, due to the cross-sectional nature of the study, neither a causality hypothesis nor mechanistic models can be inferred. In addition, present results are derived from patients consulting an Andrology Clinic for couple infertility, and could have different characteristics from the male general population.

Wider implications of the findings: Although no associations between TH and sperm parameters were observed, this is the first study supporting a positive effect of TH on SV size and a permissive role on the ejaculatory machinery. However, our results do not support a systematic evaluation of thyroid function in males of infertile couples.

Trial registration number: not applicable.

O-287 Seminal carnitine and DNA fragmentation index (DFI) impact progressive sperm motility in oligoasthenospermic men treated with metabolic and essential nutrients, with moderate accuracy

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[5]*American Center for reproductive Medicine, Andrology Center, Cleveland, U.S.A.*

Study question: The aim of the study was to correlate sperm motility with seminal carnitine level and DFI, after 3 and 6 months therapy of oligoasthenozoospermic men

Summary answer: Study showed significant improvement in progressive motility, lower DFI and high seminal carnitine level after 6 months therapy with metabolic and essential nutrients.(

What is known already: L –carnitine (L-C) plays an essential role in long – chain fatty acids oxidation, by providing a shuttle system for free fatty acids. It is taken up by the epididymal cells and realised into the epididymal lumen by difusion. Many nutrients and metabolic compounds affect mitochondrial function. Both L-C and acetyl-L-carnitine(ALC) also have important roles in the postgonadal maturation of spermatozoa

It has been showen that sperm DNA fragmentation is associated with male infertility. Intact sperm DNA is essential for fertilization and genetic trasmission. Abnormal fragmented sperm DNA can be found in 25% of men with abnormal semen parameters.

Study design, size, duration: This study was randomized, double blind, placebo controlled and it examined the effect of test formulation, Proxeed Plus, L-C 2 g and ALC 1 g, as well as vitamins and minerals, in men with oligo- or asthenoteratozoospermia. The protocol was 2 months "wash out" and 6 months therapy (125 patients) or placebo (50 patients) and 2 months follow up (T-2, T0, T+3,T+6). The control group (placebo) 50 patients were administered in a similar way with the placebo

Participants/materials, setting, methods: Men (age group 18 – 50 years), with idiopathic oligospermia, with history of difficulty conceiving > 12 months were randomized to receive treatment or placebo in a double blind protocol. Compliance was assessed at visits. Analysis of ejaculate was done according to WHO 5th guideline. Progressive sperm motility (A+B grade of rapid, progressive) was done manually. DFI was evaluated by Halosperm kit (Halotech DNA, S.L,) and seminal carnitine by enzymatic UV test

Main results and the role of chance: In the treated group there was statistically significant difference, $p = 0.004$ by McNemar-Boweker test, in the values of progressive sperm motility in 3 different time periods: T0=28.50% (12.00 ± 38.00), T3 = 30.00% (12.00 ± 39.00) and T6=31.00% (20.00 ± 41.00). The seminal plasma carnitine at T0 was 700.50 µmol/L (625.50 ± 800.00) and at T6=751.50 µmol/L (671.10 ± 896.80), and this difference was significant ($p = 0.014$, by Wilcoxon signed-rank test). DFI (%): T0=38,50 (32,00-48,75), T3=35,50 (25,50-44,00) andT6=31,00 (25,00-41,00) (Friedman test, $p < 0.001$). The Spearman's rank-order correlation test showed that the increase of seminal carnitine level influenced the progressive sperm motility ($R = 0.274$; $p = 0.023$). Thus the correlation of seminal plasma carnitine and progressive sperm motility showed that in man, an increase of seminal carnitine of 7.7%, after 6 months therapy, would impact progressive sperm motility >10% with moderate accuracy (AUC=0.713). The other point, if DFI drops by more than 3%, after 6 months of therapy, it can be expected, with moderate accuracy, that men have sperm motility greater than 10% (AUC=0.793; $p < 0.001$). DF reduction (odds ratios = 1.106 with 95% confidence intervals) independently of elevation carnitine, increases the likelihood that sperm motility is >10%.

There was no significant difference in placebo group, in sperm motility, seminal carnitine level and DFI, between T0 and T6

Limitations, reasons for caution: no technical limitations

Wider implications of the findings: This study showed, after six months therapy, that increase of seminal carnitine positively impacted upon the patient progressive sperm motility. On the other hand, the percentage of change in DFI with moderate accuracy can be used in detection of men with better sperm motility after therapy.

Trial registration number: PXP-001-B

O-288 Sperm telomere length is not related to reproductive outcomes up to live birth when analyzed independently from female variables

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Study question: What is the relationship between sperm telomere length (STL) and embryo quality and reproductive outcomes, independently of the oocyte characteristics?

Summary answer: STL of normozoospermic men has no effect on embryo quality, pregnancy rates and live birth rates in IVF/ICSI.

What is known already: Telomeres are non-coding hexameric tandem repeats of DNA located at the ends of eukaryotic chromosomes. STL has been proposed as novel biomarker of sperm fitness as its variations are associated to altered sperm parameters, increased DNA fragmentation, poor embryo quality, and lower pregnancy rates. However, in all studies reported so far, each man's STL was necessarily linked to his female partner characteristics, and an assessment of STL independently form these variables was impossible. Studying STL in sperm donor samples, each used for multiple female patients, allows to isolate and determine the effect of STL alone on reproductive outcomes.

Study design, size, duration: Basic study including 36 sperm donor samples used each for at least 10 fresh ET cycles. Study outcomes were embryo quality, biochemical, clinical, and rates ongoing pregnancy, and live birth (LB). Genomic DNA was isolated from each sample and the relative amount of telomere DNA was determined by quantitative PCR (qPCR). STL was calculated as the ratio between telomere DNA and a single copy gene (RPLP0).

Participants/materials, setting, methods: Univariate analysis was used to evaluate the correlation between STL and embryo quality, and to determine STL differences between leading or not to LB. The probability of pregnancy and LB across STLs was evaluated by LOWESS regression. The effect of STL on

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reproductive outcomes was analyzed by multilevel regression adjusted for sperm concentration and motility, woman age and BMI, number, stage and morphological score of transferred embryos, and addressing the hierarchical data structure.

Main results and the role of chance: The average STL was 4.23 (SD 3.9; range 2.6-9.6, arbitrary units). Reproductive outcomes were 48.6%, 38.4% and 31.3% for biochemical, clinical and ongoing pregnancy rates, respectively, while LB rate was 24.2%. LOWESS regression did not return relevant correlations between STL and reproductive outcomes. However, there was a significant correlation (Rho-spearman coefficient –0.069; p = 0.029) between STL and mean morphological embryo score, albeit the magnitude of the correlation is not clinically relevant. STL was not different between cycles ending or not in LB (Mann Whitney U-test p>0.05) and no significant effect of STL on the reproductive outcomes was found, with OR for each unit increase in STL of 0.98 (95%CI 0.87-1.1), 1.04 (95%CI 0.93-1.18), 1.03 (95%CI 0.91-1.17) and 1.04 (95%CI 0.93-1.18) for biochemical, clinical, ongoing pregnancy and LB, respectively. The multilevel analysis confirmed that the effect of STL on embryo morphological score, biochemical, clinical, ongoing pregnancy and LB was not significant (p>0.05).

Limitations, reasons for caution: Only normozoospermic men were included in this study, caution should be exerted when generalizing these results to non-normozoospermic men.

Wider implications of the findings: This is the largest study of STL to date, and the only one addressing STL independently from female variables. STL was not related to any of the reproductive outcomes analyzed and, regardless of its relationship to other sperm parameters, might be of little relevance as a predictor of ART success.

Trial registration number: NA

SELECTED ORAL COMMUNICATIONS
SESSION 74: ENDOMETRIUM AND EMBRYO CROSSTALK

Wednesday 5 July 2017 Room W+X 14:00–15:15

O-289 Feto-placental discrepancies in the analysis of products of conception in clinical miscarriages reveals that mosaicism is not restricted to trophoblast cells

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Study question: In Feto-Placental Discrepancies (FPD), is mosaicism always confined to placenta (MCP)?

Summary answer: Hysteroembryoscopy allow us to detect accurately FPD (2%), with the presence of the aneuploid cell line in the fetal tissue.

What is known already: During pregnancy, feto-placental discrepancies (FPD) are due the presence of two different chromosomal cell content usually presenting the fetus a normal karyotype and the placenta an abnormal one that is known as mosaicism confined to placenta (MCP). Despite that MCP is described in normal pregnancies, chromosomal abnormality present in the placenta could lead to clinical miscarriage or even a poor perinatal outcome. Nevertheless, if these abnormalities were present in the fetus, the consequences for the perinatal outcome could be even worse, furthermore a prenatal non-invasive test could lead to false negatives.

Study design, size, duration: The objective of the study was to determine the incidence of FPD in products of conception (POC) from first trimester clinical miscarriages (n = 46) carefully sampled separately from embryo and trophoblast guided by hysteroembryoscopy. We include 46 first trimester miscarriages in which two tissue types (fetal and extraembryonic) were successfully collected by hysteroembryoscopy from January 2014 to September 2016.

Participants/materials, setting, methods: POC specimens were collected in sterile tubes containing saline solution. Direct DNA extraction was performed without the previous cell culture required for classical cytogenetic studies. Chromosome status was determined by genetic molecular methods: genome hybridization (CGH array) (Illumina, San Diego, USA) or Next Generation sequencing (NGS) (Thermofisher, MA USA). STRs analysis of maternal and POC DNA were included to fully discard maternal cell contamination (AmpFlSTR Identifiler Plus (Applied Biosystems, CA, USA).

Main results and the role of chance: In 45 out of the 46 cases, the results were concordant between trophoblastic tissue and its corresponding embryonic tissue. Only in one case a discrepancy was reported, trophoblast show an euploid result (46,XX), and fetal sample was aneuploid (47,XY,+4), discarding maternal contamination. This case corresponded to a 30 years old patient with recurrent pregnancy loss that underwent preimplantation genetic screening by aCGH with transfer of an euploid blastocyst previously biopsied at day-3.

Limitations, reasons for caution: Limitations for the present study are the reduced number of samples included and the fact that not the whole tissue sample could be analyzed.

Wider implications of the findings: In FPD, abnormalities are frequently confined to placenta, but aneuploid cells can be also found exclusively in the fetus (2.1%). This FPD might be the reason for previous unknown PGS false negatives.

Trial registration number: CEIC201120007.

O-290 Clinical results following the transfer of mosaic blastocysts-impact of different aneuploidy types to ongoing implantation rates

S. Munné[1], J. Blazek[2], M. Large[2], P. Martin[3], E. Liu[4], N. Tarozzi[5], A. Baker[6], J. Zhang[6], S. Maxwell[7], J. Grifo[7], D. Wells[8], E. Fragouli[8]

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Study question: Does the type of mitotic chromosome abnormality present in a trophectoderm (TE) biopsy affect the implantation potential of mosaic blastocysts?

Summary answer: Complex mosaics had lower implantation rates, but % of abnormality, monosomy vs. trisomy, full or segmental chromosome mosaicism had no significant effect on pregnancy rates.

What is known already: Next Generation Sequencing (NGS) can detect mosaicism, and approximately 15-20% of blastocysts are then classified as such. Mosaic blastocysts miscarry more and implant less than euploid blastocysts, resulting to lower ongoing implantation rates. However, some mosaic blastocysts can become normal live births. It is therefore paramount to determine which mosaic embryos have a higher chance to reach term. Recent guidelines recommend that in the absence of euploid embryos to prioritize replacing mosaic ones with low % of abnormal cells in the TE biopsy, monosomies over trisomies, and certain aneuploidies over others. However, there is little evidence to support this position.

Study design, size, duration: Retrospective study comparing concurrent PGS cycles during which either mosaic (n = 143) or euploid embryos (n = 1045) were replaced in four fertility centers. Implantation, miscarriage rates and ongoing implantation rates were compared between euploid and mosaic blastocysts, and among different mosaic subgroups. These included: presence of 20-40% and >40% mosaicism, with 1, 2 or 3 chromosomes affected, monosomy, trisomy, complex or segmental mosaics, and mosaics involving large (1-10, X) or small chromosomes (11-22, Y).

Participants/materials, setting, methods: Blastocysts from PGS cycles were biopsied and send to three PGD Laboratories to be processed by NGS

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using the same protocol and equipment (MiSeq, Illumina). Embryos were classified as mosaic if they had the equivalent of between 20-80% abnormal cells. This was calculated from mixing experiments using cell lines (euploid: trisomies X, 13, 18, or 21) in 20% increments. All euploid and mosaic embryos were replaced after thawing, mostly as SETs.

Main results and the role of chance: Of the four centers, two had significant differences in ongoing implantation rates (OIR) between mosaic and euploid transfers. However, these differences or lack of them were attributed to the different composition of sub-groups of mosaics replaced per center, with one of them replacing only <40% mosaic embryos. Overall, implantation rates were higher (70% vs 53%, $p < 0.001$), miscarriage rates lower (10% vs 25%, $p < 0.001$) and OIR higher (63% vs 40%, $p < 0.001$) after euploid embryo transfers. Regarding sub-types of mosaics, those involving 3 or more chromosomes (Complex mosaics) had a 10% OIR compared to the rest of the group (45%, $p < 0.005$). There was a tendency for mosaics with many abnormal cells (40-80% in the TE sample) to have lower OIR than those with <40% (22% vs 56%, $p < 0.06$) but few 40-80% embryos were replaced. There was no difference between mosaic monosomy and mosaic trisomy outcomes (OIR of 47% vs. 55%), or between entire chromosome mosaicism or segmental mosaicism (OIR of 50% vs. 41%). Large chromosomes (1-10, X) implanted equally well with smaller ones (11-22, Y) (45% vs. 41%), but with significantly more segmental mosaic abnormalities occurring in larger than smaller chromosomes (47 vs. 13, $p < 0.001$).

Limitations, reasons for caution: Different fertility centers replaced different types of mosaic embryos. Overall few mosaic blastocysts with 40-80% abnormal cells in the TE sample were replaced precluding finding differences with those with <40% abnormal cells. A randomized controlled trial (RCT) would be impractical, but the current study is nonetheless retrospective in nature.

Wider implications of the findings: We concluded that complex mosaic blastocysts have lower OIR than other mosaics, and hypothesize that those with 40-80% abnormal cells in the TE sample also will. However, mosaic monosomies perform as well as mosaic trisomies and mosaic segmental aneuploidies. Guidelines should therefore be revised accordingly.

Trial registration number: Not Applicable.

O-291 Comparative analysis between endometrial stromal proteomes of repeated implantation failure (RIF), recurrent pregnancy loss (RPL) and normal fertile (NF) women

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Study question: Are there any proteomic differences between endometrial stromal cells of RIF, RPL and NF women, and is there differential protein expression upon decidualization?

Summary answer: The main proteomic difference was observed in decidualized stromal cells of RPL, where serotransferrin was expressed 1.5-fold higher than in decidualized stromal cells of NF.

What is known already: A successful pregnancy requires the endometrium to obtain structural and functional competence during the midluteal phase. Failure of the endometrium to express this receptive phenotype is thought to be a major cause of RIF, and is also considered to be linked to RPL. Insight in the biological mechanisms underlying this receptive phenotype is crucial if we wish to improve embryo implantation. While a few studies have attempted to profile the endometrial proteome, current innovative high-throughput mass spectrometry approaches allow the analysis of a substantially higher number of proteins, not only predominantly high abundant proteins (Altmäe S et al., 2014).

Study design, size, duration: We performed an exploratory study investigating the proteome of *in vitro* cultured endometrial stromal cells of in total 11 women (included in the period from March 2014 until May 2016). The aim was to compare the proteome between women with repeated implantation failure (RIF; n = 4), women with recurrent pregnancy loss (RPL; n = 3) and normal fertile women (NF; n = 4). Moreover, the differentially expressed proteins (DEPs) upon decidualization were also studied between these groups.

Participants/materials, setting, methods: Endometrial biopsies were processed for primary cultures of endometrial stromal cells and were collected at day 0 or after 5 days of decidualization. Total proteins were extracted from cell lysates using phenol/ethanol extraction, trypsin digested and analyzed by label-free quantitative High Definition Mass Spectrometry (HDMSE) using a nano-scale UPLC system coupled to a Synapt G2-Si mass spectrometer. Data analysis was performed using ANOVA ($p < 0.05$) in Progenesis QI, and SAM ($p < 0.05$; FDR 10%) in R.

Main results and the role of chance: A total of 1424 proteins were identified across all samples. For the RIF group 127 DEPs were upregulated and 138 DEPs were downregulated in the decidualized versus the non-decidualized condition, in the RPL group 39 DEPs were upregulated and 42 DEPs were downregulated and in the NF group 183 and 134 DEPs respectively ($p < 0.05$). SAM analysis comparing ratios of expression of decidualized over non-decidualized samples, revealed 6 DEPs when comparing the different groups (FDR 10%, $p < 0.05$): DUX4L2, CNPY4, PDE7A, CTSK, PCBP2 and PSMD4. Comparison of expression profiles of RPL samples versus NF samples in the non-decidualized condition showed no DEPs (FDR 92%). But comparison of expression profiles of RPL samples versus NF samples in the decidualized condition revealed TF to be differentially expressed ($p < 0.001$; FDR 3%). However, comparing expression profiles of RIF samples versus NF samples in the non-decidualized, as well as in the decidualized condition did not yield statistically significant DEPs (FDR 50% and 61% resp.).Comparison of the expression profiles in the non-decidualized or the decidualized condition between these three groups of patients could in principle identify proteins that predict differences in clinical outcome. However, this SAM analysis did not yield statistically significant DEPs (FDR=89%).

Limitations, reasons for caution: This was an exploratory study using a label free high throughput proteomics platform, ona limited number of samples. Furthermore, the proteomics data need to be validated.

Wider implications of the findings: With this innovative label-free quantitative HDMSE study, a total of 1424 proteins were identified and quantified, presenting an overview of the endometrial stromal proteome of women with RIF, RPL and NF women. These results provide a window into the mechanisms of endometrial receptivity and possible mechanisms of its impairment.

Trial registration number: Ethical approval was obtained from the Ghent University Hospital Institutional Review Board under reference EC2013/887.

O-292 Cytoskeleton and exosome trafficking proteins of epithelial endometrial cells during secretory phase interact with trophoblast proteins

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Study question: Which proteins and molecular mechanisms are involved in epithelial endometrial and trophoblastic cells crosstalk during the secretory phase?

Summary answer: Extracellular proteins of epithelial endometrial cells related to cytoskeleton organization and exosome trafficking potentially interact with trophoblast during implantation.

What is known already: Endometrium is receptive for blastocyst during a limited period within mid-secretory phase coinciding with the maximal differentiation state of epithelial endometrial cells (EECs). In this phase a complex and not well-understood crosstalk between EECs and trophoblast cells (TCs) is established for initiating embryo implantation. Recent studies provided the EECs proteome during secretory phase (Hood et al 2015) and TCs

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transcriptome derived from blastocysts (Yan et al 2013) that enable the study of their putative and functional protein interactions. The identification and characterization of these biomolecular interactions will provide new insights into cellular mechanism during embryo implantation.

Study design, size, duration: Here we show an integrative approach to study and characterize the biomolecular interactions between expressed genes in EECs and TCs respectively. This analysis was carried out using network methods on the human interactome (Rolland et al. 2014) in combination with 1.216 proteins from EECs mass spectrometry proteomic data during secretory phase (Hood et al 2015) and 14.230 transcripts from TCs (RNAseq, Yan et al 2013).

Participants/materials, setting, methods: Combined network approaches were used to characterize the resulting subnetwork of the human interactome based on subsets of genes expressed in TCs and EECs. Different biocomputational tools were used for data integration and network building (Bioconductor) and also for network representation and data curation (Cytoscape). The resulting cluster of genes from EECs physically interacting to TCs genes were used for functional enrichment analysis (GProfiler) and for detecting cellular mechanisms overrepresented during the early embryo-maternal interaction.

Main results and the role of chance: A Protein-Protein Interaction (PPI) network was built based on the direct interactions between EECs and TCs genes. The resulting subnetwork –which contains a total of 91 and 33 genes of TCs and EECs respectively– represents the core interactions between both cell types based on putative direct PPIs. Subsequent functional analysis of resulting clusters extracted from the curated network revealed an overrepresentation of biological processes such as; cytoskeleton organization (13 genes), Fructose metabolism (3 genes) and Intermediate filament organization (3 genes). We also found proteins of different cellular components related to extracellular regions such as exosomes that involved (17 genes), cytoskeleton intermediate filament (7 genes) and the supramolecular complex fibers (12 genes).

The network analysis showed two EECs proteins KRT13 and KRT15 that are part of the cytoskeleton and were connected with a high number of genes of TCs, 28 and 45 respectively. Other genes related to cytoskeleton organization were found moderately interconnected in the subnetwork such as GFAP (9 connections), KRT6A (7), EMD (7), PPL(6), KRT5 and DES (1).

Limitations, reasons for caution: The present study is based on published data and represents an approach to interrogate features of the interactome between expressed EECs proteins and TCs genes. The absence of a functionally validated protein representation of TCs could lead to decrease accuracy of assumptions compared with validated *in vivo* PPIs

Wider implications of the findings: EECs proteins that potentially interact with TCs have been revealed and put light over specific biological process during implantation. Our findings suggest a representative role of cytoskeleton and intermediate filament organization as well as exosome in the extracellular region, where could have a direct contact with the implanting blastocyst.

Trial registration number: N/A.

O-293 Does application of auto-cross linker hyaluronic acid in women following D&;C for miscarriage with at least one D&C in history improve reproductive outcome?

A. Hooker[1,2], R. Leeuw de[2], H. Brolmann[2], J. Huirne[2]

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[2]*VU University Medical Center, Department of Obstetrics and Gynaecology, Amsterdam, The Netherlands*

Study question: Does intrauterine application of auto-crosslinked hyaluronic acid (ACP) gel following dilatation and curettage (D&C) for miscarriage, in women with at least one previous D&C in history improve reproductive performance?

Summary answer: Application of ACP gel following D&C for miscarriage in women with at least one previous D&C seems to have a favourable effect on reproductive performance.

What is known already: IUAs are reported in 19% of women after miscarriage; women with more than one D&C had statistical significant more IUAs compared to women with one D&C, OR 2.05. IUAs is asocciated with menstrual disturbances, cyclis pain and infertiliy. In the Prevention of Adhesion Post Abortion (PAPA-study), intrauterine application of ACP gel following D&C for miscarriage in women with at least one previous D&C significantly reduced the incidence and severity of IUAs. Athough, the process of adhesion formation is not completely eliminated. It remains unclear if application of ACP gel improves fertility and reproductive performance.

Study design, size, duration: The present prospective, non-interventional long-term follow-up study was conducted in the Netherlands. 149 women with a (incomplete) miscarriage of less than 14 weeks with at least one D&Cin history were preoperatively randomised to D&C plus ACP gel (intervention group) or D&C alone (control group) in the PAPA-study. The participants received questionnaires three, six and twelve months after the D&C-procedure. The aim of the current study was to evaluate reproductive performance after the D&C-procedure

Participants/materials, setting, methods: All women who participated in the PAPA-study were eligible to participate. Questionnaires were send to 149 women, 77 women assigned to the intervention group and 72 to the control group, three, six and twelve months after the D&C-procedure, The questionnaires consisted of questions concerning demographics, complication, treatment received, menstrual pattern, contraceptive use, conception and reproductive performance. In women willingly to conceive, conception, miscarriage and ongoing pregnancy rates were assessed.

Main results and the role of chance: Of the 149 women elligible to participate, six women were lost to follow-up after three months and nine women after six and twelve months. The overall response rate for the questionnaires was 96% at three months and 94% at six and twelve months. The response rate in the intervention group was 94.8% after three, six and twelve months and respectively 97.2%, 93.1% and 93.1% after three, six and twelve months in the control group, there were no diference between the groups. In both groups 62 women attempted to conceive. After twelve months 44 women (71%) conceived in the intervention group compared to 40 women (64.5%) in the control group (Fisher exact test, $p = 0.56$). One pregnancy was terminated on social indication in the control group on social grounds and in the intervention group a woman had a extra uterine pregnancy, requiring surgical management.The cumulative miscarriage rate in the intervention group was 14.5% compared to 22.5% in the control group (Fisher exact test, $p = 0.79$). The cumulative ongoing pregnancy rates were respectively 71.0% and 64.5% (Fisher exact test, $p = 0.79$).

Limitations, reasons for caution: The sample size was of the current follow-up study is small and was not powered for reprodcutive outcomes. The result are based of the ansers provided by the participants, making it not possible to drawn solid conclusions. It remains unclear whether known and unknwon factors could have influenced the results.

Wider implications of the findings: Application of ACP gel following D&C for miscarriage reduces the incidence and severity of IUAs in women with at least one D&C in history. Prevention of IUAs is essential because of the association between IUAs and complications while application of ACP gel seems to improve reproductive performance.

Trial registration number: NTR3120 (Dutch Clinical Trail Registry).

SELECTED ORAL COMMUNICATIONS
SESSION 75: FROM LABORATORY TO CLINIC
Wednesday 5 July 2017 Room C 14:00–15:15

O-294 Personalised scheduling of embryo transfer at the moment of highest endometrial receptivity according to ER Map® test significantly improves clinical outcomes

J. Sarasa[1], J. Aizpurua[2], I. Jurado[1], B. Rodriguez-Estrada[1], M.C. Garcia-Poyo[1], A.B. Climent[3], P. Martínez-Ortiz[3], M. Enciso[1]

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Study question: Does identifying the window of implantation using the molecular tool ER Map® improve ART outcomes on patients with previous IVF failed cycles?

Summary answer: The use of ER Map® test for endometrial receptivity evaluation and personalised scheduling of embryo transfer during the moment of highest receptivity improves ART outcomes.

What is known already: The endometrium reaches a receptive status for embryonic implantation around day 19-21 of the menstrual cycle. During this period, known as the window of implantation (WOI), the endometrium shows a specific gene expression profile suitable for endometrial function evaluation. ER Map® is a molecular diagnostic tool able to accurately predict endometrial receptivity status by analysing this gene expression profile on an endometrial biopsy by high-throughput RT-qPCR. In this study the experience of the application of ER Map® for WOI identification and personalised scheduling of embryo transfer is studied for the first time.

Study design, size, duration: This is a retrospective study comparing pregnancy rates of patients with and without displaced WOI when embryo transfer was scheduled on the moment of highest endometrial receptivity (WOI timeframe) as recommended by ER Map® (group A), or according to standard endometrial evaluation and deviating more than 12 h from ER Map® recommendation (group B). ER Map® clinical results comparison was performed on 204 couples undergoing egg donation treatment between March and November 2016.

Participants/materials, setting, methods: Women with 2 or more previously failed IVF cycles were included in the study. Endometrial biopsy samples were obtained in an HRT cycle at P_4+5.5. Samples were classified by ER Map® into 'Receptive", 'Pre-receptive" or 'Post-receptive". In cases where a WOI displacement was detected, a second biopsy to confirm the displacement and to schedule embryo transfer on the moment of highest endometrial receptivity was performed. Once the WOI was confirmed, embryo transfer was recommended accordingly.

Main results and the role of chance: Preliminary results indicate that a total of 57 out of 204 patients (27.95%) with previous failed cycles were found to have a displaced WOI. Within this group of patients, a significantly higher pregnancy rate was achieved when embryo transfer was scheduled according to ER Map® prediction compared to transfers that followed traditional endometrial evaluation methods and deviated more than 12 h from ER Map® recommendation (75.51% vs 33.33%, X^2 Test with Yates' correction $p < 0.01$). More than double pregnancy rates were achieved when these patients' WOI displacement is identified and managed adequately.

No difference in pregnancy rates was observed in patients whose WOI was not displaced (i.e. patients whose endometria were classified as 'Receptive" at P_4+5.5) when embryo transfer schedule was guided by either ER Map® or traditional evaluation methods (73.20% vs 70.00%). No positive nor negative effect of the application of ER Map® was observed on these patients. The effectiveness of ER Map® strategy depends on patient endometrial receptivity diagnosis.

Limitations, reasons for caution: The retrospective and non-randomised design of the study may be a reason for caution. A wider spectrum of infertility conditions and patient characteristics is desirable to define more precisely the group of patients that could potentially benefit from the assessment of endometrial receptivity and personalised embryo transfer using ER Map®.

Wider implications of the findings: The application of ER Map® as a routine tool for the identification of cases of WOI displacement and personalised embryo transfer scheduling is an effective strategy for improving ART outcomes and achieving pregnancies on challenging repeated IVF failure cases.

Trial registration number: Not applicable.

O-295 Endometrial preparation: Impact of estrogen duration of administration before frozen-thawed blastocyst transfer on live birth rate

F. Kefelian[1], P. Santulli[1], M. Bourdon[1], L. Vienet-Legue[1], C. Maignien[1], V. Gayet[1], K. Pocate-Cheriet[2], L. Marcellin[1], J. Gonnot[1], C. Cervantes[1], V. Blanchet[1], C. Chapron[1]

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Study question: To investigate the impact of estrogen (E2) duration of administration before frozen –thawed blastocysts transfer on life birth rate.

Summary answer: Live birth rate significantly decreases as estrogen duration of administration increases before frozen-thawed blastocyst transfer.

What is known already: Different cycle regimens for endometrial preparation are used prior to frozen embryo transfer. Currently, one effective method is the hormonal replacement therapy with a sequential regimen with estrogen (E2) and progesterone, which aims to mimic the endocrine exposure of the endometrium in the normal cycle. There nonetheless remains a lack of knowledge concerning the optimal duration of exogenous E2 administration before transfer.

Study design, size, duration: This cohort study was conducted in a tertiary care university hospital between 01/01/2012 and 31/12/2015. Main inclusion criteria's were having a single frozen-thawed blastocyst transfer with an artificial endometrial preparation using exogenous estrogen (E2).

Participants/materials, setting, methods: A total of 1377 frozen – thawed blastocysts transfer were allocated to 4 groups according to the duration of E2 administration: ≤ 21days (Group A, n = 330), 22-28 days (Group B, n = 665), 29-35 days (Group C, n= 289) and 36-48 days (Group D, n= 93). The main outcome measured was the live birth rate following frozen – thawed blastocysts transfer. Statistical analysis were conducted using univariate and multivariate logistic regression models.

Main results and the role of chance: Live birth rates significantly decrease with the increase of E2 duration before frozen –thawed blastocysts transfer (group A: n= 98 (29.70%), OR 1; Group B: n= 185 (27.82%), OR 0.91 CI95% 0.68-1.22; Group C: n= 63 (21.80%), OR 0.66 CI95% 0.46-0.95 and Group D: n = 16 (17.20%), OR 0.49 IC95% 0.27-0.89). In contrast, early pregnancy loss rate significantly increase with the increase of E2 duration before frozen – thawed blastocysts transfer (group A: n= 41(28.47%), OR 1; Group B: n= 89 (31.79%), OR 1.17 CI95% 0.75-1.82; Group C: n = 35 (34.31%), OR 1.31 CI95% 0.76-2.27 and Group D: n= 17(48.57%), OR 2.37 IC95% 1.12-5.05).

Obstetrical and neonatal outcomes were not significantly different among groups. After multivariate logistic regression, E2 duration of administration longer than 28 days before frozen-thawed blastocyst transfer was found to be an independent predictive factor of life birth rate (29-35 days versus ≤21days: OR 0.68 95%CI 0.460-0.989; 36-48 days versus ≤21days: OR 0.51 95%CI 0.269-0.953).

Limitations, reasons for caution: One limitation is linked to the observational design of this study: randomized clinical trials are needed to confirm these results.

Wider implications of the findings: In order to give patients the best chance to obtain a live birth after frozen –thawed blastocysts transfer, it seems important to limit estradiol duration of administration before ET. This study brings new insight to endometrial preparation using hormonal replacement therapy before frozen blastocysts transfer.

Trial registration number: NA.

O-296 Antioxidant Gene Expression of Peroxiredoxin (PRDX) decreases in granulose cells from oocytes of young women with low ovarian reserve

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Study question: Is gene expression of antioxidant peroxiredoxin altered in cumulus (CCs) and granulose cells (GCs) from young women with reduced ovarian reserve compared with oocyte donors?

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Summary answer: Our results show that the expression of PRDXs was higher in donors' GCs suggesting that these are important oxidative stress regulators in low responder patients.

What is known already: Oxidative stress has been implicated in various aspects of female infertility. Our previous studies have shown that the initiation of apoptotic cell death in cumulus and granulosa cells is initiated by increased reactive oxygen species.

Peroxiredoxin (PRDX), a family of peroxidases, are associated with various biological processes such as protection against DNA damage, oxidative stress and cell apoptosis. On the other side, the upregulation of some PRDX in cells and tissues by various stress agents is considered an important biological response to prevent oxidative damages. Nevertheless, little attention has been given to the role of PRDX on female infertility.

Study design, size, duration: This observational, prospective study compared the mRNA expression of PRDX1, PRDX2, PRDX3, PRDX4, PRDX5, PRDX6 and caspase 3 in 56 oocyte-cumulus and 62 oocyte-granulose complexes retrieved from 4 healthy fertile oocyte donors <35 years old and 4 patients <35 years old who had a low response (≤ 5 oocytes retrieved) after gonadotropic stimulation from July to December 2016. Only donors with at least a previous cycle with pregnancy were included.

Participants/materials, setting, methods: All patients were stimulated with the same protocol (FSHr and triggering with GnRH analogues). GCs and CCs were collected from each patient and immediately fresh-frozen. mRNA was extracted using commercial kits. mRNA expression of PRDXs genes and endogenous controls was measured by qRT-PCR using TaqMan probes. Relative changes in gene expression were calculated using the 2-ΔΔCT method. No parametric tests were used to identify any significant difference between groups. Statistical significance was set at $p < 0.05$.

Main results and the role of chance: We found that PRDX2, PRDX3, PRDX4, PRDX5 and PRDX6 are expressed in CGs and CCs. Results obtained from comparative RT-PCR analysis revealed that the mean relative levels of mRNA coding for PRDX2, PRDX3, PRDX4, PRDX5 and PRDX6 were significantly decreased in GCs from young women with low response compared with oocyte donors: (PRDX2 p= 0.03; PRDX3 p= 0.022; PRDX4 p= 0.014; PRDX5 p= 0.02; PRDX6 p= 0.014). No significant differences were found in the levels of mRNA coding for PRDX2, PRDX3, PRDX4, PRDX5 and PRDX6 in CCs from young women with low response compared with oocyte donors.

The levels of caspase 3 mRNA in GCs were also significantly increased in patients compared with donors (p<0,001) whereas there were not differences in CCs between groups.

Limitations, reasons for caution: The main limitation of our study is the small number of replicates. The results observed in this study should be confirmed on a larger sample number.

Wider implications of the findings: These results suggest that cellular antioxidant defense capabilities are diminished during low ovarian response, leading to an increase in oxidative damage in the ovary in a similar way as in age-related oxidative damage. These findings could open up to new therapeutic strategies for the treatment of low ovarian reserve.

Trial registration number: 2.

O-297 Zika virus infects the female reproductive tract of mice via subcutaneous route

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Study question: Does Zika virus (ZIKV) infect the female reproductive tract and are there any damaging effects.

Summary answer: After inoculation with ZIKV, viral RNA is found in the ovary, oviduct, uterus, cervix, and vagina and inflammatory cells invade the ovary and follicular compartment.

What is known already: Since its emergence in the Americas, Zika virus (ZIKV) has been linked to the development of congenital birth defects and viral persistence for extended periods of time in semen, urine, and vaginal washes of humans. Mouse models have been used to confirm ZIKV infection in the male reproductive tract including testes, epididymis, and sperm. However, the effects of infection on the female reproductive tract is poorly understood.

Study design, size, duration: This was an observational, basic science experiment with inbred mice

Participants/materials, setting, methods: Using a mouse adapted ZIKV strain, we evaluated tissue tropism in the female reproductive tract in C57Bl/6 mice after subcutaneous inoculation. Hematoxylin and eosin staining was performed as was in situ hybridization looking for viral RNA expression. qRT-PCR was conducted on all tissues to detect the titers of the virus in the tissues. Immunohistochemistry was performed to locate CD45 positive leukocytes in the tissues. The infection studies were repeated in ovariectomized mice vs shams.

Main results and the role of chance: ZIKV was detected in the ovary, oviduct, uterus, cervix, and vagina. The highest titers were close to 10^6 FFU equivalents/gram in the cervix and vagina of mice on day 7 post infection. This was confirmed through in situ hybridization. Preliminary results suggest that the presence of estrogen enhances Zika infectivity. Bilateral oophorectomy reduced viral titers, however, these titers increased with add back estradiol. We further investigated the effect of ZIKV on the ovary and found no significant differences in ovarian weight or in primary or secondary follicular development at 14, 21 or 90 days post infection. ZIKV infected mice, however, did demonstrated significant infiltration of CD45 cells at all time points. This was localized to the follicular compartment, specifically antral fluid and the zona pellucida. Robust CD45 follicular staining was seen in 3/7 (43%) mice at the day 14 time point and 4/4 (100%) mice at the day 21 time point. This could represent evidence of systemic ZIKV infection in the follicular fluid, or could be indicative of a tissue-specific effect of ZIKV on the ovary.

Limitations, reasons for caution: This is a mouse study however woman to man transmission of ZIKV has been reports and would be predicted by the results of these studies.

Wider implications of the findings: ZIKV can infect all female reproductive tract tissues by the subcutaneous route, which includes mosquito bites.This infection leads to inflammatory infiltration of the ovary and could potentially lead to later tissue destruction. Women may be more sensitive to ZIKV during different times in the menstrual cycle due to estrogen.

Trial registration number: Not Applicable.

O-298 Infection with high-risk Human Papillomavirus (HPV) and the risk of female infertility

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[2]*Danish Cancer Society, Virus- Lifestyle and Genes, Copenhagen, Denmark*

Study question: Does known infection with high-risk HPV, both registered as a single HPV positive test or HPV persistence, increase the risk of female infertility?

Summary answer: Infection with high-risk HPV does not seem to increase the risk of female infertility in later reproductive life.

What is known already: HPV positivity at the time of fertility-treatment has been associated with a decreased success-rate in both IVF and IUI outcomes, but the role of high-risk HPV as a cause of female infertility is unclear. No previous studies have investigated the risk of infertility subsequent to infection with high-risk HPV.

Study design, size, duration: This study was based on a large national population-based cohort study, including 10.898 women (20-29 years of age) randomly drawn from the general female population living in Copenhagen, Denmark and enrolled during 1991-93. All women were tested for cervical high-risk HPV and again after 2 years. The study cohort was linked to the Danish Infertility Cohort, which consists of all Danish women referred for fertility problems to private or public fertility clinics.

Participants/materials, setting, methods: Follow-up for each study participant was the period from the date of enrollment until diagnosis of infertility, first conception, death, emigration, disappearance, or end of study period (Dec 2010). Data were analyzed by means of a Cox proportional hazards regression model estimating hazard ratios (HRs) and its corresponding confidence intervals (CIs). Potential confounders such as maternal age, smoking, educational level

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and previous genital infections were selected a priori according to existing literature and availability.

Main results and the role of chance: Of the 10.898 women eligible for study, 1.863 (17.0%) were high-risk HPV positive at the first visit, 2802 (25.7%) were positive at either or both first and the next visit, and 324 (3.0%) were high-risk HPV positive for a specific subtype at both the first and the second visit. In all, 1.282 women (11.8%) were diagnosed with infertility in the cohort.

Neither one (HR 0.87, 95% CI 0.75-1.02), or two following positive tests for high risk HPV (HR 1.00, 95% CI 0.88-1.13), or two tests positive for the same subtype of high risk HPV (persistence) (HR 0.90, 95% CI 0.64-1.26) was associated with an increased risk of infertility later in reproductive life. Adjusting for potential confounding factors did not change the estimates.

Limitations, reasons for caution: A limitation of the study is that an unknown number of the unexposed women in our study could have had a high-risk HPV infection before or after the test at enrolment and follow up.

Wider implications of the findings: Our results are reassuring since infection with high-risk HPV is widespread and female infertility is increasing in the industrialized countries.

Trial registration number: Not applicable

POSTER VIEWING SESSION

ANDROLOGY

P-001 Choosing the appropriate insemination method in assisted reproductive treatments based on sperm chromatin fragmentation

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Study question: We question if sperm chromatin fragmentation (SCF) can guide couples with unexplained infertility and poor intrauterine insemination (IUI) outcomes to the appropriate subsequent reproductive treatments.

Summary answer: Couples with prior IUI failures can be offered a SCF-based treatment algorithm comprising of conventional IVF or ICSI with ejaculated or surgically retrieved spermatozoa.

What is known already: Ovulation induction (OI) with IUI is the mainstay of treating couples with unexplained infertility. However, some couples may have poor IUI outcomes despite an adequate number of treatments attempts and normal semen parameters. Recent evidence has suggested that sperm DNA integrity is closely associated with fertility potential. During the later stages of spermiogenesis, breakage of a sizable amount of single- or double-stranded DNA occurs to allow tight chromatin compaction and, in ideal conditions, only those spermatozoa with fully repaired chromatin would reach the ejaculate. Thus, high SCF in the ejaculate, may have an inverse relationship with IUI success.

Study design, size, duration: Over a 36-month period, couples diagnosed with unexplained infertility, based on normal female infertility screening and semen analysis, underwent treatment with ovulation induction and IUI. Those with a history of 2-4 failed IUI attempts underwent SCF assessment of the ejaculate. The couples were then allocated to conventional IVF or ICSI with ejaculated or surgically retrieved sperm according to the level of SCF. The clinical pregnancy rate (CPR) was stratified according to the SCF-based insemination method.

Participants/materials, setting, methods: Infertile couples with unexplained infertility underwent SCF assessment by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay or sperm chromatin structure assay (SCSA). In-house TUNEL assessment evaluated at least 500 spermatozoa under fluorescent microscopy and the SCF was deemed abnormal above a threshold of >15%. SCSA assessment was also performed by an external laboratory, which analyzed at least 5000 spermatozoa. The SCSA was considered abnormal above a threshold of >25%.

Main results and the role of chance: A total of 631 couples underwent 1464 IUI cycles. The female and male age in the study cohort was 37.6 ± 4 years and 39.8 ± 5 years, respectively. The overall average sperm concentration was 50.4 ± 32 × 10^6/mL, with a motility of 46.6 ± 12% and morphology of 3.1 ± 2%. The CPR with IUI at our center in an age-matched cohort is 17.9%; however, the study cohort had CPR of 5.1%. The mean TUNEL and SCSA values of the men in study cohort was 21.7 ± 8% and 39.5 ± 26%, respectively. Couples with failed IUI attempts but normal SCF in the male partner underwent conventional IVF, resulting in a CPR of 24.4%. In women <35 years undergoing conventional IVF, the CPR increased to 38.2% ($P < 0.001$). Couples with abnormal SCF underwent ICSI with ejaculated sperm, leading to an overall CPR of 24.1% ($P < 0.001$); when controlling for female age <35 years, the CPR rose to 27.8%. Couples who failed to conceive with ICSI using ejaculated sperm were offered testicular sampling. Eighty-six men consented for the surgical retrieval of testicular sperm and were found to a have a SCF of 9.28 ± 6%, which was significantly lower than the SCF of the ejaculate ($P < 0.001$). The CPR in couples undergoing ICSI with surgically retrieved sperm was 29.8%.

Limitations, reasons for caution: Surgical sampling of the vas deferens, epididymis, or testis in men with high SCF in their ejaculates samples or those with poor IUI outcomes should only be performed after extensive individualized counseling. Moreover, such an approach is preliminary and requires further prospective analysis.

Wider implications of the findings: IVF is successful in men with intact sperm chromatin. When sperm SCF is compromised in the ejaculate, ICSI is the most suitable insemination method. In men with high SCF in their ejaculate who fail ICSI treatment, surgical sampling yields spermatozoa with lower SCF and higher changes of pregnancy.

Trial registration number: Not Applicable.

P-002 Impact of intracytoplasmic morphologically selected sperm injection (IMSI) on blastocyst quality: a prospective blinded randomized sibling oocyte study of IMSI vs. intracytoplasmic sperm injection (ICSI)

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[3]Nordic IVF Center, Malmö, Limhamn, Sweden

Study question: To evaluate the impact of IMSI on blastocyst development.

Summary answer: No difference in number of good quality blastocysts on day 5/6 was found between IMSI and ICSI.

What is known already: Motile sperm organelle morphology examination (MSOME) at magnification up to 6600x enables assessment of sperm nuclear morphology. Spermatozoa with as few vacuoles as possible can then be selected for microinjection (IMSI). However, a decade after its introduction the clinical implication of MSOME is unclear.

Study design, size, duration: The study was a prospective oocyte sibling study performed at 3 IVF centers, where the patients were blinded to randomization of treatment. When 8 or more oocytes were retrieved the oocytes were individually and randomly 1:1 allocated to either ICSI or IMSI.

Participants/materials, setting, methods: Infertile couples for ART treatment with severe male infertility (sperm concentration < 1 million sperm/ml and/or sperm motility grade less than 2.5 after gradient centrifugation), previously at least two failed ICSI attempts and at least 8 retrieved oocytes were selected for the study. All couples gave their signed informed consent.

Main results and the role of chance: A total of 58 patients were enrolled in the study with a total of 644 oocytes randomized to ICSI or IMSI. No statistical difference was found between the treatments in day 5/6 good quality blastocysts per injected oocyte or fertilized oocyte (2PN): the percent of good quality blastocysts was 19.6% (SD 27.7%) and 21.1% (SD 28.2%) in the IMSI and ICSI groups, respectively (Wilcoxon signed ranks test, P = 0.71). The fertilization rate was significantly lower in the IMSI group (54.0%; SD 23.7%) compared to the ICSI group (65.3; SD 28.5) (P = 0.004).

Limitations, reasons for caution: Time for selecting an optimal sperm could affect the quality of the embryos, which might explain the impaired fertilization rate in the IMSI group. Furthermore, many men enrolled in the study

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suffered from severe oligozoospermia and the IMSI technique cannot guarantee that spermatozoa with normal morphology were selected for microinjection.

Wider implications of the findings: In literature, IMSI is not considered "state of the art" for treating male infertility. The current study included couples with male infertility and at least two previous failed ICSI cycles, which is only one of several potential indications for IMSI. Further studies are needed to elucidate the role of IMSI.

Trial registration number: Not applicable when study was commenced in 2012.

P-003 Superior clinical pregnancy rates after microsurgical epididymal sperm aspiration

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[4]*Asada Ladies Clinic, Gynecology, Nagoya, Japan*

Study question: Since reports on the use of microscopic epididymal sperm aspiration (MESA) are limited, we assessed normal fertilization and live birth rates after use of MESA.

Summary answer: MESA is a beneficial procedure and should be given priority over TESE.

What is known already: Testicular sperm extraction (TESE) is now widely applied as a method of sperm retrieval surgery owing to its technical simplicity. Nevertheless, testicular sperm retrieval requires surgical intervention and hence carries associated risks such as bleeding, infection, and impaired testicular function.

Study design, size, duration: Of 438 subjects who underwent surgical sperm retrieval in our clinic between April 2004 and January 2016, 160 who underwent MESA were evaluated.

Participants/materials, setting, methods: MESA was performed under local anesthesia by using a micropuncture method with a micropipette. In cases where motile sperm were not obtained after repeated bilateral puncturing of the epididymis, conventional or micro TESE was used.

Main results and the role of chance: Adequate motile sperm were retrieved in 71 subjects by using MESA, and in 59 subjects by using TESE. Of the patients, 123 underwent intracytoplasmic sperm injection (ICSI). After MESA, the normal fertilization rate was 73.5%, and the clinical pregnancy rate per cycle was in 43%. Healthy deliveries resulted after MESA in 65 cases and after TESE in 38.

Limitations, reasons for caution: small number of subjects

Wider implications of the findings: MESA specimen collection dose not have any special requirement such as mincing tissue disposition. MESA can also reduce the laboratory work such as that required for cryopreseravation. Moreover, a MESA specimen is easily applied for ICSI in both cases where the sperm is fresh or frozen-thawed.

Trial registration number: none.

P-004 Influence of dietary patterns on semen quality among patients undergoing infertility treatment

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Study question: Are there any associations between food intake and semen quality in male patients undergoing an assisted reproductive technology (ART) treatment?

Summary answer: Higher antioxidant intake was associated with higher semen quality and ART success.

What is known already: Around 40% of infertility cases are caused by male factor due to significant declines in semen quality. While genetic, endocrine, congenital and demographic factors such as age, smoking and heavy alcohol use are risk factors for decreased sperm quality, there is increasing evidence that nutritional status could be a critical determinant of normal reproductive function. Dietary intake of antioxidants, such as vitamins C and E, β-carotene, and micronutrients such folate and zinc, have been demonstrated to be critically important for normal semen quality and reproductive function in humans.

Study design, size, duration: Cross-selectional study. 28 normozoospermic, 73 asthenozoospemic, 62 oligoasthenozoospermic and 33 oligoasthenoteratozoospermic patients involved in ART were recruited between March 2015 and October 2016 at Hospital Universitari i Politècnic La Fe of Valencia (Spain). 33 cycles were underground by intrauterine insemination (IUI) and the rest, by *in vitro* fecundation (IVF) or intracytoplasmic sperm injection (ICSI).

Participants/materials, setting, methods: A total of 196 men of subfertile couples undergoing IUI and IVF/ICSI treatment were studied. Semen samples were analysed according WHO 2010 criteria. Semen parameters including sperm concentration (SC), total sperm motility (TSM), progressive motility (PRM) and normal sperm morphology (NSM) were recorded. All them were analysed by an Integrated Sperm Analysis System (ISAS). Diet was assessed using food frequency questionnaire. Lineal regression models analysed associations between dietary patterns, sperm parameters and pregnancy rate (PR).

Main results and the role of chance: Attending to dietary patterns of patients it is possibly to differentiate two ones: Western and Mediterranean diet. While the Mediterranean diet is high in whole grains, fruits and vegetables, the Western diet is high in refined carbohydrates, sugars and red meats. SC, TSM and PRM are positively influenced by the consumption of vegetables, fruits, cereals and polyunsaturated fats (*P*-value <0.05). However, a high intake of saturated fatty acids declines SC and TSM (r = -0.32 and r = -0.47, respectively). Higher intake of selenium, omega-3 and omega-6 polyunsaturated fats was associated with higher normal sperm morphology. Men in the highest tertile of selenium intake had a 1.4 (95% CI: 0.9 to 2.8) higher percentage of morphologically sperm than men in the lowest tertile of intake. Men in the highest third of omega-3 fatty acids had 3.2% (95% CI: 1.1 to 3.9) higher normal morphology than men in the lowest third (P_{trend} = 0.01). The Mediterranean diet was associated with a significant higher PR (*P*-value = 0.02).

Limitations, reasons for caution: This was a cross-sectional and observational study, which limited our ability to determine causality of diet on semen quality parameters.

Wider implications of the findings: There are many studies that analyse the relationship between dietary patterns and semen quality but only a few of them study its influence on pregnancy rates. Our findings suggest that a diet rich in antioxidants, like Mediterranean diet, may improve semen quality and increases the chance of getting pregnant.

Trial registration number: This is a basic science.

P-005 Sperm preparation after freezing improves sperm count, motility, viability and morphology in frozen-thawed sperm compared with sperm preparation before freezing-thawing process

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Study question: The purpose of this study is to evaluate which cryopreservation protocol, semen preparation before or after freezing, improved sperm count, motility, viability and morphology results

Summary answer: Semen preparation after cryopreservation resulted in higher total motile sperm count, viability and morphology, so it is recommended in patients with poor sperm baseline

What is known already: Indications for sperm cryopreservation include donor insemination, cryopreservation prior to surgical infertility treatment and malignancies and avoiding additional surgery in couples undergoing repeated IVF/ICSI cycles. However, dramatic changes during cryopreservation have

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detrimental effects on the sperm membrane, resulting in a large increase in the percentage of poorly motile sperm or sperm with abnormal morphology. The negative effects related to rapid temperature decrease, such as osmotic injury, cellular dehydration, intra-cellular ice crystal formation, and oxidative stress can also lead to damage the sperm function affecting the reproductive outcome. Seminal plasma could perform a protective function during the freezing-thawing process

Study design, size, duration: Comparative study between two cryopreservation protocols performed since January 2016 until August 2016 in the Assisted Reproduction United (La Fe Hospital). Semen samples from 40 normozoospermic men and 40 men presenting abnormal sperm parameters according the 2010 WHO were equally divided into two aliquots, one of which was processed by swim-up before cryopreservation (SF) while the other was prepared following cryopreservation (FS). Sperm count, motility, viability and morphology were evaluated in each group

Participants/materials, setting, methods: Semen samples presenting more than 4 ml of volume were considered. A 0.5 ml aliquot of the same semen sample was used for each protocol. Sperm count, progressive and total motility and morphology were performed using CASA system. Sperm vitality was assessed by HOS-test. Slow freezing was used for sperm cryopreservation. Sperm preparation was performed by swim-up. Multivariate linear regressions and T-test of dependent samples were performed. P-values<0.05 were considered significant

Main results and the role of chance: The progressive (PM) and total motility (TM), the total motile sperm count (TMS) and the percentage of viable sperm (VS) in the semen samples prepared after cryopreservation (FS) were higher than that in the pre-freezing preparation group (SF) (PM: 37.38% vs7.64%; TM: 38.71vs13.97%; TMS: $5.41 \cdot 10^6$vs$1.61 \cdot 10^6$; VS: 38.93%vs14.93%, in all cases $P < 0.01$). Regarding the morphology (M), percentage of normal sperm in fresh samples seemed to decrease when semen preparation was performed prior cryopreservation (4.05%vs3.05%, $P = 0.079$). The semen samples behavior after freezing-thawing process was not influenced by the initial semen quality since no differences were observed between samples presenting normal or abnormal initial seminal parameters. Total motility in both, prepared and non-prepared samples, is more related to HOS-test results than progressive motility: HOS=0.38+0.97•TM

Limitations, reasons for caution: Future studies should include more patients in order to validate these results testing other storage devices apart from straws and other freezing techniques should be investigated. Fertility potential of the spermatozoa could not be assessed since ICSI outcomes had not been followed

Wider implications of the findings: FS protocol resulted in better sperm quality than SF. Therefore, it is demonstrated that sperm preparation after freezing should be considered to increase the available sperm number, especially in patients with poor sperm baseline undergoing repeated ICSI cycles. TM could assess sperm viability in a simpler way than HOS-test

Trial registration number: .

P-006 Zinc nanoparticles prevents freeze-thaw-induced DNA damage in human spermatozoa

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Study question: To assess the beneficial role of Zinc Oxide nanoparticles (ZnO NP) in preserving the genetic and functional characteristics of human spermatozoa during cryopreservation

Summary answer: ZnO nanoparticles stabilizes the DNA of human spermatozoa and decreases the freeze-thaw-induced damage

What is known already: Semen cryopreservation is an integral part of the assisted reproductive technology and reproductive medicine field. Despite the importance of semen cryopreservation importance in infertility treatment, even today we do not have an ideal semen cryopreservation medium which can preserve the structural and functional characteristics of the spermatozoa. A recent study conducted in our laboratory has demonstrated that addition of Zinc to fresh human ejaculate before cryopreservation can be an efficient approach to prevent the DNA damage during freeze- thaw process.

Study design, size, duration: This is a prospective study involving 30 normozoospermic semen samples. The semen samples were stored in liquid nitrogen with commercial freezing medium containing ZnONPs (100 µg/mL). After 7 days of storage in liquid nitrogen, the semen samples were thawed and assessed for sperm functional parameters.

Participants/materials, setting, methods: Men attending Manipal Assisted reproduction center, Kasturba Medical College, Manipal University, Manipal for routine semen evaluation were recruited for the study. The motility was assessed manually, DNA integrity of the spermatozoa was assessed by TUNEL assay, acrosome intactness was studied by using PSA-FITC staining method and lipid peroxidation in the spermatozoa was assessed by estimating Malonedialdehyde level.

Main results and the role of chance: A significantly higher progressive motility ($p < 0.05$) was observed in ejaculates cryopreserved with ZnONP's at 1 and 24 h. In addition, the sperm DNA damage was significantly lower ($p < 0.01$) and number of spermatozoa with intact acrosome were non-significantly higher in this group. The sperm pellet had significantly lower ($p < 0.01$) level of lipid peroxidation as indicated by decrease in malondialdehyde level in ZnONP group. However, the transmission electron microscopic (TEM) imaging of the spermatozoa cryopreserved with ZnONP's revealed that these nanoparticles do not permeate across the membrane which was also confirmed by fluorescence imaging with fluorescent ZnONP's.

Limitations, reasons for caution: The sample size used in the study is relatively small. The beneficial effect of ZnONP on cryopreservation of poor quality ejaculates and fertilization outcome of spermatozoa needs to be studied further to assess its clinical application.

Wider implications of the findings: This is a novel approach to stabilize the DNA of spermatozoa which may help us in extracting superior quality spermatozoa for ART and thus help in improving the fertilization outcome with frozen-thawed spermatozoa.

Trial registration number: Not applicable.

P-007 Structural modification of pentoxifylline to improve the sperm function and reduce embryo toxicity

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Study question: Is it possible to modify the structure of pentoxifylline (PTF) to lower its adverse effects on oocyte and embryos without compromising with its efficacy on enhancing the motility in spermatozoa?

Summary answer: We synthesized two safe and potent modified pentoxifyline (mPTF) compounds which significantly improved the survival and functional competence of human spermatozoa *in vitro*

What is known already: Poor sperm motility is the common cause for fertilization failure in assisted reproductive technology (ART). Various strategies have been implemented in improving the success in IVF fertilization rate by chemical stimulation of spermatozoa such as treating spermatozoa with non-specific inhibitors of phosphodiesterase (PDEIs). Even though pentoxifylline is used as a potent sperm motility enhancer in ART, due to its adverse effect on fertilization and embryonic development, search for alternate molecules is of great clinical significance.

Study design, size, duration: This is a prospective study. We first synthesized four mPTF compounds (mPTF1, mPTF2, mPTF3, mPTF4) and characterized them by infrared and mass spectroscopy. These compounds were assessed for beneficial effect on human sperm motility by using fresh normozoospermic samples (n = 12). The selected mPTF compounds were further assessed for sperm function tests (n = 12). Cytotoxicity and genotoxicity of the selected compounds were assessed *in vivo* by injecting Swiss albino mice (n = 7) with PTF and mPTF compounds.

Participants/materials, setting, methods: Ejaculates were collected from men attending for routine semen analysis at the Manipal Assisted reproduction center, Kasturba Medical College, Manipal University, Manipal. Manual motility assessment, computer- assisted sperm analysis (CASA), mitochondrial function using rhodamine 123 staining, DNA integrity by TUNEL assay and ionophore-induced acrosome reaction was studied in human spermatozoa. Cytotoxicity

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and genotoxicity of these compounds were assessed in mouse bone marrow by assessing micronucleus incidence.

Main results and the role of chance: We have successfully synthesized four derivatives of pentoxifylline, mPTF1, mPTF2, mPTF3, and mPTF4 by adding 6 methoxy naphtholdehyde, 5 bromobenzaldehyde, 4 nitrobenzaldehyde, 2 and 4 dichlorobenzaldehyde to methoxy group of pentoxifylline. Pentoxifylline, mPTF1 and mPTF4 increased the progressive motility in comparison to control at all intervals. At 24 h intervals, the motility dropped in all the groups which was more predominant in control and PTF groups. However, in mPTF1 (0.25 mM) and mPTF4 (0.3 mM), significantly higher percentage of motile spermatozoa was observed ($P < 0.01$). In addition, the mPTF had slower acrosome reaction rate compared to PTF. Prolonged incubation of processed spermatozoa up to 24 h did not result in any increase in chromatin instability compared to control or PTF group. mPTF1 and mPTF4 had lower percentage of micronucleated cells in mouse bone marrow cells compared to PTF suggesting that their lower genotoxic effect.

Limitations, reasons for caution: This study involves *in vitro* assessment of the modified compounds to improve sperm motility. The effect of these mPTF compound on ART outcome is yet to be tested. The motility enhancement property of these agents in asthenozoospermic or testicular samples is not studied.

Wider implications of the findings: This experiment confirms the structural modification and synthesis of pentoxifylline compounds that are able to enhance sperm. The present study helped us in identifying novel compounds with lower toxicity and higher motility enhancement property compared to PTF. This will have potential clinical application in ART set up in future.

Trial registration number: Not applicable.

P-008 The influence of the storage temperature on the semen parameter: storage at 37°C might not be suitable

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Study question: What is the most suitable temperature for sperm storage?

Summary answer: The most suitable temperature to preserve sperm may be 25°C.

What is known already: In the assisted reproduction technologies, oocyte are stored in the suitable condition by controlling temperature and gas using equipment such as incubators. Meanwhile, the sperm are stored at room temperature or 37 °C in incubators, but the suitable conditions of the storage of sperm are undetermined and not standardized among the facilities. Therefore, we investigated the most suitable condition of the storage of semen by focusing on the storage temperature.

Study design, size, duration: In this study, 5 men who fathered within 2 were included.

Participants/materials, setting, methods: The standard semen analysis tests, the sperm vitality test, and the measurements of static oxidation-reduction potential (sORP) of sperm and sperm DNA fragmentation index (DFI) were conducted immediately after ejaculation. Then, 700 µl of semen were divided into 2 ml tubes, and incubated in the water bath whose temperatures were set at 4, 25, or 37 °C. The examinations described above were conducted after incubations for 6, 12, and 24 hours.

Main results and the role of chance: Sperm concentration were not significantly different among 4, 25 and 37 °C groups at any time point. Sperm total motility of 25 and 37 °C groups were significantly higher compared with 4 °C group after 3 hours incubation, and that of 25 °C group was significantly higher than those of 4 and 37 °C groups after 6, 12 and 24 hours incubation. Progressive sperm motility of 25 and 37 °C groups were significantly higher compared with 4 °C groups after 3 hours incubation, and that of 25 °C group was significantly higher than those of 4 and 37 °C groups after 6 and 12 hours incubation although those of each group were not significantly different after 24 hours incubations. As for the sperm vitality tests, that of 25 °C group was significantly higher than those of 4 and 37 °C groups after 6, 12 and 24 hours incubations. The sORP of 25 °C group was significantly lower than that of 37 °C group after 12 and 24 hours incubations and higher than that of 4 °C group after 6, 12 and 24 hours incubations. DFI was not significantly different among groups at any time point.

Limitations, reasons for caution: Small sample size Included only fertile males

Wider implications of the findings: The storage at 25°C lead to keeping sperm quality, it is improving treatment outcome of reproductive therapy.

Trial registration number: none.

P-009 Correlation of Sperm DNA fragmentation and Seminal oxidation reduction potential in infertile men

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Study question: Is Sperm DNA fragmentation really correlated to seminal oxidative stress?

Summary answer: Sperm DNA fragmentation is positively correlated with seminal oxidative stress measured by Seminal oxidation reduction potential

What is known already: Oxidative stress is a proposed cause for sperm DNA fragmentation. Oxidation reduction potential is a new accurate measure for oxidative stress as it measures the balance between oxidants and reductants

Study design, size, duration: This is a prospective study performed on 312 patients attending the male infertility clinic at a tertiary medical center between February and August, 2016.

Participants/materials, setting, methods: Patients receiving medical or surgical treatment for infertility prior to their presentation or who had a sperm concentration less than 5 millions/ml sperm were excluded. Patients were subjected to history taking, clinical examination as well as semen analysis and SDF assessment using Halosperm kit (cut off value <30%) and static oxidation reduction potential (sORP) using MiOXSYS system. Patients were divided according to SDF result (normal/ high) and to age (<40 years / > 40 years).

Main results and the role of chance: A total number of 312 patients were included in the study. Patients with high SDF had significantly higher age than those with normal SDF. The mean total and progressive motility was significantly higher in the normal SDF group while sORP was significantly lower in the normal SDF group compared with the high SDF group. Presence of varicocele did not significantly affect SDF level (Table 1). SDF was negatively correlated with total (-0.526, $p < 0.001$) and progressive motility (-0.415, $p < 0.001$) and positively correlated with abnormal morphology, sORP and age (0.351, 0.222 and 0.192 respectively, $p < 0.001$ for all). SDF was significantly lower while total motility was significantly higher in patients <40 years compared with patients > 40 years of age.

Table I Comparison between normal and abnormal sperm DNA% result.

	Normal DNA%	High DNA%	p Value
Age	**35.11 ± 0.47**	**38.14 ± 0.95**	**0.01***
Count	35.45 ± 1.71	30.99 ± 2.62	0.14
Total motility	**57.53 ± 0.89**	**40.92 ± 1.78**	**0.00***
Prog.	**13.8 ± 0.73**	**5.56 ± 0.83**	**0.00***
Abnormality	92.94 ± 0.69	95.86 ± 0.78	0.12
sORP	**2.14 ± 0.14**	**4.03 ± 0.61**	**0.00***
Varicocele	77 (68.1%)	36 (31.9%)	0.56

Limitations, reasons for caution: The main limitation of the study is absence of normal fertile controls.

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Wider implications of the findings: Sperm DNA fragmentation and seminal oxidation reduction potential should be included in assessment of male infertility. Using ORP testing can help in detecting the target patients for antioxidant therapy.

Trial registration number: This is not a clinical trial.

P-010 Indices of global DNA methylation status of sperm from smokers and non-smokers men

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Study question: Are there Global DNA methylation variations in sperms of smokers that negatively affect sperm quality and male fertility?

Summary answer: Cigarette smoking significantly correlated with sperm global DNA methylation and sperm quality.

What is known already: Abnormal sperm DNA methylation was connected with male infertility in patients with poor sperm quality.

Study design, size, duration: A prospective study compares sperms from 109 men; 55 smokers and 54 non-smokers whose female partners attended an assisted reproduction and andrology laboratory were evaluated.

Participants/materials, setting, methods: A total of 109 sperm samples collected from 55 smokers and 54 non-smokers patients were involved in this study. DNA was extracted from purified sperms. A colorimetric quantification of global DNA methylation measures specifically the levels of 5-methylcytosine (5-mC) with an Enzyme Link Immunosorbent Assay (ELISA)-like method. Sperm's chromatin condensation, vitality and membrane integrity were evaluated according to the World Health Organization guidelines.

Main results and the role of chance: Results are showing highly and significantly ($p = 0.000$) lower levels of global DNA methylation in non-smokers than smokers (4.85 ± 2.72 and 7.08 ± 1.77 ng/ µL, respectively). Also, global DNA methylation levels are found to be significantly ($r = 0.220$; $P = 0.021$) correlated with non-condensed chromatin (CMA3 positive). In addition, global sperm DNA methylation are correlated negatively and highly significantly ($p < 0.010$) with sperm count (r=-0.273), motility(r=-0.301), and vitality (r=-0.265).

Limitations, reasons for caution: The results of this study were created from males whose wives attended the IVF and Andrology laboratory pursuing fertilization. However, comparison between smokers and non-smokers supports the idea that smoking negatively affects sperm, but additional studies are required to reveal the impacts of DNA methylation on male fertility.

Wider implications of the findings: The study results may act as an evidence for the negative effect of smoking on male fertility. Furthermore, DNA methylation from smokers may serve as a marker for male fertility.

Trial registration number: NA.

P-011 Management of infertility in men with spinal cord injury: an educational program for practitioners

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Study question: Can practitioners obtain formal training in the management of infertility in men with spinal cord injury?

Summary answer: Training is provided at University of Miami, Florida. Trainees' travel to Miami supplemented by grant from Craig Neilsen Foundation. Course approved for Continuing Education Credit

What is known already: Spinal cord injury (SCI) occurs most often to young men. Infertility is a major sequela of SCI. Anejaculation and abnormal semen parameters are seen in 90% of cases. Currently, there is no established standard of care for the management of infertility in these men, largely due to a lack of education of healthcare providers at all levels. Consequently, couples with SCI male partners are often over-treated with expensive and unnecessary procedures. A three-year educational program funded by the Craig Neilsen Foundation has been developed to train practitioners in methods and techniques for managing infertility in this population.

Study design, size, duration: A teaching curriculum was developed based on our 27 years of experience in the management of infertility in men with SCI. The training is performed at our center at the University of Miami Miller School of Medicine in Miami, Florida. Training can also be provided at outside centers, if requested. The program offers Continuing Medical Education (CME) credits for physicians (*7.5 AMA PRA Category 1 Credits™*). Certificates of training are provided to all participants.

Participants/materials, setting, methods: The 1.5 day course includes:

(1) A half-day of lectures and videos.
(2) Two half-days of hands-on training in the techniques of penile vibratory stimulation (PVS) and electroejaculation (EEJ) as well as management of autonomic dysreflexia, and retrograde ejaculation.
(3) Syllabus, PowerPoint presentation, and instructional videos are given to trainees for incorporation into their own teaching.
(4) Limited travel reimbursement (up to $2,250) is available for professionals who train at our center in Miami.

Main results and the role of chance: During the first 5 months of this program (January to May 2016), educational materials (syllabus and comprehensive 30 minute instructional video) were developed. Training began in June 2016. A total of 9 physicians from the USA, Canada, Spain, Brazil, and Israel have been trained to date at our center. An additional 20 practitioners have been trained at the San Antonio Veterans Administration Hospital. Requests for training are ongoing, and scheduling is in progress. Training sessions scheduled at our center in February, March and April, 2017 are full. The curriculum was presented to 150 professionals at the 2016 Academy of Spinal Cord Injury Professionals meeting in Nashville. The curriculum has been selected for presentation at the 2017 meetings of the Barrow Neurological Institute in Phoenix, the American Spinal Injuries Association meeting in Albuquerque, the American Society of Andrology meeting in Miami, and the 2017 American Urological Association meeting in Boston. This program is open to trainees from outside the United States, including physicians and allied health personnel. At the conclusion of the funded training period, (2018), a communication network of providers will be established. A website will be created providing information to professionals and patients.

Limitations, reasons for caution:

(1) The 1.5 day training course is comprehensive, but cannot address every possible case that a practitioner may encounter.
(2) Training is available only in the English language.

Wider implications of the findings: This educational program addresses a gap in knowledge among health care professionals on the topic of management of infertility in men with SCI. Interested professionals are encouraged to take advantage of this training opportunity, which will be available through December 2018.

Trial registration number: Not applicable.

P-012 Effect of antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: a double blind place controlled (dbpc) study

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Study question: Is effective an antioxidant supplementation on sperm quality of patients suffering oligo-astheno-teratozoospermia? Is there any difference in the effect between those with varicocele and those without?

Summary answer: Antioxidant supplementation is effective and safe when used for treating patients with oligo-astheno-teratozoospermia. The effect is stronger when patients are affected by varicocele.

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What is known already: The male factor in worldwide infertility incidence is 20-30%. Sperm has high energy requirement for maturation, capacitation and motility. Many factors affecting sperm quality act through decreasing energy and increasing reactive oxygen species (ROS) by causing mitochondrial dysfunction. Sperm is vulnerable to ROS causing sperm immobilization, impairment of acrosomal reaction, abnormal morphology, DNA fragmentation and cell death.

Study design, size, duration: To determine the effect of antioxidant supplementation containing L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, vitamin B12 and zinc on sperm quality in subjects with oligo- or astheno-teratozoospermia, with and without varicocele and history of difficulty conceiving.

Participants/materials, setting, methods: This was a monocentric, randomized, DBPC with a total of 104 patients, 52 in the supplementation and 52 in the placebo arm, that were recruited in 6 months. The enrollment was divided in 52 patients with varicocele and 52 patients without. We evaluated efficacy of 6 months of supplementation versus placebo. Spermogram evaluation, according to the WHO guidelines, was done at the beginning of treatment and at the end of the 6 month treatment.

Main results and the role of chance: Sperm count (number $x10^6$/mL) in patients with varicocele was 39.3 +/- SD 16.8 in placebo group and 49.4 +/- 18.9 in supplementation group (percentage change 25.7% t=2.04 $p < 0.05$ Student test); in patients without varicocele 47.5 +/- 7.9 in placebo group and 52.3 +/- 9.1 in supplementation group (percentage change 9.9% $t = 2.01$ $p < 0.05$). Total sperm motility in patients with varicocele was 33.9+/- 6.9 in placebo group and 38.3+/- 8.0 in supplementation group (percentage change 18.6% $t = 2.10$ $p < 0.05$); in patients without varicocele was 35.0 +/- 7.5 in placebo group and 39.9 +/- 8.0 in supplementation group (percentage change 13.8% t=2.19 $p < 0.05$). Progressive sperm motility in patients with varicocele was 23.1 +/- SD 6.7 in placebo group and 27.4 +/- 7.9 in supplementation group (percentage change 18.6% t=2.10 $p < 0.05$); in patients without varicocele was 25.1 +/- 7.0 in placebo group and 29.7 +/- 9.1 in supplementation group (percentage change 18.6% t=2.07 $p < 0.05$).

Limitations, reasons for caution: We didn't compare the effect of this treatment with varicocele surgical treatment and we didn't evaluated DNA fragmentation. Furthermore latest evidences report that evaluating oxidative-stress can help in better understanding the best strategy for male infertility treatment.

Wider implications of the findings: In our study, at the end of the treatment we observed a marked increase in quality parameters of sperm especially in varicocele patients. The supplementation was safe without adverse events. On this basis it can be established that functional substances can form part of an efficacious strategy to handle infertility.

Trial registration number: PXP-001 A.

P-013 Perspectival study about Testicular sperm aspiration as easy, outpatient procedure in support to Assisted Reproduction Technique in Obstructive Azoospermia: confrontation with Testicular Sperm Extraction

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Study question: Is Testicular Sperm Aspiration (TeSA) a suitable technique for "fresh" sperm retrieving in ART, with the same results (or better) than cryopreservation from TESE?

Summary answer: TeSA has shown to be an easy, cheap and feasible technique to retrieve fresh spermatozoa with same results (possibly better) than cryopreservation from TESE.

What is known already: TESE and cryopreservation is most used technique before ART in OA, for sperm retrieving. TeSA is an alternative, costless, outpatient procedure, providing fresh spermatozoa.

Study design, size, duration: Since September 2013,all OA cases underwent TeSA contextually to ovuli pick-up for partners (group A, n= 33, data perspectivally stored, TESE and cryopreservation had been carried on during previous months, to avoid impossibility of fertilization in hypothesis of absence of spermatozoa from TeSA). Control group were couples that previously underwent ICSI (B, n= 31, only TESE). Average age was 41 and 35; 37,5 and 34 (respectively group A and B, patients and partners, p>010).

Participants/materials, setting, methods: During TeSA, the aspiration has been repeated until an adequate amount of spermatozoa has been obtained (range 1-4 aspirations, average 2.03) for ICSI procedure.

Main results and the role of chance: In all cases of group A, spermatozoa have been found. No complication of procedures has been reported. Average fertilization rate for single couple has been 56% of obtained ovuli. In only one couple, no fertilization occurred in group A (4%). In group B, fertilization rate has been 34%. In 4 cases we didn't obtain fertilization (19,5%), 2 complications occurred. In particular one patients reported pain for 10 days (grade 1), one patients has been recovered for orchyepididimitis (grade 2). TeSA demonstrated in all cases to provide an adequate amount of spermatozoa by a very low number of aspirations and low pain (average 2, VAS during procedure 1-7, average 1.66). TeSA associated to use of fresh spermatozoa showed much higher fertilization rate than TESE (56% vs 34%, p = 0.001, MannWhytney test). In addition, in only one case TeSA didn't show fertilization (embryo-transfer carried on in 96% of procedure), TESE with cryopreservation in 4 cases (80,5%).

Limitations, reasons for caution: The sample is not big, results are only preliminary.

Wider implications of the findings: By now, considering the higher fertilization rate, we are suggesting and using it in II and III level ART, also with aim to reduce costs of these procedures. In EAU guideline, TeSA is considered a second choice in confrontation to TESE. We think in future this could be changed.

Trial registration number: No registration number.

P-014 Influence of cigarette smoking on DNA methylation levels of sperm from the current smokers

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Study question: Are there an influence of cigarette smoking on DNA methylation patterns of sperm from the current smokers males.

Summary answer: Cigarette smoking causes alterations in sperm DNA methylation level at CpGs in *MAPK8IP* and *TKR* gene-related amplicon. Besides its impact on some semen parameters.

What is known already: Sperm DNA methylation can be influenced by several factors including environment/lifestyle like chemicals known as mutagens and carcinogens found in cigarettes and cigarette smoke.

Study design, size, duration: A prospective cohort study. A total of 108 human semen samples were collected during the period of February 2016 to May 2016. 57 samples were obtained from never smokers, and 51 from current smoker's males.

Participants/materials, setting, methods: As a screening study, 450 K BeadChip arrays were used, to identify genomic regions that show differences in DNA methylation patterns between 15 current smokers and 15 never smokers males. Subsequently, local deep bisulfite sequencing was used to validate two differentially methylated CpGs (cg07869343 and cg19169023), which have the highest difference in the methylation level, and directly linked to *MAPK8IP3* and *TKR* genes, respectively; on 78 sperm DNA samples (42 never-smokers versus 36 current smokers).

Main results and the role of chance: The results of 450 K BeadChip arrays identified 8 CpGs have an alteration in DNA methylation patterns, and only 5 of those CpG do not overlap common annotated SNPs. According to the

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results of validation, the variation in methylation levels was found in more than one CpGs: The results of *MAPK8IP* amplicon showed a significant difference at six from twenty-two CpGs was tested (CpG3, CpG5, CpG6, CpG7, CpG8, and CpG21) in cases compared to controls group ($P \leq 0.043$; $P \leq 0.005$; $P \leq 0.002$; $P \leq 0.003$; $P \leq 0.045$ and $P \leq 0.040$, respectively). Besides, one from four CpG tested in the *TKR* amplicon (CpG4, $P \leq 0.002$). On the other hand, the results of this study indicate the presence of a significant correlation between methylation levels at CpG4, CpG8, CpG9, CpG10, CpG15 and CpG22 in the *MAPK8IP* gene-related amplicon and percentage of total motility ($P \leq 0.005$, $P \leq 0.001$, $P \leq 0.013$, $P \leq 0.0001$, $P \leq 0.021$, and $P \leq 0.003$, respectively). Furthermore, a significant correlation between the methylation level at CpG13 and CpG16 and CpG18 in the *MAPK8IP* gene-related amplicon and the sperm count ($P \leq 0.014$, $P \leq 0.032$, and $P \leq 0.030$, respectively).

Limitations, reasons for caution: The number of samples is small to obtain a strong conclusion, so more samples is needed to elucidate the effects of these alterations in sperm DNA methylation pattern on male fertility.

Wider implications of the findings: The results of the present study suggest that the cigarette smoking affects sperm DNA methylation patterns. Besides, this finding may explain some causes of male infertility.

Trial registration number: No.

P-015 The effect of BMI on semen quality with an assessment of the new 2010 WHO Laboratory Manual for the Examination of Human Semen- a meta-analysis

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Study question: To assess the effect of body mass index (BMI) on semen quality based on the new World Health Organization (WHO) manual for laboratory examination of human semen.

Summary answer: Men who are overweight (OW) or obese (OB) show decline in all semen parameters and clinical pregnancy rate achieved through ART cycles.

What is known already: Obesity is considered as a risk factor for male infertility. Its prevalence has doubled worldwide since 1980. Recent studies have suggested an increased risk of abnormal semen parameters, including concentration, motility, and morphology among overweight and obese men. However, the evidence is not unequivocal, and some studies have found no effect of obesity/overweight on sperm quality. Therefore, this study sought to assess the effect of BMI on semen parameters according to the new WHO criteria for the laboratory examination of human semen.

Study design, size, duration: A systematic search was conducted using MEDLINE/PubMed, SJU discover and Google Scholar to identify all relevant studies published from 2010 to 2017 (January) corresponding to the release of the 2010 WHO manual. Participants were from fertility/urological clinics and general population. The outcome measures were semen volume, sperm count, motility, and morphology. Clinical pregnancy rate (CPR) assessed in couples following ART was used as an outcome measure to evaluate fecundity.

Participants/materials, setting, methods: Participants were males aged 13 years and older regardless of population size and origin. Each semen parameter was evaluated separately and independently. The study groups were stratified and compared according to BMI categories based on WHO classification - Normal, Overweight, and Obese. Subgroup analysis included the comparison of WHO 2010 manual (Fifth Edition) versus 4 previous editions.

Main results and the role of chance: We used 18 studies in the meta-analysis, involving 21,011 subjects. Semen volume (MD -0.11 mL, [95% CI -0.17, -0.04], P = 0.001), and morphology (MD -1.55%, [95%CI -2.54, -0.55], P = 0.002) were significantly reduced in OW group when compared to men with normal BMI. Sperm concentration, motility, total progressive motile sperm count and CPR achieved through ART were also reduced in OW men. Obese men as compared to men with normal BMI showed a significant decline in semen volume (MD -0.22 mL, [95% CI -0.34, -0.11], P < 0.001), sperm count (MD -8.26 × 10^6/mL, [95% CI -13.42, -3.10], P = 0.002), motility (MD -4.89% [95% CI -6.74, -3.04], P < 0.001), morphology (MD -1.91%, [95%CI -3.16, -0.65], P = 0.003), total progressive motile sperm count (MD -20.27 × 10^6 [95% CI -38.26, -2.29], P = 0.03) and CPR achieved through ART (OR 0.78% [95% CI 0.62, 0.98], P = 0.04). The recent changes in the 2010 WHO reference values for semen parameters had no observed negative effect on an association between BMI and sperm quality in overweight and obese men, but motility results in obese men were equivocal. Subgroup analyses based on the study participant type did not affect heterogeneity estimates

Limitations, reasons for caution: The meta-analysis did not include any randomized trials since a randomized trial to assess the impact of BMI is not possible. We conducted a thorough bias evaluation of the included studies.

Wider implications of the findings: Pooled results from evaluated studies suggest that BMI negatively affects sperm quality and fecundity. The full clinical implications of the 2010 WHO criteria on the association between BMI and human semen quality deserves further investigation as does the effect of BMI on natural fecundity rates.

Trial registration number: Not applicable.

P-016 The downstream signaling pathways of perfluorooctanesulfonate (PFOS)-induced disruption of blood-testis barrier – disorganization of F-actin-based and microtubule-based cytoskeleton in Sertoli cells

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Study question: We sought to investigate the involvement of Akt (Ak strain transforming) pathway in the PFOS-induced Sertoli cell injury and to explore the rescue process.

Summary answer: PFOS exerts its disruptive effects on Sertoli cell function downstream through Akt1/2. The PFOS-induced male dysfunction can be managed through an intervention on Akt1/2 expression.

What is known already: Earlier study in our laboratory has shown that the PFOS-mediated Sertoli cell injury that impedes blood-testis barrier (BTB) function is through a disruption of actin-based cytoskeleton in Sertoli cells involving an activated form of FAK (p-FAK-Y407). This notion was further confirmed by using an endogenous miRNA specific for FAK, miR-135b, which was found not only perturbed the Sertoli TJ-permeability barrier alone, but also worsened PFOS-induced TJ-barrier disruption. Studies in other mammalian cells have shown that FAK is the upstream signaling protein of Akt, and these two signaling proteins often work as close partners to modulate a number of cellular functions.

Study design, size, duration: In order to better understand the signaling pathway of FAK-mediated rescue function during PFOS-induced Sertoli cell injury, we sought to examine the involvement of Akt in PFOS-mediated Sertoli cell injury, and its functional relationship with p-FAK-Y407 using an in vitro model of Sertoli cell blood-testis barrier.

Participants/materials, setting, methods: Sertoli cells isolated from 20-day-old rat testes were cultured in vitro for 3 days to form a cell epithelium with an established functional TJ-barrier. Thereafter, these cells were treated with PFOS at 10, 20 or 50 μM for 24 hr.

Main results and the role of chance: PFOS was found to induce Sertoli cell injury by perturbing actin cytoskeleton through changes in the spatial expression of actin regulatory proteins. Specifically, PFOS caused mis-localization of Arp3 (actin-related protein 3, a branched actin polymerization protein) and palladin (an actin bundling protein). These disruptive changes thus led to a disorganization of F-actin across Sertoli cell cytosol, causing truncation of actin microfilament, thereby failing to support the Sertoli cell morphology and adhesion protein complexes (e.g., occludin-ZO-1, CAR-ZO-1, and N-cadherin-ß-catenin), through a down-regulation of p-Akt1-S473 and p-Akt2-S474. The use of SC79, an Akt1/2 inhibitor, was found to block the PFOS-induced Sertoli cell injury by rescuing the PFOS-induced F-actin dis-organization. These findings thus illustrated that PFOS-mediated Sertoli cell BTB disruption can be rescued by the use of an Akt activator SC79, which apparently exerts its effects by re-

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establishing the underlying actin- and MT-based cytoskeletons, restoring the localization of BTB-associated adhesion protein complexes. This thus reseals the disrupted Sertoli cell TJ-permeability barrier induced by PFOS.

Limitations, reasons for caution: In summary, we have demonstrated that PFOS exerts its disruptive effects on Sertoli cell function downstream through Akt1/2. As such, PFOS-induced male reproductive dysfunction can possibly be managed through an intervention on Akt1/2 expression.

Wider implications of the findings: Supported by General Research Fund 771513 of Hong Kong Research Grants Council and NSFC/RGC Joint Research Scheme N_HKU717/12.

Trial registration number: N.A.

P-017 Assessment of sperm ploidy status by flow cytommetry correlates to embryo quality

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Study question: To assess, by using flow cytometry, the effect of sperm ploidy status on embryo quality following an *in vitro* fertilization treatment

Summary answer: Sperm ploidy condition constitutes a critical parameter for the evaluation of semen samples before an assisted reproductive treatment

What is known already: Male infertility is a contributing factor in up to 50% of all infertility cases. New and improved diagnostic methods that reduce inter/intra variability should be evaluated. Flow cytometry has been an outstanding tool to evaluate sperm cells for achieving a better reproductive condition. The implementation of this technique in order to evaluate sperm ploidy, is contributing to the discrimination of spermatozoa from subfertile men in an objective way, leading to a reproducible assessment of male infertility, especially in candidates for an assisted reproductive treatment

Study design, size, duration: Prospective and observational study. Sixty seminal samples coming from sperm donors (n = 30) and subfertile patients (n = 30) were analyzed by flow cytometry in IVI Madrid from June to December 2016. Furthermore, in order to analyze if there were any correlation between sperm ploidy and embryo quality, we evaluated clinical outcomes from 30 couples included in our oocyte donation program; all these couples underwent an ICSI procedure and a fresh embryo transfer

Participants/materials, setting, methods: Semen analysis was performed according to World Health Organization guidelines. Ploidy determination was performed by using propidium iodide (PI) staining in combination with sperm flow cytometry; the resulting cell suspension (n = 20000 cells/sample) was examined. For describing the correlation between ploidy and other sperm and embryo parameters, linear functions were approximated and the Pearson's correlation coefficient (r) was determined. Differences were considered to be significant if the probability of their occurrence by chance was <0.05.

Main results and the role of chance: We found significant differences in some of the analyzed variables between patients and donors respectively. From semen analysis, data were as follows: ejaculation volume (ml) 2.8 vs. 3.5, $p = 0.027$; sperm concentration (million/ml) 43.6 vs. 70.9, $p < 0.001$; total sperm number (million) 122.4 vs. 234.6, $p < 0.001$; progressive motility (%) 36.7 vs. 58.5, $p < 0.001$; total motility (%) 47.8 vs.139.0 $p = 0.001$. Data derived from sperm ploidy showed significant differences in the percentage of subploid cells between patients (20.4%) and donors (8.1%), $p < 0.001$; similar results were obtained for haploid cells (75.6 % vs. 84.3%, $p = 0.011$)

According the correlation between sperm ploidy and some embryo features, we observed a positive correlation in the fertilization rate ($r = 0.398$) and in the number of viable embryos ($r = 0.454$) as well as the percentage of haploid cells increased in the seminal sample. On its behalf, seminal samples with a higher rate of subploid cells were significant negative correlated with both variables, $r = 0.265$ and $r = 0.459$ for the fertilization rate and viable embryos respectively.

Limitations, reasons for caution: Flow cytometry is a screening method in which is not possible to perform a precise determination of aneuploidies of specific chromosomes; moreover, we cannot quantify the degree of DNA damage within a single cell

Wider implications of the findings: Although conventional semen analysis maintain its central role in assessing male fertility, is often insufficient to provide definitive diagnosis. Implementing flow cytometry in the daily's practice due to its higher sensibility and lower cost could help many couples undergoing unexplained infertility to improve clinical outcomes within an assisted reproductive treatment.

Trial registration number: Does not apply.

P-018 Loss of both OSR1 and SPAK in Sertoli cells manifest Sertoli cell-only-like syndrome in mice

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Study question: What is the modulatory role of oxidative stress-responsive kinase-1 (OSR1) and STE20 (sterile 20)/SPS1-related proline/alanine-rich kinase (SPAK) in Sertoli cells?

Summary answer: The OSR1 and SPAK are vital in Sertoli cell support spermatogenesis. Loss of both OSR1 and SPAK in Sertoli cells caused spermatogenesis arrest.

What is known already: OSR1 and SPAK are upstream regulators of Na^+-K^+-$2Cl^-$ cotransporters. Most studies have revealed their roles in the regulation of blood pressure and cell volume. We previously generated Sertoli cell-specific OSR1 combined with global SPAK double knockout mice. These male mice are infertile with defective spermatogenesis, indicating that Sertoli cell function is impaired.

Study design, size, duration: This time, we generated Sertoli cell-specific OSR1 and SPAK double knockout mice to further elucidate the physiological roles of OSR1 and SPAK in Sertoli cells.

Participants/materials, setting, methods: We assessed male fertility in these tissue-specific double knockout mice. Testes of adult mice were evaluated by histology. Immunofluorescence staining and RT-qPCR were performed to further identify the cellular composition of these double knockout mice. Moreover, the first wave of spermatogenesis was analyzed to evaluate key time points for germ cell development.

Main results and the role of chance: These male Sertoli cell-specific double knockout mice were infertile. The adult testicular histology revealed Sertoli cell-only-like syndrome. Immunofluorescence staining supported that most cells within seminiferous tubules showed Sertoli cell-specific markers, GATA-1 and SOX9. Moreover, most germ cell-specific genes were barely detected in RT-qPCR. Furthermore, no obviously round spermatids were found in these mice during the first wave of spermatogenesis, indicating that absence of OSR1 and SPAK in Sertoli cells caused spermatogenesis arrest.

Limitations, reasons for caution: This study is mainly performed in the mouse model. Application of this mutant model in the patients with Sertoli cell-only syndrome needs further studies.

Wider implications of the findings: This study provides that OSR1 and SPAK are potential biomarkers for the assessment of human male infertility.

Trial registration number: All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC) in Taiwan.

P-019 Differential sperm DNA fragmentation of X- and Y-chromosome bearing human spermatozoa

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Study question: Do X- and Y- chromosome bearing spermatozoa have different levels of DNA fragmentation?

Summary answer: Sperm DNA fragmentation level of Y- chromosome bearing spermatozoa was significantly higher than X- chromosome bearing spermatozoa in pathozoospermic subjects, but not in normozoospermic subjects.

What is known already: Sperm DNA fragmentation could be induced by suboptimal testicular and epididymis environment. The Y chromosome of sperm has been considered particularly vulnerable to DNA damage, partly because of its genetic structure and partly because it cannot correct double-stranded DNA fragments by homologous recombination. An early mice study demonstrated that X- chromosome bearing spermatozoa are more robust to irradiation induced DNA damage than Y- chromosome bearing spermatozoa. However, direct evidence is lacking regarding whether X- and Y- chromosome bearing sperm has different susceptibilities to DNA fragmentation.

Study design, size, duration: This is a cross sectional study, which included 100 men who underwent routine fertility assessment between November 2015 and June 2016.

Participants/materials, setting, methods: Fifty normozoospermic men (Group I) and fifty pathozoospermic men (Group II) were recruited in this study. All participants received routine semen analysis at the IVF unit of Prince of Wales Hospital, the Chinese University of Hong Kong. DNA fragmentation states and sex chromosome complement were assessed by a combined test of sperm chromatin dispersion (SCD) and fluorescence in situ hybridization (FISH), followed by an observation of at least 300 spermatozoa/sample under florescent microscope.

Main results and the role of chance: We found, for the first time, in our study that the median level of DNA fragmentation in Y- chromosome bearing spermatozoa was significantly ($P < 0.05$) higher than that of X- chromosome bearing spermatozoa when all subjects were considered together (23.5% and 20.4% respectively) or in Group II (25.9% and 22.5% respectively), but not ($P>0.05$) in Group I (17.2% and 17.3% respectively). We also observed several findings which were in line with previous studies: (1) DNA fragmentation level is correlated with both sperm concentration [regression coefficient (RC) 95% confidence interval (CI): 0.08 (0.01 to -0.15); $P = 0.03$] and motility (RC (95% CI): -0.33 (-0.48 to -0.17); $P < 0.001$); (2) DFI and sex chromosome disomic rate of subjects in the pathozoospermic group [median(IQR): 4.81%(2.73%-8.98%)] was significantly higher ($P = 0.01$) than subjects in the normozoospermic group [median(IQR): 2.40%(1.79-4.78%)].

Limitations, reasons for caution: We did not assess the autosomes complement of spermatozoa in our study. As a result, we cannot determine the impact of autosomes aneuploidy on sperm DNA fragmentation.

Wider implications of the findings: Y- chromosome bearing spermatozoa are more susceptible to DNA damage under suboptimal testicular environment, especially in pathozoospermic subjects. The clinical relevance of the finding requires further investigation.

Trial registration number: N/A.

P-020 The role of sperm surface galectin-3 on spermatozoa-zona pellucida binding in humans

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Study question: Is galectin-3 involved in human spermatozoa-zona pellucida (ZP) interaction?

Summary answer: Is galectin-3 involved in human spermatozoa-zona pellucida (ZP) interaction?

What is known already: A spermatozoon acquires its fertilization capacity within the female reproductive tract by capacitation. Spermatozoa-ZP receptor is suggested to be a composite structure that are assembled into a functional complex during capacitation. In the male reproductive tract, galectin-3, a carbohydrate binding protein, has been identified in Sertoli cells, epididymis, seminal plasma and ejaculated spermatozoa.

Study design, size, duration: The origin and cellular localization of sperm galectin-3 were studied. The roles of galectin-3 on sperm functions were assessed by functional blocking anti-galectin-3 antibody and galectin-3 competitive carbohydrate substrate. Ejaculated sperm were obtained from semen samples from normozoospermic men. For epididymal sperm samples, donated cryopreserved epididymal spermatozoa from men who have undergone assisted reproduction with microsurgical epididymal sperm aspiration and have completed their family were used. Human oocytes were obtained from an assisted reproduction program.

Participants/materials, setting, methods: Extracellular vesicles (EVs) in seminal plasma were purified by ultrafiltration. The expressions of galectin-3 in seminal plasma/EVs and spermatozoa were studied by transmission electron microscopy, western blotting or immunostaining. Sperm functions including motility, viability, acrosome reaction and ZP-binding capacity were assessed by standard assays.

Main results and the role of chance: The expression of galectin-3 on ejaculated spermatozoa (uncapacitated or capacitated) was much higher than that of epididymal spermatozoa, indicating that galectin-3 is acquired after leaving the testis. This possibility is supported by the presence of galectin-3 in the EVs of human seminal plasma, which are capable of transferring proteins to the sperm plasma membrane. Galectin-3 immunoactivity was further demonstrated to be localized in the post-acrosomal region of uncapacitated spermatozoa. It was translocated to the apical region of spermatozoa after capacitation, a region known to bind ZP. Blocking of galectin-3 function by specific antibody or competitive substrates against galectin-3 reduced the ZP-binding capacity of human spermatozoa.

Limitations, reasons for caution: The mechanisms by which EV-derived galectin-3 transfer to the sperm surface have not been depicted.

Wider implications of the findings: The results indicate the possible use of galectin-3 content in seminal plasma and sperm surface as test for prediction of fertilization potential of semen samples.

Trial registration number: NA.

P-021 Sperm morphology and motility alterations do not predict bipolar spindle assembly defects in the egg cytoplasm

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Study question: Is there a relationship between sperm morphology/motility and their ability to support bipolar spindle assembly?

Summary answer: Spermatozoa were able to build bipolar spindles regardless of their morphology/motility.

What is known already: Despite significant advances in the in vitro culture of human embryos, around 30% of fertilized oocytes arrest their development during early cell divisions. This suggests that functional defects of the sperm cell, such as the failure to fuse the pronuclei or to build the bipolar spindle, could be partially responsible for these arrests. To test this possibility, we first established an ex-vivo system with *Xenopus laevis* egg extract (XEE) and then used it to obtain quantitative data on the ability of human sperm sample with different diagnosis to support bipolar spindle assembly.

Study design, size, duration: We analyzed 20 human sperm samples with different clinical outcomes, and different morphology and motility: normozoospermic (N, n = 11), astenozoospermic (A, n = 2), teratozoospermic (T, n = 4), astenoteratozoospermic (AT, n = 3). Each sperm sample was incubated in 4 different XEE preparations. The sperm capacity to build bipolar spindles in XEE was analyzed by immunofluorescence, and the contribution of the sperm centrosome was dissected by interfering with one of the microtubule assembly pathways.

Participants/materials, setting, methods: The cytoplasmic layer of MII *Xenopus* eggs were isolated and 3000 Xenopus or human spermatozoa, treated with Triton X-100 and DTT, were incubated in XEE at 20°C for up to 150 min. The presence and overall morphology of spindles formed upon incubation of 200 human spermatozoa were correlated with the corresponding clinical data. To study more specifically the chromosomal and the centrosomal microtubule assembly pathways, the system was perturbed by cold treatment or recombinant protein addition.

Main results and the role of chance: We established a quantitative ex-vivo experimental system based on the use of XEE and human spermatozoa for functional studies. A time-course analysis showed that the human sperm cells

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incubated in XEE require a longer time (40 minutes) than the control *Xenopus* sperm nuclei (10 min) to promote microtubule assembly and this regardless of their clinical diagnosis. Complementation assays showed that the sperm centrosome become competent for nucleating microtubules. During interphase, the human sperm centrosome duplicates and the sperm DNA undergoes a partial and rapid decondensation during the first 10 minutes of XEE incubation (area from 17,3 ± 5,40 to 55,9 ± 16,26 μm^2 on average), followed by a 50 min of steady state, before undergoing full nuclear decondensation (156,2 ± 38,80 μm^2 on average). Upon entry into mitosis, the duplicated centrosomes localize correctly to the spindle poles even for samples with low motility (*Xenopus*=41%, N = 42,6%, A=41,1). Moreover samples with a variety of diagnosis assemble bipolar spindles with similar efficiency (Bipolar spindles%: N = 62,4 ± 8,64; A=58,1 ± 3,01; T=52,0 ± 16,34; AT=58,1 ± 2,66. Abnormal spindles%: N = 24,2 ± 10,66; A=31,06 ± 1,32; T=31,2 ± 4,02; AT = 29,0 ± 6,50, p>0,05).

Overall, our results suggest that human spermatozoa can drive bipolar spindle assembly when incubated in oocyte cytoplasm even when they present defects in morphology and motility.

Limitations, reasons for caution: In these experiments we did not address directly the functionality of the centrosome. Since during the early embryonic divisions spindle assembly is mainly dependent on the chromosomal dependent microtubule assembly pathway, defective centrosomes (reflected, for example, in defects in pronuclei apposition) may not be detected.

Wider implications of the findings: We established a heterologous system to obtain quantitative data on the human sperm functional capacity to support bipolar spindle assembly. Our data suggest that sperm morphology and motility defects do not interfere directly with the assembly of the bipolar spindle in the human embryo.

Trial registration number: NA.

P-022 Sperm affinity for hyaluronic acid relates to sedimentation properties in density gradients and to sperm chromatin integrity

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Study question: Is sperm selection by hyaluronic acid (HA) binding at least as good as selection by density gradient centrifugation (DGC, 90%:45%) in relation to chromatin integrity?

Summary answer: 90% and HA binding sperm have lower levels of DNA fragmentation and higher levels of chromatin compaction than 45% and HA non binding sperm.

What is known already: Many ART laboratories rely on differential density gradient centrifugation (DDGC) or swim-up to obtain sperm suitable for IVF or ICSI. Sperm preparation by hyaluronic acid (HA)-binding could be an appropriate alternative if it was shown to be as effective and had any advantages over these standard methods. HA binding proteins (HABPs) appear on the matured sperm plasma membrane and bind HA in the extracellular calyx surrounding the cumulus-oopherous complex. This phenomenon can be considered part of a 'capture' mechanism that may 'select' for higher quality sperm with lower levels of DNA fragmentation and high chromatin compaction.

Study design, size, duration: An investigation into DNA fragmentation, chromatin compaction and the location of HABPs in sperm obtained by DGC or after HA-binding of 16 washed semen. The study is part of a three year PhD programme sponsored by the EU Marie Curie Training network.

Participants/materials, setting, methods: DNA fragmentation and chromatin compaction were assessed using acridine orange (AO) and aniline blue (AB) staining, respectively in samples from males with unproven fertility (N = 16) resolved into 90% and 45% fractions or in samples binding or not to hyaluronic acid. HA binding affinity was assessed in pelleted and interface fractions and HABPs were evaluated by immunocytochemistry.

Main results and the role of chance: Higher capacity for sperm hyaluronic-acid-binding was shown to correspond with their DDGC sedimentation profiles.

Strong, statistically significant relationships between AO/AB staining and sperm sedimentation and hyaluronic-acid-binding were revealed. Sperm resolving in the 90% fraction or binding to HA had lower levels of DNA fragmentation and higher levels of chromatin compaction than sperm resolving in the 45% fraction or not binding to HA. Sperm capacitation enhanced HA binding of both 90% sperm and sperm washed free of seminal fluid that may reflect associated changes in the expression of HA-binding proteins.

Immunocytochemistry showed that the HABP CD44 was located mainly on the sperm acrosome and equatorial segment and became more restricted to the equatorial segment in capacitated spermatozoa. Hyaluronic acid-TRITC, a generic probe for detection of HABPs labelled the membrane and the neck region, and capacitation intensified the signal.

The results show that by measures of DNA fragmentation and chromatin compaction, standard, correctly done DGC is good at enriching for good quality sperm for IVF or ICSI or quid pro quo, excluding poor quality sperm from a sample. Under circumstances where sperm count is very low or DGC cannot be carried out, HA binding should be considered. Investigations into sperm HABPs are continuing.

Limitations, reasons for caution: The study used 16 normozoospermic men of unproven fertility. While not diminishing the study findings, future inclusion of men with proven fertility or idiopathic infertility would be helpful.

Wider implications of the findings: These data support the argument for further research into HA-based sperm selection, particularly for ICSI, where the choice of sperm is more critical.

Trial registration number: The study is not a clinical trial.

P-023 The beneficial role of CoQ10 supplementation in male infertility: fact or fiction?

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Study question: To evaluate if oral supplementation with CoQ_{10} improves semen parameters and male fertility potential.

Summary answer: The evidence supporting the use of CoQ10 in male infertility is inadequate, but its oral intake appears to augment mitochondrial α-tocopherol content.

What is known already: Men with high levels of oxidative stress (OS) may be subfertile or infertile, expose their female partners to higher risk of miscarriage, and pass on *de novo* genetic and epigenetic changes to their offspring. To combat OS, many natural antioxidants have found widespread use in the management of male infertility but often without sufficient scientific or clinical evidence. Naturally synthesized by all animals, Coenzyme CoQ_{10} (CoQ_{10}) is a critical component to the mitochondrial electron transport chain. The bioenergetic and antioxidant role of this molecule suggest possible involvement in sperm biochemistry and therefore the male fertility potential.

Study design, size, duration: This review focuses on the latest evidence from human clinical trials but also closely examines the evidence from biochemical studies documenting the importance of CoQ_{10} molecule to male reproductive health.

Participants/materials, setting, methods: A systematic and detailed review of the scientific literature including analysis of human clinical trials, *in vitro/vivo* studies and a stringent set of medicinal and pharmacological criteria were used to critically examine the role of CoQ_{10} in the management of male infertility.

Main results and the role of chance: The beneficial use of exogenous CoQ_{10} supplementation in the management of male infertility remains controversial. To date, 15 low-medium grade clinical trials have been performed. Only three trials were randomized, placebo-controlled, double-blind studies but only one reports minor improvement in sperm motility with no rise in pregnancy or live-birth rates. Additionally, the oral intake of CoQ_{10} is allied with a rise in mitochondrial concentration of α-tocopherol (common form of Vitamin E). Interestingly, as an antioxidant, CoQ_{10} only has $1/10^{th}$ the reactivity of α-tocopherol with much poorer oral bioavailability. These observations suggest that the minor, beneficial effect seen with CoQ_{10} supplementation reported in some trials may be incidental and attributable to an increase in cellular Vitamin E content.

Limitations, reasons for caution: Our findings are largely based on the results of many low quality clinical trials conducted with CoQ_{10} oral supplementation. To definitively determine the potential value of CoQ_{10} in the

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management of male infertility, a large, placebo-controlled randomized clinical trial in a 2 × 2 factorial-blinded design with Vitamin E is required.

Wider implications of the findings: The evidence surrounding the beneficial use of CoQ10 supplementation in the management of male infertility is minor and circumstantial, so its widespread use among fertility experts should be deferred until data from high quality clinical trials becomes available.

Trial registration number: n/a.

P-024 Sperm DNA fragmentation and semen hyperviscosity in human samples

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Study question: Is there a possible correlation between sperm DNA fragmentation and semen viscosity?

Summary answer: Semen hyperviscosity wouldn't seem to be related with higher DNA fragmentation values.

What is known already: It has been shown that semen hyperviscosity is associated with poor outcomes in Assisted Reproduction Techinques (ART). Moreover fertilization rate, positive β-HCG, clinical pregnancy and implantation rates after intra cytoplasmatic sperm injection (ICSI) are significantly lower in patients with hyperviscous semen. In literature hyperviscous samples are characterized by a significant lower percentage of sperm concentration and progressive motility. This could explain, at least in part, the lower fertilization rates. In addition, it has been reported that in hyperviscous samples, oxidative stress is increased, as well as chromatin integrity and packaging defects.

Study design, size, duration: From January 2009 to December 2016, data from 6846 semen samples were collected and retrospectively analyzed. Evaluated semen parameters were sperm viscosity, volume, concentration, motility and morphology according to WHO 2010. DNA fragmentation was detected by TUNEL, using the commercially available In situ Cell Death Detection Kit (Roche).

Participants/materials, setting, methods: Seminal viscosity was assessed by aspiration of the sample into a glass Pasteur pipette. Ejaculates with normal viscosity had a thread length of ≤ 2 cm. Sample's parameters were evaluated according to the WHO 2010 guidelines. A TUNEL positive semen was defined as a sample with DNA fragmentation > 20%. Correlations between seminal fluid viscosity and TUNEL value, sperm volume, concentration, motility and morphology were tested with t-student test (level of significance was $P< 0.05$).

Main results and the role of chance: Of the 6846 semen samples, 4951 showed normal viscosity (Normal Viscosity Group, NVG), whereas 1895 had high viscosity (High Viscosity Group, HVG). Mean men age was 40.1 ± 7.9 and 40.5 ± 7.9 in NVG and HVG, respectively (NS). Sperm concentration (million/ml) was statistically higher in NVG (30.7 ± 20.1) then in HVG (29.1 ± 19.2) ($P < 0.05$). Sperm motility was significantly higher in NVG (58.1 ± 16.7) then in HVG (53.9 ± 17.6), ($P < 0.05$). Sperm morphology was statistically different between the two groups (4.5 ± 3.4% in NVG and 4.2 ± 3.1% in HVG) ($P < 0.05$). In NVG, 472/4951 semen samples examined (9.5%) were TUNEL positive whereas, in HVG, 174/1895 (2.5%) semen samples examined were TUNEL positive (NS). According to our data, there is no correlation between hyperviscosity and fragmentation in examined semen samples. As for motility and morphology it is clear that hyperviscous samples present lower values of these parameters.

Limitations, reasons for caution: Tunel test is a diagnostical test and it is not applicable in the practical routine. In this study patients' medical history is not known; previous infections, prostatic affection or medical therapy may interfere with semen viscosity. Also age may affects sperm viscosity and sperm concentration, motility and morphology.

Wider implications of the findings: Since TUNEL detects only necrosis and final apoptosis effects, and not earlier ones, further studies are needed on sperm chromatin structure and oxidative stress in hyperviscous human semen such as Mitocondrial Membrane Potential Study (JC-1) or Magnetic Cells Sorting (MACS).

Trial registration number: Not applicable.

P-025 Ejaculate vs surgical spermatozoa in severe male factor: which is the best choice?

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Study question: Could conventional testicular sperm extraction (cTESE) be a good strategy after one or more previous cycles without implantation in couples with a severe male factor?

Summary answer: In couples where male-partner present severe oligoasthenoteratozoospermia (OAT), proceeding to intracytoplasmatic sperm injection with ejaculated or testicular sperm would seem to lead to similar outcomes.

What is known already: Severe male factor constitutes about 30% of the infertility causes. Couples undergoing ICSI with severe OAT, risk a failure of the treatment because of the low fertilization and cleavage rates. To reduce cycle cancellation rate, a cleavage stage embryo-transfer is usually performed. The reasons of the low success rate could be a DNA damaged by Reactive Oxygen Species (ROS), improper DNA-packaging, nuclear decondensation, varicocele, infections or iatrogenic causes. Testicular sperm in this patients could give a better outcome, since this spermatozoa present a shorter time of exposition to ROS compared to ejaculated, a lack of infection and better DNA-packaging.

Study design, size, duration: In this prospective study, performed from June 2013 to December 2016, 18 couples which underwent a second or third cycle of assisted reproduction with Pre-implantation genetic screening (PGS) after previous implantation failure, were enrolled. All male-partners were affected by severe OAT (WHO 2010). All blastocysts were frozen immediately after the biopsy and a frozen-thawed single embryo-transfer (sFET) were performed in a subsequent cycle. Mean female and male ages were 34.05 ± 3.52 and 34.77 ± 3.94 years old, respectively.

Participants/materials, setting, methods: Mature oocytes retrieved were injected and cultured individually until blastocyst stage at 37°C, 6%CO^2, 5%O^2. Male partners were asked a single or double semen collection. On the day of oocytes pick-up, males underwent cTESE. Both ejaculated and testicular sperm samples were processed by washing. After denudation, approximately half of mature eggs were injected with ejaculated sperm and half with testicular one. In all patients histological analysis was performed and they all resulted hypospermatogenesis.

Main results and the role of chance: A total of 207 mature oocytes were injected; 99 with ejaculated (EG) and 108 with testicular (TG) spermazoa. Fertilization rates were 62.6% (N = 62) and 57.4% (N = 62) in EG and TG, respectively (NS). An amount of 5.0% (N = 5) and of 3.7% (N = 4) of the injected oocytes presented an irregular fertilization (3PN) in EG and TG, respectively (NS). Degenerated eggs after ICSI were 4.0% (N = 4) and 3.7% (N = 4) in EG and TG, respectively (NS). Blastocyst formation rates were 46.8% (N = 29) and 53.2% (N = 33) in EG and TG, respectively (NS). After biopsy, 31.0% (N = 9) and 30.3% (N = 10) of the blastocysts resulted euploid in EG and TG, respectively (NS). In EG, 4 euploid blastocysts were transferred in 4 sFET resulting in a clinical pregnancy rate (CPR) of 75.0% (N = 3) and an implantation rate (IR) of 75% (N = 3). In TG, 5 sFET were performed transferring 5 euploid blastocysts obtaining the CPR and IR of 100%. One of the clinical pregnancies of this group resulted in an early abortion. The other pregnancies are still ongoing.

Limitations, reasons for caution: Sample size is still too low and it should be enlarged; furthermore a high number of mature oocytes available for the injection per patient is needed. Finally, most of the euploid obtained blastocysts have still to be transferred before drawing final conclusions.

Wider implications of the findings: In case of male with severe OAT it should be carefully evaluated if performing cTESE is an efficient strategy after previous implantation failure. Blastocysts obtained injecting ejaculated or testicular sperm would seem to have the same ploidy rate and the same implantation potential.

Trial registration number: Not applicable.

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P-026 HYDROGEN TREATMENT IS SAFETY FOR HUMAN SPERM NUCLEI

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Study question: Is the hydrogen treatment safe on the nuclei of human sperm?

Summary answer: We found that the H_2 treatment does not cause nuclear DNA fragmentation or cell death of human sperm.

What is known already: H_2 treatment remarkably improved rate of forward motility of human spermatozoa, whereas N_2 treatment did not. H_2 treatment also increased the mitochondrial membrane potential of human sperm. The frozen-thawed sperm from patients that had low motility improved the rate of forward motility after being cultured in a 75% H_2-saturated medium.

The H_2 treatment increases ATP production in the human spermatozoa, and seems to act on the electron transport chain downstream of the action site A or directly acts on oxidative phosphorylation.

Study design, size, duration: We used 140 samples of human sperm with $>2.0 \times 10^6$ cells/mL and >60% motility. DNA Fragmentation of the sperm nuclei was evaluated by the TUNEL, EOSIN and Chromomycin A3 staining methods, and by the CASY cell counting system. At least 150 and 1,500 sperm per sample were examined in the staining methods and the CASY measurements, respectively.

Participants/materials, setting, methods: The sperm samples were from subjects who consented to have their samples used in research. The samples were mixed with TEST Yolk Buffer (TYB) at a ratio of 1:1 to create a suspension that was then frozen in liquid N_2. After thawing, sperm samples were separated into 2 groups; a H_2 group, which was washed in a culture medium containing 75%-saturated H_2, and a control group, which was washed in a culture medium without H_2.

Main results and the role of chance: DNA fragmentation of the sperm nuclei and survival rates were compared between the H_2 and control groups. TUNEL reactions were observed in 1.4% ± 1.9% sperm of the H_2 group and 2.5% ± 3.6% sperm of the control group. No significant difference was seen between the two groups ($n = 30$, $P = 0.98$). There was also no significant difference in EOSIN reactions (1.6% ± 4.3% in the H_2 group, and 2.3% ± 5.7% in the control group) ($n = 30$, $P = 0.069$). The rates of positive Chromomycin A3 reactions were 2.1% ± 3.6% in the H_2 group, and 4.0% ± 8.0% in the control group: the H_2 group were significantly lower than the control group ($n = 50$, $P < 0.01$). The rates of dead sperm found by CASY were 11.8% ± 2.6% in the H_2 group and 11.5% ± 2.7% in the control group, indicating no significant difference between the groups ($n = 30$, $P = 0.69$)

Limitations, reasons for caution: This is a basic study on a relatively small sample size with limited conditions. The confirmation using larger samples under various conditions may be required. Furthermore, we need to check the safety of H_2 treated sperm use in ART.

Wider implications of the findings: The findings of this study indicate that H_2 treatment is a safe method of increasing sperm motility and ATP content with minimal damage to cells and their nuclei. It may also be useful in the selection of good quality sperms for ICSI.

Trial registration number: No.

P-027 First case report of successful deliveries of an oligoasthenoteratozoospermia patient and of a repeated fertilization failure patient using ICSI sperm selected by hydrogen treatment

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Study question: Can oligoasthnoteratozoospermia (OAT) patients and repeated fertilization failure patients take home healthy babies using ICSI sperm selected by hydrogen treatment?

Summary answer: We performed ICSI for patients using sperm selected by hydrogen treatment and transferred fertilized eggs. The patients conceived and delivered healthy babies.

What is known already: The selection of a good sperm is key to the success of ICSI, therefore the development of a safe method that ensures accurate selection is urgently needed.

Empiric treatments such as pentoxifylline have met with limited success and their efficacy and safety are yet to be verified. We have reported that hydrogen treatment elevates the mitochondrial membrane potential and increases the ATP production of OAT sperm. As a result, hydrogen treatment improves the motility of the OAT sperm which has no DNA damage.

Study design, size, duration: The first case involves a 36-year-old male with OAT. ICSI-ETs were performed several times with the couple but all treatments failed. The second case involves a 31-year-old male whose semen analysis showed normal. However, fertilization did not occur with IVF nor ICSI. After obtaining informed consents from these couples we performed ICSI using sperm selected by hydrogen treatment and transferred the egg to the female partner.

Participants/materials, setting, methods: The patients' semen were divided into two groups: the hydrogen treatment group and the control group. Each sample was mixed with 1.5 ml culture medium saturated with 75% hydrogen molecules and separated with density-gradient centrifugation. The pellet was resuspended with 6 ml medium saturated with 75% hydrogen molecules and centrifuged again (hydrogen molecule treatment). The collected sperm were used for ICSI. The control group was processed with a medium not containing hydrogen.

Main results and the role of chance: In both cases the sperm motility rates were significantly improved in the hydrogen molecule treatment groups compared to the control groups. In the first case (OAT case), the female partner's oocyte were not fertilized by ICSI in the control group. The oocytes were fertilized (100%, 5/5), cleaved (100%, 5/5) and developed to the blastocyst stage (75%, 3/4) by ICSI in the hydrogen molecule treatment group. The blastocyst obtained were cryopreserved. In the transfer cycle, the blastocyst was thawed and transferred to her. She conceived and delivered a healthy baby (male, 3184 g). In the second case (fertilization failure case), the female partner oocytes were not fertilized in the control group. The oocytes were fertilized (50%, 1/2), cleaved (100%, 1/1) and developed to the blastocyst stage (100%, 1/1) by ICSI using hydrogen molecule treated sperm. The blastocyst was cryopreserved. The egg was thawed and transferred in the transfer cycle. She conceived and delivered a healthy baby (male, 3440 g).

Limitations, reasons for caution: This study is a case report of an OAT couple and a fertilization failure couple receiving hydrogen molecule treatment as a sperm enhancer and successfully giving birth to healthy babies. More trials are needed to confirm the effectiveness and safety of hydrogen molecule treatment.

Wider implications of the findings: This is the first report of the delivery of a healthy baby for an OAT couple and a fertilization failure couple treated by hydrogen molecules as a sperm enhancer. Hydrogen molecule treatment could be an effective and safe choice for selecting good sperm for ICSI, IVF and IUI.

Trial registration number: No.

P-028 Impact of MTHFR isoform C667T on fertility through sperm DNA fragmentation index (DFI) and sperm nucleus decondensation (SDI)

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Study question: Are MTHFR isoforms involved in the subfertility pathologic aspects through sperm nucleus structure parameters (DFI and SDI)?

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Summary answer: Based on the threshold values, 40% of the patients tested had a DFI over 20% and 22% had a SDI over 20%.

What is known already: The study of sperm DNA structure is essential in the evaluation of the causes of infertility. DFI is involved in ART failures (Bungum) and afterwards in children's psychic disorders (Evenson). SDI anomalies lead to developmental arrest at early stages and to recurrent ART failures (Junca). According to a recent meta-analysis (Gong), MTHFR polymorphism increases the male infertility risks. MTHFR is a step in the 1-Carbon Cycle (1-CC): it allows the synthesis of major anti-oxidant molecules and correct DNA methylation process, part of epigenetics and imprinting.

Study design, size, duration: This is a retrospective study involving subfertile couples controlled for C677T isoforms in an IVF unit. Male partners were tested for DFI and SDI during the year 2015-2016. Eighteen patients were controlled as carrying the isoform on a heterozygote state. Nine were found to be homozygote for the C677T isoform: these patients faced 3 to 9 IVF/ICSI failures and/or miscarriages. Their DFI and SDI were compared to our control group involving more than 1400 patients.

Participants/materials, setting, methods: MTHFR isoforms detection is now part of our assessment in couples after repeated failures of IVF/ICSI failures. Determination of C677T isoform was performed with Real Time Polymerase Chain Reaction. DFI and SDF is measured using Acridine orange/Flow cytometry according to a technique recently described (Hamidi).

Main results and the role of chance: In our general sub-fertile population, 40% of the patients have a DFI > 20% and 23% have a SDI>20%; 20% being considered as the threshold value. Out of the 18 heterozygote patients, 3 have high DFI and SDF (16%). For the homozygote patients, 8/9 have pathological values: two have both high DFI and SDI (average: 41% and 31% respectively). 4 have an isolated high SDI (average: 29%), 2 have only a high DFI only (average: 28%). One patient has correct DFI and SDI values. The homozygote MTHFR C677T induces pathologies of nucleus structure measured by SDF and DFI.

Limitations, reasons for caution: The low number of homozygote patients involved in this study. We evaluated only one mutation (C677T), which is the most frequent though. The influence of potential confounding factors has to be taken into consideration: modification of life style, diet, etc…as well as environmental factors (endocrine disruptors, pollution…).

Wider implications of the findings: In case of repeated ART failures, patients with very high DFI/SDF should be tested for MTHFR. Treatments involving folic acid are useless and has to be replaced by 5 Methyl tetrahydrofolic acid, a compound of the 1-CC downstream the MTHFR.

Trial registration number: Not applicable.

P-029 Examination of the effect of seminal plasma on uterus contraction from two different donor collectives with normozoospermia

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Study question: Is there a difference in the capability to induce uterine contractions of seminal plasma of men with an infertility history and those without infertility history?

Summary answer: Application of seminal plasma into porcine uteri from voluntaries significantly increases uteri contractility more than from patients of the IVF laboratory, although both had normozoospermia.

What is known already: Adequate uterine contractility plays an important role in successful fertilization and embryo implantation. Uterine contractility is regulated by various substances such as oxytocin and prostaglandins, which cause an increase in myometrial contractility and progesterone, and that leads to a decrease in uterine contractility. The fact that these substances are ingredients of the seminal plasma stresses its relevance for the reproductive function. Examinations in animals as well as in humans showed seminal plasma's impact on conception. However seminal plasma composition and reciprocity of the substances seem to be very complex and not completely understood yet.

Study design, size, duration: In this experimental in-vitro study, the influence of human seminal plasma was evaluated on its effect on contractility by using an extracorporal perfused non-pregnant pig uteri. Ejaculate of men with normozoospermia was taken either from patients of the IVF laboratory or voluntary donors. The ejaculates were injected into the perfused uterus of 54 pigs to analyze whether a dose-dependent effect of the different seminal plasma on the uterine motility exists by repetitive intraluminal bolus application.

Participants/materials, setting, methods: After measuring the basic activity of 54 perfused uteri over 60 minutes, three 0.6 ml doses of Krebs-Ringer solution or seminal plasma from patients or voluntary donors were administered, with an interval of 45 minutes between each application. Uterine pressure as the area under the curve (AUC) was measured by a precision pressure catheter with two measure points near to the ovaries (IUP 1) and near to the cervix (IUP 2).

Main results and the role of chance: The seminal plasma application into the porcine uterus significantly increases contractility in the form of AUC, contraction frequency as well as average and maximal values of the contraction amplitude. The ovarian and the cervical measure point showed no significant difference concerning maximal and average pressure amplitude after application of 0.6 ml seminal plasma but revealed significantly higher values at IUP 2 after administration of 0.6 and 1.2 ml seminal plasma. The administration of Krebs-Ringer solution also led to a discrete rise in uterine contractility. However, the effect was clearly less pronounced than that caused by administering seminal plasma. A significant difference in the increase of myometrial activity between the two groups was identified. It was demonstrated that seminal plasma from voluntary donors led to a significantly more pronounced increase in contractility than patients' seminal plasma. This difference became especially evident after repeated administrations of the test substances. The data suggest a dose-dependent effect.

Limitations, reasons for caution: Physical disturbance variables potentially influencing the results were limited as much as possible. In avoidance of an increase in uterine contractility due to the temperature, seminal plasma was warmed up to the nutrition solution temperature. By applying a slow uniform injection, steady seminal plasma distribution and low pressure were attainable.

Wider implications of the findings: The results demonstrate the importance of physiologic seminal plasma composition and introduce new potential options in diagnostic and treatment for couples with an unfulfilled wish for a child. Further investigations about the exact composition of seminal plasma and the interaction of the ingredients are necessary.

Trial registration number: None.

P-030 How do habits and life style affect IVF-TESE outcomes?

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Study question: to examine the effect of serum FSH level, BMI and smoking on TESE-ICSI and pregnancy outcomes

Summary answer: No adverse effects related to male BMI, male FSH and smoking on sperm parameters and pregnancy and delivery rates

What is known already: high serum FSH, smoking and high BMI has an adverse effect on make fertility

Study design, size, duration: Retrospective study, data were extracted from files of 52 azoospermic men who underwent TESE and IVF-ICSI in our IVF unit. Demographic information, treatment cycle follow-up and pregnancy outcomes were assessed.

Participants/materials, setting, methods: Azoospermic males requiring TESE. Retrospective cohort study

Main results and the role of chance: Fifty-two patients underwent 79 TESE due to azoospermia. There were 143 IVF cycles. Smoking was found to significantly affect sperm motility in TESE specimens before freezing (45.5% vs. 14.8%; $P < 0.001$). However, this did not influence the pregnancy rate. Male FSH was negatively correlated with testicular volume ($r = -0.595$, $p < 0.0001$). Weight did not affect semen parameters after TESE or ICSI outcome.

Limitations, reasons for caution: It was retrospective and the sample size was the relatively small. All azoospermic patients who underwent TESE were

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included, with no subgroup analysis according to the etiology of azoospermia. Moreover, there are some confounders that affect the patient selection, clinical and laboratory techniques to find spermatozoa.

Wider implications of the findings: Azoospermic males has poor quality and quantity sperm so smoking, high BMI and elevated srum FSH do not have additive adverse effect

Trial registration number: 0024-15-HYMC

P-031 Sperm DFI improvement only dependent on motility improvement after antioxidant therapy: A large cohort study

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Study question: Is sperm DFI improvement independent from semen parameters after antioxidant therapy or not?

Summary answer: Sperm DFI improvement correlated only with total and progressive motility improvement after antioxidant therapy, and independent from concentration and morphology improvement.

What is known already: Antioxidant therapy used for treatment of men with high degree of DFI and resulted in DFI improvement and semen parameters. But there is a challenge that this improvement correlated with each other or DFI and semen parameters improved separately.

Study design, size, duration: This study design as a large Prospective Cohort and lost to follow-up study. Semen sample collected during January 2016 to January 2017 in Nobel Laboratory, Isfahan, Iran. 1200 infertile men participated in this study that 505 of them finished the antioxidant therapy period.

Participants/materials, setting, methods: Semen samples from infertile individual collected and semen parameters analyzed based on WHO 2010 guideline. Sperm DFI assessed with Sperm Chromatin Structure Assay(SCSA) with flow cytometric method. Patient with high level of DFI(> 27%) under supervisor of an urologist, treated with Antioxidant supplementation including Vitamin E, Vitamin C and Q 10 during 3 month. Then patient repeated spermogram and SCSA analysis. Only improvement rate >15% accounted for analysis after Antioxidant therapy.

Main results and the role of chance: From total 1200 individuals, 468 men had normal sperm parameters. from this 468 men, 22% (103/468) had DFI > 27%. Also from 732 abnormal semen parameters, 63% (461/732) had DFI> 27 %.

DFI improvement after antioxidant thrapy was 40.7% (42/103) in men with normal sperm parameters and 45.8 % (147/321) in men with abnormal parameters. Only total motility improvement (p Value = .0.017 and r = 0.331) and progressive motility improvement (p Value=0.013, r = 0.349) correlated with DFI improvement after Antioxidant therapy.

Limitations, reasons for caution: Lack of regular consuming antioxidant supplementation. Not participating individual in study after antioxidant therapy.

Wider implications of the findings: SCSA as a reliable test offer for all normal semen parameters and abnormal semen parameters individual. Antioxidant therapy is useful for men with high sperm DFI. But it must be noted that DFI improvement not correlated with concentration and morphology and only dependent with total and progressive motility improvement.

Trial registration number: Not available.

P-032 A novel solution for freezing small number of spermatozoa by a sperm vitrification device

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Study question: Does a sperm vitrification device (VD)provide an efficient method for freezing a small number of human spermatozoa?

Summary answer: The use of a spermVD can provide an efficient method for freezing a small number of spermatozoa and can be used after testicular sperm extraction.

What is known already: Cryopreservation of a small number of human spermatozoa can provide an appropriate solution for cases of severe male infertility. Mainly after testicular biopsy an efficient method for sperm preservation is needed. Over the years, different methods for cryopreservation of small numbers of human spermatozoa were proposed (e.g. mini-straws, ICSI pipette, cryoloop, alginate beads. Each of these methods had drawbacks which prevented widespread use, mostly due to cumbersome preparation and sperm retrieval procedures. Moreover, in most of the previous methods the thawed spermatozoa were not immediately available for micromanipulion and required additional treatment which may cause harm to the thawed spermatozoa.

Study design, size, duration: A total of 15 patients with virtual azoospermia and 5 patients with frozen IMSI selected spermatozoa before oocyte donation were recruited for a prospective study between 2015-2016.

Participants/materials, setting, methods: Sperm was collected from patients diagnosed with virtual azoospermia. After centrifugation and washing the re-suspended pellet was distributed into 10 μL droplets on a Petri dish. The droplets were searched and any single spermatozoa found was transferred to 1 μL of washing medium/cryoprotectant on the spermVD. The spermVD was then plunged into LN ^{2}. At oocyte pick-up day, the spermVD was thawed and placed on the ICSI dish and spermatozoa were retrieved from the droplets and injected.

Characteristic/Outcome	Average, SD, %
Male Age	37.5 ± 8.6
Female Age	33.6 ± 8.6
No. of motile frozen sperm	17.1 ± 11.1
No. of thawed sperm	16.9 ± 10.2
Spermatozoa retrieval rate (No of thawed/No of frozen)	96.3%
% Motile sperm	29%
Fertilization rate	57%
Pregnancy rate	60%
Delivery rate (including ongoing pregnancies)	58%

Main results and the role of chance: Linear regression was used for continuous variables and logistic regression was used for categorical variables. No correlation was found between motile thawed spematozoa and fertilization rate (p = 0.661) and pregnancy rate (p = 0.083).

Limitations, reasons for caution: No limitations or reasons for caution.

Wider implications of the findings: Our efficient and simple method for freezing a small number of spermatozoa may allow the routine use of frozen spermatozoa after TESE and decrease the number of unnecessary OPU procedures.

Trial registration number: The study was approved by the ethics board No 2013042 and registration was not done because of intellectual property.

P-033 Effect of sperm DNA fragmentation in men living in desert climate on outcome of ICSI and its correlation with semen and social characteristics

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Study question: What is the effect of sperm DNA fragmentation (SDF) in men living in desert climate on ICSI outcome?

Summary answer: No pregnancy resulted in the group when male partner's SDF was 38.54 ± 10.14 %.

What is known already: One of the important male factors affecting success after assisted reproduction is SDF. The fertility potential of men with higher than 30 % SDF is very poor, however, high SDF can be corrected by lifestyle changes and medication.

Study design, size, duration: It is a retrospective study of 94 men tested for SDF by sperm chromatin dispersion test before going to ICSI at a private infertility clinic in Riyadh, Saudi Arabia from Jan 2015 to June 2016. The study parameters were SDF, male age, body mass index, smoking, semen values and fertilization rate. The % SDF categories were; low (SDF ≤15%), moderate (SDF >15-30%) and high (SDF >30%). The Institutional review board approval was obtained.

Participants/materials, setting, methods: The 94 study couples underwent SDF test and ICSI. The SDF was tested by Halosperm G2® kit (Halotech®, Spain). The female partner's were prepared by standard ovarian stimulation protocols. The oocyte collection, ICSI and embryo culture were performed as per routine of the laboratory. The embryos were cultures at 37 °C in humidified environment of 6% CO2 in air. The pregnancy rates among groups were compared and correlations among various parameters analysed by SPSS.

Main results and the role of chance: The SDF was low (≤15%) in 53.19 % men, moderate (>15-30%) in 33 % men and high (>30%) in 14 % men. The Mean ± SD values for low, moderate and high SDF categories were; 10.46 ± 3.31%, 20.80 ± 4.68 % and 38.54 ± 10.14 %, respectively. There was no significant correlation between % SDF and semen parameters except in between the progressive motility and low SDF ($r = -0.36$, $P = 0.008$). For the social characteristics, higher body mass index, smoking and increased age were positively correlated with SDF. In high SDF category, no couple achieved pregnancy.

Limitations, reasons for caution: The study was conducted on 94 men and over a period of Jan 2015 to June 2016 at one private clinic. Larger multi center study is needed. This study suggests the need to test SDF before ICSI and to investigate any impact of SDF on congenital anomalies in the offspring.

Wider implications of the findings: Some social characteristics like high body mass index, smoking and age were significantly correlated with SDF. The patient counseling to adapt a healthy lifestyle and SDF test prior to initiation of treatment are needed.

Trial registration number: None.

P-034 evaluation of testicular tissue vitrification by ectopic transplantation and in vitro culture

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Study question: Does this simple vitrification procedure maintain testicular tissue function and structure?

Summary answer: Vitrification resulted in a success rate in maintaining testicular cell proliferation and differentiation capacity up to round spermatid and testicular tissue structure same as control.

What is known already: Fortunately survival rate of cancer in pre-pubertal males has been increasing. Infertility, as one of the adverse effects of treatments, has become an important concern for patients. Sperm freezing is the only efficient method for fertility preservation. As there are no mature spermatozoa in young patients then efficient testis tissue cryopreservation followed by transplantation or in vitro maturation is a realistic measure for preserving the fertility. Although there are a lot of findings regarding different tissue cryopreservation methods various researches are ongoing to reach simpler vitrification protocol which maintains tissue developmental function.

Study design, size, duration: In this experimental study, immature mice testicular tissue fragments were vitrified-warmed. Vitrified (n = 42) and control (fresh, n = 42) fragments were ectopically transplanted into the mature mice (n = 14) for 7 weeks in order to assess vascularization of the grafts, germ cell differentiation, proliferation and apoptosis frequency. Vitrified (n = 42) and control (n = 42) tissues were cultured for 1 and 6 days.

Participants/materials, setting, methods: 6-days postnatal BALB/c as testes donors and 10-weeks of the same strain as recipients were used. Immature mice testicular tissue fragments (0.5-1 mm^2) were vitrified-warmed by maximum cooling-warming rate and minimized vitrification media volume with a special metal grid carrier. Histological staining by H&E, immunohistochemical staining by PCNA, and TUNEL assay were used. After 1 and 6 days culturing, testicular tissue structure was evaluated by H&E staining.

Main results and the role of chance: Vascularization of the vitrified graft (59%) did not significantly differ with the control (71%). The percentage of the most advanced germ cells present in the tubules of control and vitrified grafts recovered respectively was as follow: Sertoli cell (6.5% and 6.82%), spermatocyte (56% and 62.056%), spermatogonia (8.35% and 7.56%), and round spermatids (29% and 23.434%). The frequency of intratubular cell per microscopic field (50 µm=×40) was respectively included in spermatocyte (51.52% and 54.0), spermatogonia (18.18% and 19.26%), Sertoli cell (8.13% and 11.12%) and round spermatids (22.15% and 15.59%) in control and vitrified grafts. We observed no statistically significant difference in the most advanced germ cells and frequency of intratubular cells between control and vitrified groups. Apoptosis in vitrified (8.3%) was comparable to control (6.5%) graft. Proliferation in vitrified (17.6%) and control (22.8%) tissue did not reach statistical significance. Moreover, compared to the control group, the numbers of intact (71.82% vs 64.58%), medium (17.86% vs 20.33), and degenerated tubules (10.31% vs 15.1%) were not significantly different with the vitrified group at first day. After 6 days, the numbers of intact (55% vs 49.66%), medium (21.33% vs 22.33%), and degenerated tubules (23.66% vs 28%) in control samples were similar to vitrified group.

Limitations, reasons for caution: The human testis is very different in physiology from the mice testis; further investigations are still needed to optimize the vitrification protocol and culture system for future use in humans.

Wider implications of the findings: The successful differentiation of spermatogonia using the testicular tissue transplantation might be employed in future to produce mature spermatozoa from human spermatogonia as a clinical tool for fertility preservation. Further studies would be necessary in order to demonstrate how useful is culturing testicular tissue in media before transplantation.

Trial registration number: N/A.

P-035 Gene polymorphic variants in taste receptors (TAS) genes and male fertility: a possible correlation

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Study question: We analyzed possible associations between *TAS2Rs/TAS1R* and *GNAT3* (gustducin alpha-3 chain) gene variability and male fertility status, and investigated TAS genes expression in human spermatozoa.

Summary answer: Some genetic variants in taste related genes are associated with sperm parameters, and several components of taste receptor transduction cascade are expressed in mature spermatozoa.

What is known already: Male infertility is estimated to affect about 7% of men and the causes are often unknown. About 40% of infertile men have

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normal reproductive hormone production and any known cause of infertility can be diagnosed. Previous studies showed the expression of taste receptors in testis and sperms, suggesting their possible role in germ cells development and sperm production. Taste sensitivity varies among individuals, being associated with functional polymorphisms in taste receptor genes. The vast genetic variability in taste genes results in a large degree of phenotypic diversity. We hypothesis that polymorphisms in taste receptors might influence sperm functionality.

Study design, size, duration: We analyzed a total of 452 individuals undergoing semen evaluation during an infertility diagnostic screening, from October 2014 to February 2016, at the Centre of Couple Sterility, Siena University Hospital.

Participants/materials, setting, methods: Enrolled subjects were characterized for main sperm parameters, according to WHO (2010) guidelines: concentration, morphology, progressive and total motility. All subjects were genotyped for 24 single nucleotide polymorphisms (SNPs) in *TAS2Rs/TAS1R* and *GNAT3* genes, using the KASPar SNP genotyping system. To assess the possible functional associations between SNPs and sperm parameters, different bioinformatic tools were used. Additionally we analyzed the expression of selected genes involved in taste receptor transduction cascade, using quantitative real time PCR.

Main results and the role of chance: Our results suggested a correlation between: TAS2R3-rs11763979 and sperm morphology; TAS2R3-r rs11763979 and sperm concentration; TAS2R14-rs3741843 and sperm motility. This last association was supported by a high statistical significance and by compelling *in silico* prediction of the SNPs effects on the gene nearby. The minor allele was associated with a decreased sperm motility and with an increased expression of the genes at the locus, suggesting that the increase in expression decreases the sperm motility. These data confirm the pleiotropic role of taste receptors, without information on the molecular mechanisms underlying these associations. Gene expression analysis of transduction cascade elicited by taste receptors was aimed to shed light on the role of these genes and their associated proteins in sperm cells. We highlighted that some these genes are not expressed in sperms *(CALHM1, USP20, GCM1, TAS1R1, TAS1R2)*, whereas *PLCβ2, CAMKK1, TRPM5, TAS2R14, TAS1R3, GNAT3, SLC2A2, PDE4A,* and *MZF1* genes are expressed in sperm, although at different levels, demonstrate a high variability in mature sperm cells.

Limitations, reasons for caution: The major limitation is the sample size because some taste receptors (TAS) genes polymorphic variants have a low frequency in the population, and therefore a larger study needs to be carried out in order to validate the findings.

Wider implications of the findings: Further studies might contribute in better understanding the genetic components of male infertility; they should be crucial to clarify the cause of reproductive failure in men affected by idiopathic infertility.

Trial registration number: NONE.

P-036 Sperm DNA fragmentation test (TUNEL) and male infertility assessment. Setting a cut-off value

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Study question: Which is the cut-off value for TUNEL assay according to the collected data from sperm DNA fragmentation studies in fertile and infertile men?

Summary answer: The ROC curve analysis reported that the cutoff value for TUNEL assay was 15% (AUC: 0.78), with 53% of sensitivity and 100% of specificity.

What is known already: Studies have shown a negative correlation between sperm DNA fragmentation and fertility. High values of DNA fragmentation may have an adverse impact in ART outcomes, reducing the fertilization rate, poor embryo developing and recurrent miscarriages. Therefore, sperm DNA fragmentation may be important factor to be assessed for male infertility evaluation. Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay is one of the principal techniques for measuring sperm DNA damage. It detects single/double strand fragmentation. However, protocols are not well established. Some laboratories apply flow cytometry for fluorescence detection while others use fluorescence microscopy, been results difficult to compare.

Study design, size, duration: It is a retrospective cohort study. Forty-eight infertile men that performed a semen analysis between 20132015 were enrolled. Twenty-eight healthy male volunteers with proven fertility (< 12 months) were selected as the control group.

Participants/materials, setting, methods: From 500 initially recruited patients, just 48 were enrolled due to pure male factor infertility, it means that couples with female factor (>37, poor ovarian response, tuboperitoneal factor, polycystic ovarian syndrome, altered karyotype) were excluded. Semen analysis was performed according to the procedures established by WHO (2010). Sperm DNA fragmentation was assessed over motile sperm (selected by swim up) by TUNEL assay. Ttest and ROC curve analysis were used for statistical analysis.

Main results and the role of chance: The average age for fertile men was 32.8 ± 4.7 and 36.5 ± 5.2 for infertile men (p: 0.99). According to sperm concentration, the average rate was 55.1 ± 22.4 mill/mL for fertile men and 45.5 ± 40.6 mill/mL for infertile men, showing no significant differences (p: 0.22). Progressive motility was 57.2 ± 6.1% for control group and 47.8 ± 9.8 for infertile men (p: 0.01). Strict morphology was 13.2 ± 2.5% for fertile men compared to 6.5 ± 3.8% for infertile men (p: 0.01). Neutrophils concentration was 0.5 ± 0.01 and 1.8 ± 0.2 mill/mL respectively (p: 0.99). Finally, TUNEL assay value was17.8 ± 10.8% for infertile men and 7.6 ± 3.9 for control group (p: 0.01).

From these seminal results, it is shown that sperm progressive motility, strict morphology and DNA fragmentation were significantly higher in patients with pure male factor compared to controls ($p < 0.05$). The cut-off value calculated from ROC curve analysis was 15% (AUC: 0.78), with 53% of sensitivity and 100% of specificity.

Limitations, reasons for caution: The cut-off value of 15% for TUNEL test was calculated from our own studies, performed by our own protocol. The application of this cut-off value in other laboratories must be done with caution.

Wider implications of the findings: A cutoff value of 15% for TUNEL assay with 100% specificity can differentiate infertile men with DNA damage from fertile men. TUNEL test for the evaluation of sperm DNA fragmentation is a useful test in the evaluation of male factor in couples with fertility problems.

Trial registration number: None.

P-037 Polymorphic chromosome variants, human inversions, and their functional consequences in male infertility

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Study question: To assess the effect of polymorphic chromosome variants in male infertility and assisted reproduction treatment (ART).

Summary answer: The chromosome involved in the polymorphism is crucial in determining its effect on male seminal quality and the probability of success after ART.

What is known already: Polymorphic chromosome variants are still been considered "normal" as it was thought that heterochromatin has no coding potential. However, it has been described that some genes involved in fertility reside in heterochromatin, and they could have an impact on fertility potential and miscarriage rate. An increase in the frequency of polymorphic variants and inversions have been observed among infertile males, related to sperm aneuploidies, poor seminal quality and failures in ART. There is no absolute consensus on the chromosomes mainly involved in polymorphisms or their implication in seminal quality or fertility.

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Study design, size, duration: Retrospective study performed in 11 private clinics belonging to IVI group from January 2012 to December 2016. We included 1193 men, of which 29.2% (n = 348) had normal karyotype and were considered as a control group; 48.7% (n = 581) were carriers of a polymorphism; and 22.1% (n = 264) showed an inversion on chromosome 9 or Y. All men underwent a fresh autologous ICSI cycle. Statistical analysis was performed by ANOVA and chi-square where applicable.

Participants/materials, setting, methods: The cytogenetic study was performed by culture of peripheral blood lymphocytes stimulated with phytohemagglutinin and subsequent staining with trypsin-Giemsa (GTG bands). 15 metaphases were evaluated for each case and the banding resolution was 400–550 bands per haploid set. Polymorphisms were included as a variant when the chromosome region was greater or smaller than the same region on the homologous chromosome; as a minimum, twice the size on the other homologue.

Main results and the role of chance: Polymorphic variants for chromosomes 1 (46,XY,1qh+), 9 (46,XY,9qh+), 13 (46,XY,13 ps+), 14 (46,XY,14 ps +), 15 (46,XY,15 ps+), 16 (46, XY,16qh+), 21 (46,XY,21 ps+), 22 (46, XY,22 ps+) and Y (46,XYqh+) were obtained, as well as inversion of chromosomes 9 or Y, being the most frequent 46,XY,inv(9)(p11q12).

When analyzing whether the chromosome involved in the polymorphism could influence clinical results, we did not found differences related to seminal quality (volume, sperm concentration, progressive motility) or clinical data like implantation, pregnancy and miscarriage rates. Male carriers of polymorphisms implying the acrocentric chromosomes showed significant lower fertilization rate compared to males with normal karyotype. Fertilization rates were as follows: 64.7% for control group; 55.2% for chromosome 13; 52.0% for chromosome 14; 64.2% for chromosome 15; 51.0% for chromosome 21; 57.8% for chromosome 22 and, 61.9% for chromosome Y (p = 0.026) meaning that somehow, polymorphism for these specific chromosomes impair the fertilization ability of their gametes. In contrast, we did not found differences related to fertilization rate but a significant increase in implantation rate for male carriers of chromosome 9 inversion (36.5%) compared to control group (29.9%), p = 0.059. Patients with chromosome 9 inversion behave similarly to the control population or even better. No significant differences were found for other analyzed parameters.

Limitations, reasons for caution: Despite the advantages that our data set confer the analysis, limitations still remain. One consequence of a retrospective study is that not all pertinent risk factors are likely to have been identified and subsequently recorded. Therefore, only association, and not causation, can be inferred from the results.

Wider implications of the findings: As "normal" polymorphic variants could play a significant and yet unknown role in clinical outcomes in infertile couples, it would be of great interest to know the individual implication of each chromosome in each analyzed parameters, to counsel carrier couples about their expected clinical outcome and the most appropriate treatment.

Trial registration number: It does not apply.

P-038 Signaling proteins as biomarkers for sperm quality evaluation

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Study question: To determine the correlation between basic semen parameters and the expression and activity of signaling proteins.

Summary answer: In this study, we unraveled signaling pathways involved in regulating human sperm function and correlated the activity of signaling proteins with clinical data.

What is known already: Sperm cells are incapable of genetic expression and thus highly dependent upon post-translational modifications and signal cascades to execute their function. However, not much information is available on the biological significance of specific signaling pathways in human spermatozoa or their possible roles in male infertility.

Study design, size, duration: Thirty-seven random donors provided semen samples for *in vitro* studies with human spermatozoa carried out in an academic research institute in collaboration with a hospital.

Participants/materials, setting, methods: Thirty-seven human semen samples, obtained from a randomized group of donors, were included in this study. Basic semen parameters were analyzed according to the WHO's guidelines. Sperm DNA fragmentation (SDF) was measured using a Sperm Chromatin Dispersion (SCD) test. Antibody-based arrays were carried out to determine the expression patterns of 18 well-characterized signaling molecules when phosphorylated or cleaved. A commercial Kinetworks™ Protein Kinase Screen was used to analyze the levels of 75 protein kinases.

Main results and the role of chance: The results indicated that the phosphorylated levels of several proteins [Bad, GSK-3β, HSP27, JNK/SAPK, mTOR, p38 MAPK and p53], as well as, cleavage of PARP (at D214), and Caspase-3 (at D175)] were significantly correlated with motility parameters. Additionally, the percentage of morphologically normal spermatozoa demonstrated a significant positive correlation with the phosphorylated levels of p70 S6 kinase and, in turn, head defects and the teratozoospermia index (TZI) showed a significant negative correlation with the phosphorylated levels of Stat3. There was a significant positive correlation between SDF and the TZI, as well as, the presence of head defects. In contrast, SDF negatively correlated with the percentage of morphologically normal spermatozoa and the phosphorylation of Akt and p70 S6 kinase. Subjects with varicocele demonstrated a significant negative correlation between head morphological defects and the phosphorylated levels of Akt, GSK3β, p38 MAPK and Stat1. Additionally, 34 protein kinases were identified as expressed in their total protein levels in normozoospermic samples. From those, 8 were identified for the first time in human spermatozoa.

Limitations, reasons for caution: The major limitation of this study is the relatively small sample size. Still, *the* dataset size was considered reasonable since based on the central limit theorem it was possible to approximate the distribution of variables to a normal distribution.

Wider implications of the findings: This study contributed towards establishing a biomarker "fingerprint" to assess sperm quality based on molecular parameters.

Trial registration number: NA.

P-039 The (Pro)renin receptor is expressed in human sperm cells and is related to sperm quality and embryo development

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Study question: To investigate the presence of (pro)renin receptor (PRR) in human spermatozoa and its association with basic sperm parameters, human embryo development and reproductive success.

Summary answer: The PRR is present in human spermatozoa. Higher percentages of PRR-positive spermatozoa are associated with worst sperm quality, embryo development and embryo viability.

What is known already: There is increasing evidence that sperm molecular features, such as RNA and proteins, are involved in fertilization and embryo development; suggesting that male infertility could be caused by different deficiencies, distinct from those related with basic semen analysis. Understanding those sperm characteristics will allow to develop functionally diagnostic or tools to maximize reproductive success. Sperm fertility ability may be modulated by different molecular systems, such as the renin-angiotensin system (RAS). Several components of RAS are described in human spermatozoa. However, despite to renin is one of its most relevant peptides, the presence and role of the PRR is completely unknown.

Study design, size, duration: This prospective cohorts study (conducted February 2014 to July 2015) analyzed 120 human semen samples from the Clínica IVI Bilbao. Of these, 23 samples from normozoospermic patients were used to determine the presence and location of PRR in human spermatozoa; and a total of 97 normal and/or pathologic semen samples of couples undergoing oocyte donation cycles were used to analyze the association among the PRR

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and basic sperm parameters, human embryo development and reproductive success.

Participants/materials, setting, methods: A total of 120 human semen samples and 755 embryos were examined. Sperm samples and embryo quality were examined following WHO and "Asociación Española para el Estudio de la Biología de la Reproducción" (ASEBIR) guidelines, respectively. To determine the presence of PRR and its transcript we performed expression assays by western blot, immunoflurorescence and RT-PCR. The levels of PRR were measured by flow cytometry. Statistics: Spearman´s rank correlation, Kruskal-Wallis and Mann-Whitney U-test.

Main results and the role of chance: We demonstrated the existence of PRR and its transcript in human sperm by western blot and RT-PCR techniques. Immunofluorescence studies showed that PRR is mainly located in the proximal region over the acrosome and in the postacrosomal region of sperm head, and to a lesser extend along the sperm tail. On the other hand, the presence of PRR in spermatozoa is related to sperm quality. Concretely, in prepared sperm cells, the percentage of PRR-positive spermatozoa was negatively correlated with semen concentration ($p < 0.05$), and positively with the percentage of non-progressive (NP) spermatozoa ($p < 0.01$). Regarding human embryo development, semen samples with higher percentages of PRR-positive spermatozoa are associated with worst embryo development on day 6 ($p < 0.01$) and with not viable blastocysts on day 5 ($p < 0.01$).

Limitations, reasons for caution: Only high quality embryos used in fresh transferences have been considered in this study.

Wider implications of the findings: PRR is present in human spermatozoa. It may be negatively involved in sperm physiology and also it may condition human embryo quality. In-depth understanding of sperm proteins can help elucidates their role in male infertility as well as establish biomarkers for sperm selection to use in assisted reproduction techniques.

Trial registration number: CEISH/61/2011.

P-040 The efficacy and adaptation of microTESE in the day before of oocyte collection: superior motile sperm recovery and cycle planning

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Study question: What is the efficacy of performing microdissection of testicular sperm extraction (MicroTESE) for non-obstructive men in the day before oocyte collection of the female partner?

Summary answer: MicroTESE in the day before of oocyte collection allow to identify motile-sperm in about 50% of samples that showed immotile-sperm in the day of procedure.

What is known already: Non-obstructive azoospermia count for 60% of all azoospermic men, who require an invasive procedure to retrieve sperms from testicles plus ICSI. The MicroTESE has been shown to be the best approach; however, the procedure has some inconvenient as the hospital necessity is required, and the sample must be transported to IVF clinic in enough time for ICSI. Additionally, if sperm is not found in the testicle sample, the IVF procedure has to be changed without planning and oocytes cryopreserved, or fertilized by donor sperm sample to permit excellence of the results.

Study design, size, duration: Cross-sectional study included 61 non-obstructive azoospermic men who underwent microTESE between 2012 and 2016. All procedures were done by the same surgeon, in a hospital, under anesthesia. The microTESEs were handled with microsurgical material and microscopy. During the microTESE all removed tubules were analyzed by andrologist into the surgical room to preliminary identify sperm between slides under 400X microscopy, and the surgeon provide areas of testicle to be removed.

Participants/materials, setting, methods: The samples were placed into a conical tube with HEPES plus 15% SSS and transported to laboratory at 37°C. The samples were centrifuged and the pellet transferred to a petri plate with GIFV media following dissected. The material was transferred to an ICSI plate with GIFV media covered with mineral oil. After sperm identification, samples were kept under 37°C, 5% CO2 overnight. Next day, the samples were reanalyzed and motile sperm were always preferentially injected.

Main results and the role of chance: Men were 29 to 78 years of age (40.7 ± 8.7). Among 61 microTESEs performed, 55.7% (n = 34) of samples found sperm, which is in according to literature that report 40% to 60% of sperm recuperation rate after microTESE. From those, 8.2% (n = 5) were motile sperm and 47.6% (n = 29) were immotile sperm. All samples were reanalyzed in the day after (day of oocyte collection) and the samples presenting no sperm or motile sperm in the microTESE time, kept the same result. Interestingly, from the 29 samples that presented immotile sperm in the day of microTESE, 44.8% (n = 13) presented motile sperm in the day after, which allowed superior viability for ICSI. From the 34 samples that sperm were present, oocytes were injected with microTESE sperm in 24 cases, with a mean normal fertilization rate of 47.1%. Other cases were canceled, or used donated sperm for ICSI. The normal fertilization rate was 55.1% (n = 18) when microTESE motile sperm were injected, while it was significantly lower when immotile sperm were used (33.9%, $p = 0.014$).

Limitations, reasons for caution: This is a retrospective study and all non-obstructive azoospermic men were included independently of azoospermia etiology, age or any other clinical characteristic, which could affect the microTESE success rate. The embryo development and pregnancy rates could not be accurately evaluated due to different female factors present in the study population.

Wider implications of the findings: MicroTESE in the day before of oocyte collection allow superior cycle planning and more detailed sperm searching. Sample incubated overnight permitted us to find motile sperm when just immotile were present in the day before. This approach can be an important tool to patients with non-obstructive azoospermia.

Trial registration number: not applied.

P-041 Outcome of intracytoplasmic sperm injection (icsi) using cryopreserved testicular sperm from azoospermic infertile men with varicocele

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Study question: Does varicocele decrease the pregnancy rate in infertile men with non-obstructive azoospermia undergoing ICSI?

Summary answer: Varicocele significantly decreases the pregnancy rate in infertile men with non-obstructive azoospermia (NOA) who underwent Intracytoplasmic Sperm Injection (ICSI).

What is known already: Varicocele has been implicated as a causal factor for male infertility. The exact mechanism by which varicocele can alter spermatogenesis is debatable. Intracytoplasmic Sperm Injection using testicular sperm from men with azoospermia is the treatment of choice for this category of patients.

Study design, size, duration: This is a prospective controlled clinical study conducted at Ajyal IVF/ICSI center, Sohag, Egypt between July 2014 and June

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2016. Forty infertile men with NOA who underwent ICSI with cryopreserved testicular sperm were included. Twenty two patients had clinically palpable varicocele (group 1) and 18 had no detectable abnormality in their genital examination (group2).

Participants/materials, setting, methods: Varicocele was confirmed by scrotal Doppler ultrasound. Azoospermia was confirmed by repeated absence of sperms after centrifuge. Exclusion criteria include female factor infertility, men with abnormal hormonal profiles, genetic abnormalities or genitourinary anomalies suggestive of obstructive pathology. ICSI was done using mature oocytes. The pro-nuclei was assessed at 18 hours following injection. Embryo quality was assessed on day 3 and day 5. Clinical pregnancy was diagnosed using trans-vaginal ultrasound 15 days after B-hCG.

Main results and the role of chance: Results were presented as mean ± standard deviation (SD) for continuous variables and frequency and percentage (%) for categorical variables. P-value <0.05 was significant. Men's age was 37.8 ± 5.4 years in group 1 and 34.5 ± 7.5 years in group 2 (P = 0.07). Female partner's age was 27.9 ± 5 years in group 1 as compared to 26.3 ± 3.7 years in group 2 (P = 0.35). Out of the 22 patients with varicocele, 12 (54.5%) had grade 3 and 10 (45.5%) had grade 2. Mean ± SD of number of retrieved oocytes, fertilized oocytes, good quality day 3 and day 5 embryos are shown in table 1. Clinical pregnancy was significantly reduced in group 1[7/22 cases (31.8%)] as compared to [12/18 (66.7%)] in group 2 [OR: 0.23; 95% Confidence Interval (0.06 - 0.88); P = 0.03].

Limitations, reasons for caution: One of the limitation of this study is the small sample size of the recruited patients.

Wider implications of the findings: Future research is warranted to study whether this selected category of patients would benefit from varicocele repair before recommending ICSI.

Trial registration number: NCT02306499.

P-042 Near-infrared spectroscopy assessment of testicular tissue oxygen saturation in healthy controls and male infertility

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Study question: To determine the level of testicular oxygen saturation by Near-infrared spectroscopy which we developed currently.

Summary answer: Tissue oxygen saturation of testis is significantly low compared to that of hand and neck. That of male infertility showed different values from healthy controls.

What is known already: Near-infrared spectroscopy is a non-invasive and simple method to evaluate tissue oxygen status. There have been no reports on tissue oxygen saturation of testis in male infertility by Near-infrared spectroscopy. The objective was to asses testicular tissue oxygen saturation (StO2) by transscrotal near infrared spectrometry (NIRS) for healthy volunteers and male infertility which we currently developed as examiner's mounted tissue oximetry.

Study design, size, duration: Study design: Prospective clinical study. Size: Healthy volunteers (n =5), Male infertile patients (n = 7)

Participants/materials, setting, methods: Recently we developed examiner's mounted tissue oximetry by NIRS. We measured StO2 of hand, abdomen, testis of healthy volunteers and those of patients with male infertility by the NIRS transscrotaly.

Main results and the role of chance: We could succeed StO2 in any parts of testis rapidly just after attachment of the sensor to testis. The mean StO2 for the left testis of healthy control volunteers was 43.6+/- 2.2% and that of right was 40.5%+/-1.7%. In contrast the StO2 of hand and abdomen was 60 ± 3.3%, 57 ± 2.9% respectively. The mean StO2 of left testis in varicocele patients was 45.3+/- 2.9%, and that of right was 48.7 ± 1.3. There was a significant difference of right testis between healthy volunteers and varicocele patients. The StO2 of oligospermia due to small testis of left side was 44.0 ± 2.2, that of right side was 48.0 ± 2.8. There was a significant difference of right testis between healthy volunteers and patients with oligospermia by small testis. We discovered that StO2 of testis for healthy controls was significant lower than that of hand and abdomen.

Limitations, reasons for caution: The number of the subjects is small.

Wider implications of the findings: Our data suggest that the StO2 of male infertility could be higher than that of controls especially in right testis. Our NIRS was simple and non-invasive to detect StO2 of testis indicating that it could be useful in clinical practice.

Trial registration number: N.A.

P-043 The predictive factors for successful fertilization by in-vitro fertilization assessed by new CASA (Computer aided sperm analysis) system SMAS (sperm motility analysis system)

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Study question: Which parameters are important for successful fertilization in in-vitro fertilization (IVF)? Is it possible to predict the fertilization rate by using these parameters?

Summary answer: Curvilinear velocity, total motile sperm count and sperm motility index before sperm separation can be used as the predictive factors for successful fertilization by IVF.

What is known already: Sperm quality is known to affect the outcome of assisted reproductive technology (ART). However, most study is based on the quantity or morphology of sperm and the importance of the detailed sperm motility is rarely discussed. In attempt to make the assessment of sperm quality, CASA have been developed and several studies reported the CASA motility parameters may have the predictive value for fertilization. However, the definitive criteria of semen motility parameters related to fertilization is still unknown. SMAS is a newly developed system, which focuses on individual sperm movement and can analyze the detailed motility with some unique parameters.

Study design, size, duration: To identify the predictive factors of successful fertilization, IVF cycles in our clinic between June and October 2016 were analyzed. All semen was analyzed by SMAS before and after sperm separation using Percoll solution. After identification of predictive factors by statistical analysis, another IVF cycles between October and December 2016 were prospectively analyzed to verify the collectivity of predictive factors.

Participants/materials, setting, methods: By using retrospective data, clinically acceptable threshold was calculated using ROC curve in every semen parameters. All factors were then divided into two groups based on the threshold, and compared fertilization in each factor. We then conducted logistic regression analysis to identify the factors which independently related to IVF outcome. Finally, we assessed the association between fertilization rate and number of predictive factors exceeding the threshold using prospective data.

Main results and the role of chance: A total of 898 IVF cycles were evaluated to identify predictive factors. When compared the fertilization rate between threshold in semen parameters before sperm separation, statistically significant differences ($p < .01$) were observed in straight line velocity (VSL), curvilinear velocity (VCL), total motile sperm count (TMS), motility rate, sperm concentration, amplitude of lateral head displacement (ALH) and sperm motility index (SMI). Similarly, significant differences were observed in parameters after sperm separation with width of lateral head displacement and straightness of sperm motility in addition to VSL, VCL, TMS, ALH and SMI. Logistic regression analysis revealed VCL (>47 μm/s), TMS (>40 million) and SMI (>225) as independent predictive factors for IVF fertilization outcome. Adjustment of the model for female age, female anti-Mullerian hormone (AMH) levels did not change this finding. Prospective analysis of 332 IVF cycles revealed that IVF fertilization rate were significantly different between groups according to the number (0, 1, 2 and 3) of variables.

Limitations, reasons for caution: This study only evaluated IVF fertilization and there is no data about the pregnancy rate or live birth rate.

Wider implications of the findings: Among the above mentioned predictive factors, Curvilinear velocity and sperm motility index were only measurable

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by CASA system especially SMAS. The information obtained by SMAS will be beneficial when planning strategies of ART.

Trial registration number: none.

P-044 Relationship between seasonal variations in human semen and fertilization rate after IVF

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Study question: Does human semen change seasonally and if so are the changes related to fertilization potential?

Summary answer: Sperm motility correlates with ambient temperature. The fertilization rate after Conventional-IVF(C-IVF) was higher in months where sperm motility rate was low.

What is known already: We have observed in our clinical setting more cases of poor sperm motility during winter period. However, reports on relationship between seasonal variations in human semen and fertilization rate after IVF are not many.

Study design, size, duration: This was a retrospective study including 4229 ART patients treated in 10083 cycles where freshly processed semen was used. The time period was 36 months (January 2013 to December 2015). Statistically significant effects were determined at the level of $p < 0.05$ using the ANOVA or chi-square test.

Participants/materials, setting, methods: Patients were seeking infertility treatment in a private IVF clinic. We calculated the semen volume, sperm concentration, total sperm count and motility sperm rate per month, and examined for seasonal variability. Similarly, the monthly fertilization rate after C-IVF and intracytoplasmic sperm injection (ICSI) were calculated and examined for any seasonal variations. Calculation of the fertilization rate was limited to the age of the wife at oocyte collection which was = >30 to <36 years old.

Main results and the role of chance: Semen volume, sperm concentration and total sperm count did not vary greatly from month to month. However, the sperm motility rate underwent a clear change with a peak at August. There was a very strong positive correlation when comparing the monthly sperm motility rate with the monthly average temperature in Nagoya city, Japan. The monthly fertilization rate after ICSI was 81.9 to 84.7% and it showed a very stable value, also there was no seasonal variation at all. On the other hand, the fertilization rate after C-IVF varied widely from month to month (60.8~71.0%). When the fertilization rate after C-IVF was divided into three monthly periods according to the sperm motility rate (High: June July August September, Middle: April May October November, Low: January February March December), the fertilization rate after C-IVF in the group with a low motility rate was significantly higher than the other 2 group ($p < 0.05$).

Limitations, reasons for caution: The study was a retrospective study.

Wider implications of the findings: Sperm motility rate has a positive correlation with ambient temperature, and we reported that seasonal variation exists. C-IVF fertilization rate was suggested to be high in winter. It might show that higher ambient temperature causes faster capacitation and higher sperm motility, which leads to exhausted sperm before processing for C-IVF.

Trial registration number: None.

P-045 Saturated fatty acids increase intracellular lipid accumulation through a PPARγ-dependent pathway

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Study question: Do saturated fatty acids disturb the lipid metabolism of Sertoli cells, and can peroxisome-proliferator-activated receptor γ (PPARγ) agonist rescue such disturbance?

Summary answer: Both palmitic acid (PA) and stearic acid (SA), which are two representative saturated acids, increased intracellular lipid accumulation, and PPARγ agonist rosiglitazone ameliorated such accumulation.

What is known already: It has been reported that obesity impairs spermatogenesis, and diminished inhibin B production, which is secreted by Sertoli cells. A disruption of blood-testis barrier was also observed in obese mice. Therefore, obesity causes Sertoli cell dysfunction. On the other hand, obesity is accompanied by an increase in the levels of saturated fatty acids, especially PA and SA. Collectively, these findings illustrate that saturated fatty acids may participate in the decline of male fertility caused by obesity. PPARγ is a transcription factor relevant to lipid metabolism. Its agonists are applied in metabolic syndrome, and can possibly ameliorate aberrant lipid accumulation.

Study design, size, duration: High-fat diet (HFD) induced obese mice were constructed, and the accumulation of fatty acids in the microenvironment around Sertoli cells was analyzed. Primary mouse Sertoli cells or cultured TM4 cells were treated with PA or SA, and lipid accumulations were observed. Expression levels of representative genes related to lipid metabolism were also analyzed. Rosiglitazone, a PPARγ agonist, was used to treat TM4 cells in combination with PA or SA, and lipid accumulations were investigated

Participants/materials, setting, methods: Mouse testes were homogenized to test fatty acid levels, and testes sections were stained with Oil Red O (ORO). Primary Sertoli cells or TM4 cells were treated with 0.2-0.4 mM PA or SA for 24 h, and ORO staining was executed. Expression levels of FASN and CPT1b were analyzed using qPCR. Rosiglitazone at 20 or 50 μM was added in TM4 cells in combination with PA or SA to investigate the involvement of PPARγ pathway.

Main results and the role of chance: Testes of HFD induced obese mice contained higher levels of fatty acids, and lipid droplets were observed around Sertoli cells in seminiferous tubule, indicating an accumulation of fatty acids in the microenvironment near Sertoli cells in obese individuals. In vitro experiments showed that both PA and SA induced intracellular lipid accumulation in Sertoli cells, demonstrating an aberrant lipid metabolism caused by saturated fatty acids. Also, both PA and SA treatment increased the expression level of CPT1b, which is involved in lipid degradation, but did not affect the expression of lipid synthetic gene FASN. These results indicate that saturated fatty acids induced lipid accumulation and promoted the lipid degradation mechanism. Rosiglitazone pretreatment ameliorated the accumulation of lipids in TM4 cells caused by PA or SA, which proved an involvement of PPARγ pathway in the influence of saturated fatty acids on Sertoli cells.

Limitations, reasons for caution: The investigation of effects of saturated fatty acids on Sertoli cells were limited on in vitro experiments in this study. An ex vivo or in vivo study should be conducted in the future.

Wider implications of the findings: These results suggest that saturated fatty acids affect lipid homeostasis in Sertoli cells, and are possibly one of the critical causes of the declined fertility in obese males. PPARγ agonists, such as rosiglitazone, can be used as candidates to recover fertility of obese men.

Trial registration number: None.

P-046 Prospective studies on semen quality of 163060 male infertility in our hospital in China

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Study question: Is there decline in semen quality of infertile men? Do this semen abnormalities associated with age? If age thresholds for elements of semen quality exist?

Summary answer: Main type of infertility in male infertility is asthenospermia. 46-year is a watershed in dramatic changes in semen parameters of infertile men in our hospital.

What is known already: Infertility is a global problem to contend with and male factor infertility is on the increase. The decline in sperm quality reported over the last decades have raised concerns. However, it is unclear whether season、year、age would have differing effects on large-scale semen quality at

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various parameters. Therefore, their exploration may provide useful information in cases of male idiopathic infertility.

Study design, size, duration: Population-based cross-sectional and retrospective chart review. Semen samples were collected by masturbation after abstaining for 3–5 days from 163060 male patient of age between 18 and 65 years and who were also attending infertility clinic for having children in our hospital, China from 2011 to 2015.

Participants/materials, setting, methods: The semen samples of 163060 male patient in our hospital were analyzed for sperm volume、sperm concentration、sperm count、sperm progressive motility、total motile spermatozoa and immotile spermatozoa, using Neubauer counting chamber in line with world health Organization procedures (the fifth edition). And we analyzed the dynamic changes of 163060 semen samples with the seasonal variation、year、age changing, and age thresholds for changes in semen parameters.

Main results and the role of chance: There is not obvious decline in semen quality of infertile men in our hospital. The main type of infertility in male infertility is asthenospermia (20.83%-46.63%), followed by oligoasthenozoospermia (8.26%-13.28%) and azoospermia (7.54%-8.79%). 55.2% sperm progressive motility and 44.53% total motile spermatozoa is lower than the standard of WHO; 62.33% of infertile men had at least one of the semen parameters (semen volume, sperm concentration, sperm count, sperm forward movedment) lower than lower limit of WHO. With advancing age, the proportion of asthenospermia increased: ≤20 years (31.87%), 21-25 years (28.26%), 26-30 years (31.80%), 31-35 years (35.97%), 36-40 years (39.43%), 41-45 years (41.94%), 46-50 years (43.94%), 51-55 years (49.48%), 56-60 years (50.76%), ≥61 years (53.33%). 34 and 63 years old, 36 and 63 years old, 46 years old, 40 years old were respectively age threshold of sperm concentration, sperm count, sperm progressive motility and total motile spermatozoa, immotile spermatozoa.

Limitations, reasons for caution: We do not have actual number of infertile men in infertile couples of our hospital in China. And the effects of age on semen parameters are not for the same person, but for large numbers of infertile men.

Wider implications of the findings: There is not obvious decline in semen quality of infertile men in our hospital. Asthenospermia、azoospermia、oligoasthenozoospermia are associated with male infertility. Only semen parameters of 37.67% infertile men were within normal range. 46-year is a watershed in dramatic changes in semen parameters of infertile men in our hospital.

Trial registration number: Funding by national/international organization (s)-This work was supported by the National Natural Science Fund Project (81070531).

P-047 Roles of coenzyme Q10 on sperm quality in frozen-thawed samples of men with oligoasthenoteratozoospermia

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Study question: To test if coenzyme Q10 (CoQ10) can improve sperm quality in frozen-thawed samples of men with oligoasthenoteratozoospermia.

Summary answer: CoQ10 significantly improves sperm motility and sperm kinetics in frozen-thawed samples of men with oligoasthenoteratozoospermia.

What is known already: As a dietary supplement, CoQ10 can improve sperm quality parameters but has not yet been shown to increase live birth rate or pregnancy rate. Adding CoQ10 directly to sperm culture medium (in vitro) can be beneficial but data are still quite limited.

CoQ10 has been studied primarily in fresh semen samples. There has been very limited evidence on benefits of CoQ10 in frozen-thawed samples where CoQ10 could possibly reduce negative effects of freezing and thawing processes especially in those with poor sperm parameters like oligoasthenoteratozoospermia.

Study design, size, duration: Laboratory study with a control group and an intervention group. Frozen-thawed samples (N = 10) of men with oligoasthenoteratozoospermia were used to test in vitro effects of CoQ10 on sperm quality. In the control group, the culture medium was Ham's F10 with HSA and, in the intervention group, CoQ10 was added to culture medium (50 μg per ml). We collected data on sperm parameters at 30, 60 and 240 minutes after thawing.

Participants/materials, setting, methods: Semen samples were obtained from male infertility patients with oligoasthenoteratozoospermia at King Chulalongkorn Memorial Hospital's Fertility Clinic. Their mean age was 39.0 years (SD = 2.2). Their mean weight and height were 72.5 kilograms (SD = 9.8) and 170.3 centimeters (SD = 4.4), respectively. Semen samples were kept frozen in liquid nitrogen tank for 2 weeks before thawing and dividing to be incubated in the control medium and in the intervention medium.

Main results and the role of chance: Sperm parameters right after thawing were as followed: Concentration = 13.7 million per milliliter (SD = 1.1), motility = 10.1% (SD = 5.3), progressive motility = 1.0% (SD = 1.1), and normal morphology = 0.2% (SD = 0.4). Survival rate was 34.1% (SD = 9.6).

Sperm motility improved in the CoQ10 group at 30, 60 and 240 minutes; 12.1% versus 10.8%, 13.9% versus 9.8% and 14.6% versus 4.6%, respectively. Mean improvements were 1.3%, 4.1% and 10.0% (paired t-test; $p = 0.057$, 0.004 and <0.001, respectively). Progressive motility also increased in the CoQ10 group at 30, 60 and 240 minutes; 3.5% versus 0.6%, 2.8% versus 0.2% and 2.2% versus 0.0%, respectively. Mean increases in progressive motility were 2.9%, 2.6% and 2.2% (paired t-test; $p = 0.001$, 0.006 and 0.008, respectively).

Sperm kinetics (average path velocity, straight-line velocity and curvilinear velocity) were significantly improved at 30, 60 and 240 minutes (paired t-test; $p < 0.0001$ for all comparisons). TUNEL assay did not differ at 60 minutes but was better in CoQ10 group at 240 minutes (paired t-test; $p = 0.001$).

Sperm concentration and morphology did not differ between the CoQ10 and control groups (paired t-test; $p > 0.05$ for all comparisons).

Limitations, reasons for caution: Our study was conducted in a laboratory setting. We would need a larger number of samples to validate these findings before proceeding to further tests in a clinical setting.

Wider implications of the findings: While most studies report roles of CoQ10 as dietary supplement, our study shows effects of adding CoQ10 directly to culture medium. We demonstrate that CoQ10 can significantly improve sperm motility and kinetics in frozen-thawed samples of men with oligoasthenoteratozoospermia. This might be proven to be beneficial in a clinical setting.

Trial registration number: Not applicable.

P-048 Sperm DNA fragmentation and sex chromosome aneuploidy after swim-up versus density gradient centrifugation

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Study question: Which one is more effective between swim-up and density gradient centrifugation (DGC) in terms of reduction of sperms with fragmented DNA, sex chromosome aneuploidy, or abnormal chromatin structure?

Summary answer: The swim-up method is superior to DGC in enriching genetically competent sperms.

What is known already: Sperm motility, the percentage of normal form, and SDF level increase significantly after swim-up. We processed semen samples with either swim-up or DGC, and evaluated sperm DNA fragmentation, sex chromosome aneuploidy, or abnormal chromatin structure.

Study design, size, duration: Prospective cohort study using semen samples obtained from 18 healthy male (single sample per person) from January to December 2015.

Participants/materials, setting, methods: Semen sample was divided into three aliquots. The first was processed by the swim-up method. The second was processed by the DGC method. The third was not processed and served as the control. The percentage of sperms with fragmented DNA is measured using the sperm chromatin dispersion test. The percentage of normal X or Y chromosome is measured using fluorescence in situ hybridization. The percentage of abnormal chromatin structure is measured using toluidine blue staining.

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Main results and the role of chance: The percentage of sperms with fragmented DNA was significantly lower in the swim-up fraction (9.7%, P <.05), but not in the DGC fraction (27.8%, P >.05) compared with that in the unprocessed fraction (27%). The percentage of normal X or Y sperms was similar in the three fractions. The percentage of sperms with abnormal chromatin structure significantly decreased after DGC (from 15.7% to 10.3%, P <.05). The swim-up method also tended to reduce the percentage of sperms with abnormal chromatin structure, but the difference was not significant (from 15.7% to 11.6%, P >.05).

Limitations, reasons for caution: Because the results of this study obtained from semen samples of healthy male partners who attended infertility clinics, these cannot be extrapolated to patients with severe male factor. There are diverse methods to assess sperm DNA fragmentation. However only sperm chromatin dispersion test was used in the present study.

Wider implications of the findings: Even if the percentage is decreased substantially, sperms with genetic abnormality still remain after swim-up. It is expected that adding novel methods for sperm preparation to conventional swim-up will be able to select functionally and genetically competent sperms one by one just like natural selection.

Trial registration number: This study is not a clinical trial.

P-049 Heterozygous mutation of CFTR gene leads to activation of autophagy in Sertoli cells

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Study question: Sertoli cells have a strong autophagy activity to eliminate different substrates during the seminiferous epithelium cycle. There has never been a study of autophagy in testis of patients CFTR mutated.

Summary answer: CFTR mutated patients show evidence of activation of testicular autophagy.

What is known already: Autophagy is necessary for the resorption of apoptotic germ cells at all stages of their differentiation but also is essential for the regression of the tubulo-bulbar complexes and the residual bodies present during the elongated spermatid stage. Autophagy defect is known to be associated with cystic fibrosis (CF), an incurable lung disease caused by a mutation in the gene coding for cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation of CFTR gene is a deletion of three nucleotides coding for phenylalanine at the 508th position on the protein (F508delCFTR).

Study design, size, duration: Electron microscopy study of testis specimens from control and F508del CFTR+/- patients. Study of autophagy in cultured Sertoli cells (TM4) transfected with normal (wt) CFTR or mutated F508delCFTR (GFP coupled).

Participants/materials, setting, methods: Search for autophagic markers in several morphological observations of Sertoli cells by electron microscopy obtained from testicular biopsies of patients carrying a mutation of the CFTR gene.

Main results and the role of chance: We have identified by electron microscopy in F508delCFTR +/- patients the presence of compartments such as mitochondria or lipid droplets delimited by a double membrane, characteristic structures of autophagy. These structures are found in Sertoli cells but, surprisingly, also in germ cells. Confocal microscopy observation of murine TM4 Sertoli cell line expressing human F508del CFTR and wt CFTR demonstrates the presence of multiple autophagosomes stained with antibodies against autophagy membrane marker LC3 protein, in F508delCFTR cells, while in cells expressing CFTR WT they are rather rare.

Limitations, reasons for caution: The CFTR protein, besides its function of regulation of the ion channels, is involved in the modulation of the phenomena of exocytosis and endocytosis.

Wider implications of the findings: It could be a mechanism triggered in order to counteract the deleterious effect of the presence of the mutated protein.

Trial registration number: GMR 2017-01

P-050 "In vitro" outcome of different follicle-stimulating hormone preparations on pre-pubertal porcine Sertoli cells: preliminary results

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Study question: At present, there are no 'in vitro" studies on the effects of different preparations of follicle-stimulating hormone (FSH) on pre-pubertal Sertoli cells, which could provide important information in reproductive medicine.

Summary answer: We aimed to assess the effects of different FSH preparations on porcine pre-pubertal Sertoli cells (pSC) by evaluating modulation of their specific markers.

What is known already: It is known that FSH is the main regulator of spermatogenesis. The different preparations of FSH available in the market, are obtained by either recombinant technology (α and β follitropin) or post-menopausal urine (urofollitropin). The reason for the structural and functional heterogeneity of FSH lies on their different content in sialic acid C-terminus residue of the oligosaccharide chain. In the male testis, FSH-receptor (FSH-r) is exclusively localized on Sertoli cells that represent the unique testis target of this hormone. The use of FSH in oligo-zoospermic males, while representing a useful therapy in selected patients, is adversely affected by conflictual findings.

Study design, size, duration: Set-up an "in vitro" comparative study of different FSH preparations on ultrapure, viable and functional pSC to evaluate, at 48 hours of FSH stimulation, the modulation of SC specific markers by:

(1) Real Time PCR analysis of anti- Müllerian hormone (AMH), inhibin B, FSH-r;
(2) Western blotting analysis (WB) of FSH-r;
(3) ELISA assay, both in cell extract and culture medium, for AMH and inhibin B.

Participants/materials, setting, methods: pSC, obtained from 15-20 days old neonatal porcine testes, were evaluated in terms of purity by AMH (unique pre-pubertal pSC marker), INSL3 (specific Leydig cells marker), ASMI (unique peritubular cells marker) and PGP9.5 (gonocytes and spermatogonial cells marker) immunostaining under immunofluorescence and cytofluorimetric analysis. Thereafter pSC culture were treated with:

- α-follitropin, β-follitropin or urofollitropin at the same molar concentration (100 nM) for 48 hours;
- Testosteron (T): 0.2 mg/ml;
- Combinations of different FSH preparations with T.

Main results and the role of chance: In our model we observed, in the tested experimental conditions, that:

- All three preparations of α-follitropin, β-follitropin and urofollitropin induced, as expected, a reduction of AMH in terms of mRNA, cell extract and secreted protein;
- All three preparations induced an increase of inhibin B in terms of mRNA and cell extract protein and, while interestingly, only α-follitropin induced an increase of inhibin B secreted in the culture medium;

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- All three preparations induced, as expected, a reduction of FSH-r mRNA but only α-follitropin was associated with downregulation of FSH-r (WB).

These results preliminarily showed, that the three FSH preparations were associated with different effects in terms of inhibin B secretion, posing the question if, in the treatment of the infertile male, use of FSH preparations that increase inhibin B secretion (with a potential down-regulation of endogenous FSH) or those that do not induce this increase should be preferred.

Limitations, reasons for caution: Dose and time-dependent relationship.

Wider implications of the findings: The present original study, based on the use of an 'in vitro" model of pre-pubertal pSC, could help better understanding of the effects of different FSH preparations, thus providing important information on both, the conflictual findings with regard to use of FSH in oligozoospermic males and, in general, reproductive medicine.

Trial registration number: NA.

P-051 Exogenous oral combination antioxidants in idiopathic infertile males, having higher DNA fragmentation index, before ICSI cycle may improve live birth rate

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Study question: Does oral combination antioxidant supplementation correct DNA abnormality in idiopathic infertile males and thereby improve live birth rate in ICSI cycles?

Summary answer: Study confirms the efficacy of oral combination antioxidants in treating DNA abnormality in idiopathic male infertility (IMI) and improvement in live birth rate following ICSI.

What is known already: IMI associated with oxidative stress (OS) may have higher DNA Fragmentation Index (DFI) which may be responsible for failed fertilization or abnormal fertilization leading to implantation failure or pregnancy loss. Antioxidants like Vitamin C, Lycopene, Zinc and Selenium protect sperm DNA by scavenging off excess free radicals and reducing DNA oxidation rate, whereas folic acid and methyl cobalamine synthesize healthy DNA and thus help in normal fertilization and conception.

Study design, size, duration: A prospective study involving 162 men with IMI was done from August 2012 to August 2016 at Institute of Reproductive Medicine, Kolkata, India.

A combination of antioxidants containing vitamin C, Lycopene, Zinc, Selenium, Folic Acid and methyl cobalamine etc. was administered for six months before ICSI cycle in a universally recommended dosage. There was a history of at least two failed cycles or pregnancy loss. Female partners (age ≤ 38 yrs) were apparently normal.Participants/materials, setting, methods:

Group A (n = 104) males were used as controls, Group B (n = 58) had infertile males having OS and high DFI (≥ 25%)

OS was confirmed by the high level of Reactive Oxygen Species (ROS) and reduced Total Antioxidant Capacity (TAC) by Chemiluminescence Assay. Sperm count, motility, morphology and leucocyte count were evaluated. ICSI was done in 148 cycles. Embryo transfer was performed in 110 index cycles. In 38 cycles frozen embryo transfer (FET) was done.

Main results and the role of chance: In Group A there was improvement in sperm count (29.5 ± 7.96 vs 54.65 ± 11.05, NS) and motility (15.8 ± 3.13 vs 28.60 ± 4.37, p = 0.0179) after six months' therapy. Sperm count in Group B was 18.40 ± 3.15 vs 46.70 ± 5.65, p = 0.0001, Motility in Group B was 19.20 ± 2.80 vs 35.50 ± 4.35, p = 0.0019.

ROS level in Group A was 32107.15 ± 9623.52 vs 31257.0 ± 9240.0, NS.

TAC level in Group A was 1164.20 ± 99.76 vs 2010.65 ± 116.30, p = 0.0001.

ROS level was reduced, (72330.50 ± 9124.45 vs 41230.65 ± 6245.10, p = 0.005) and TAC level was enhanced (1144.50 ± 134.45 vs 1640.75 ± 175.60) significantly after therapy in Group B.

DFI decreased in Group A (24.40 ± 2.82 vs 21.75 ± 2.39, NS) In Group B there was significant decrease in DFI (29.45 ± 2.55 vs 20.30 ± 2.24, p = 0.0078) after therapy.

Improvement in sperm morphology and leucocyte count was not significant after therapy in Group B.

Pregnancy rate was 48.27% (Group B) and 30.76% (Group A),p = 0.04. Live birth rate was 32.14% in Group B and 9.37% in Group A, p = 0.04.

Other factors for implantation and pregnancy loss need to be excluded to minimize the role of chance.

Limitations, reasons for caution: Only antioxidant therapy may not be sufficient in IMI if some other causative factors for development of OS are present. Therefore, it is essential to remove the causative factors before initiation of antioxidant therapy. Moreover, ideal combination and optimum duration of treatment is still unclear.

A prospective study is needed.

Wider implications of the findings: A very simple and safe therapy by combination of antioxidants may reduce implantation failure and pregnancy loss and increase live birth rates by correcting DNA abnormality in certain percentage of IMI induced by OS.

Trial registration number: Not applicable.

P-052 Sperm DNA Fragmentation Testing and significance, where do we stand?

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Study question: Assessment of the most prevalent sperm DNA fragmentation and sperm selection techniques. Also looking into the most prevalent technique that has yielded better outcomes.

Summary answer: Among all available sperm DNA Fragmentation testing techniques, TUNNEL Assay and SCD were the most commonly used with significant difference from the others.

What is known already: Sperm DNA integrity assessment is getting more important nowadays in the evaluation of semen fertility. There are different available techniques, and each of them has its own merit. Also, sperm selection techniques are currently more widely used according to the result of the sperm DNA fragmentation test.

Study design, size, duration: This web based cross-sectional survey included 23 questions categorized into 4 categories (DNA fragmentation techniques, interpretation of results, lab procedures to deal with abnormal DFI, and related embryological factors & clinical outcome). Data was collected during January 2016 and 120 responses were received.

Participants/materials, setting, methods: Of the 120 collected responses, 100 were completed correctly, and 82 of the participants were clinical embryologists and andrologists. Data was collected and analyzed using an online survey software and questionnaire tool.

Main results and the role of chance: Fifty three percent of the responders were using sperm DNA fragmentation testing. The most prevalent Technique was TUNEL Assay. TUNNEL Assay (34.62%), and SCD (32.69%) were significantly more commonly used than the COMIT, SCSA, and other techniques combined (P = 0.0006). The indications were (multiple answers were allowed): 73% Repeated miscarriage, 71% previous ICSI failure, 55% unexplained infertility, and 51% poor semen parameters. Threshold values parameter, 44.23% said it's dependent on technique used, 30.77% center's guidelines. 25% general threshold. The preferred ART modality in cases of abnormal DFI was 84.62% ICSI (P < 0.0001) Compared To IVF, IUI, and OI. The survey revealed that there's no significant difference between IMSI, PICSI, TESE, and MACS as a preferred sperm selection technique for abnormal DFI patients. General improvement was observed in prognostic lab indices after using a sperm selection technique it included fertilization rate, cleavage parameters, and blastulation rate and quality. Recipients were asked to specify the improvement in their clinical outcome after using sperm selection technique, a higher pregnancy rate by 5% and lower miscarriage in the 1st trimester got the highest percentages. Retrospective analysis of responses whose having positive clinical outcome showed that 40% of them were using TUNEL Assay.

Limitations, reasons for caution: The number of responses is relatively low to reach a level of significance in the results and it doesn't represent a worldwide view since it's only 29 countries responded to the survey. However we've received responses from the most eminent centers worldwide and covers over 100 thousand cycles per year.

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Wider implications of the findings: Out from the received responses, Sperm DNA fragmentation testing has been used by more than 50% of the participants. Urgent studies are needed to set up a universal threshold value for each technique. The most clinically relevant technique for assessment of sperm DNA fragmentation need to be looked at.

Trial registration number: NA.

P-053 The effects of HPV infection on semen: first results from a new approach

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Study question: The effect of Human Papilloma Virus (HPV) infection on semen characteristics was investigated using a new method that allow to evaluate virus localization in the different semen components.

Summary answer: Our data indicate a correlation between HPV infection of exfoliated epithelial cells (from spermatic ducts and accessory glands) and reduction of sperm cell motility.

What is known already: HPVs are agents of the most common sexually transmitted diseases. Both in men and in women, HPVs cause a variety of clinical symptoms ranging from warts to cancer. It has been shown (IARC, 2009) that infection with 12 oncogenic HPVs is the major cause of development of cervical cancer in women and it is also related to penile cancers in men. HPV infects epithelial cells and can bind other cell types. Its presence in semen is widely known, but the effects on fertility and reproductive function are still controversial.

Study design, size, duration: From January 2015 to December 2015, twenty men were enrolled. From each subject seminal parameters were evaluated according to WHO guidelines. HPV was detected and genotyped both in total semen and in swim up-fraction. HPV detection was performed also in separated semen fractions: sperm cells from total semen and from swim-up and epithelial cells. Semen parameters of positive samples were compared with parameters of negative samples. Moreover, HPV genome wholeness in sperm cells was evaluated.

Participants/materials, setting, methods: Partners of women with high-grade squamous intraepithelial lesions. DNA was purified after differential lysis. HPV genotyping was performed by a conventional reverse hybridization and HPV detection in the different semen components was performed by nested PCR with the use of universal primers (MY09 / 11, GP5+ / 6+). HPV genome wholeness was evaluated by Real Time-PCR using six different target genes.

Main results and the role of chance: Samples were distributed into HPV positive and negative based on the conventional genotyping (total DNA extraction and reverse hybridization method) or on the new method (differential lysis, DNA extraction from separate fractions and nested PCR method) for every different fraction. In any case, we did not find any statistically significant difference on semen volume, sperm concentration, morphology. On the contrary, only when we compared HPV-positive epithelial cell samples to negative samples in the same fraction, we found a difference in sperm motility. The average percentage of sperm linear progressive motility amounted to 10% ± 11.5 in the positive samples and 54.4% ± 23.3 in the negative samples (two-tailed P value = 0.0003). Moreover the HPV genome wholeness was tested by RT-PCR. Results performed on DNA extracted from sperm cells of a sample showed that the copy number of E1 and E2 genes was lower than LCR (Long Control Region) and E6 copy numbers (copy number ratio: 0.4 ± 0.05). These data indicate that in this sample more than half viral genomes are defective, suggesting that a recombination event could be occurred into the sperm heads.

Limitations, reasons for caution: Low number of samples because of the sporadic frequency of infection with a single high-risk HPV genotype.

Wider implications of the findings: The data support the proposed role of HPV in decreased male and couple fertility. The presence of defective viral genomes rises the idea that these defective forms could be integrated in the sperm genome. In turn, this risk prompts new possible consequences of the viral infection in semen.

Trial registration number: Not required.

P-054 Does protein supplements taken in relation to workout impact sperm quality?

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Study question: Does termination of intake of workout related dietary supplements affect sperm concentration in oligospermic men?

Summary answer: A two months abstinence time elevated the sperm concentration and had an important impact in the clinical decision-making.

What is known already: The market for dietary supplements is growing and especially young men who enter fitness gyms for workout seem to have a large intake with an average of 5 times/week. Recently, it was shown that 23/24 selected dietary supplements from fitness shops contained anabolic steroids. However, these so called pro-androgenic supplements are presumably not in the same category as the more commonly used whey and soy protein supplements. Importantly, protein supplies are not medical drugs, but dietary supplements. Hence protein supplements are not tested like medical drugs and consequently, they might contain known and unknown active mediators that impact reproductive health.

Study design, size, duration: Case-series.

Participants/materials, setting, methods: A total of 11 men who used dietary supplements as part of their workout, and who were submitted to a tertiary fertility clinic were prospectively enrolled. The sperm concentration had to be below 15million/mL at time of enrollment. After 2 months abstinence from dietary supplements a new semen analysis was measured according to WHO criteria and patients underwent a clinical examination and completed a questionnaire to assess potential confounders.

Main results and the role of chance: The median difference in sperm concentration was 1.6 million/mL (Interquartile range: 0-5.1) higher at the follow-up 2 months later compared to baseline. A total of four patients did not need donor back up following the dietary abstinence which was otherwise indicated from their baseline sample. Furthermore, one couple changed from IVF to IUI treatment. Another case showed a percentage-wise large increase in sperm concentration from only 1 spermatozoa seen to 0.6 mio/mL, interestingly the size of the testes was 12 and 10 ml, respectively. One patient had azoospermia in the initial sample and no difference was observed after termination of the dietary supplement intake. Only one patient had a slightly lower sperm concentration following protein abstinence.

Limitations, reasons for caution: Although patients where prospectively enrolled, this is a case-series, emphasizing the need for future corroboration. Moreover, the study might have unknown confounders even though no patient used anabolic steroids or smoked. Furthermore, the type of dietary supplemental product, the active components, and the dose might vary for each case.

Wider implications of the findings: As there is no health benefit from protein supplies for young and otherwise healthy men, we suggest fertility doctors to advice for caution in the use of protein supplies while undergoing fertility treatment. Further research is urgently needed.

Trial registration number: N/A.

P-055 Induction of DNA damage during sperm selection for ARTs: DNA fragmentation (sDF) in the viable sperm fraction following density gradient centrifugation (DGC) and swim-up

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Study question: Is DGC-induced sperm DNA damage more sensitively detected by evaluating DNA fragmentation in the viable sperm fraction and prevented by swim-up procedure?

Summary answer: DGC induced DNA damage is detected with higher sensitivity when measured in viable spermatozoa and is partially prevented by swim-up procedure

What is known already: Although many studies reported that sperm selection improves sperm DNA integrity, some recent papers showed that sperm selection with DGC can increase sperm DNA fragmentation levels. In particular, a recent study from our group has shown that DGC induced an additional sperm DNA damage in about 50% of male partners of infertile couples who subsequently experienced a 50% lower probability to achieve pregnancy after Assisted reproduction Techniques (ARTs). These results rise concerns about the safeness of DGC and suggest that identification of the subjects undergoing sperm DNA damage during selection may allow a better management during ARTs.

Study design, size, duration: We measured sDF both in total (total sDF) and in viable (viable sDF) sperm populations before and after selection in 20 semen samples processed with DGC and in 20 processed with swim-up, collected from January 2016 to December 2016. We compared the number of subjects showing an increase of sDF during selection with both procedures and the average percentage increase of sDF, in total and viable sperm populations.

Participants/materials, setting, methods: Semen samples were from male partners of infertile couples undergoing routine semen analysis. Selection was conducted by layering semen upon a 40:80% PureSperm density gradient for DGC or on Human Tubal Fluid/Human Serum Albumin for swim-up. Total and viable sDF were simultaneously detected by a flow cytometric technique using DAPI to define the sperm population, Tunel to detect sDF and L10120 to discriminate between viable and not viable sperm.

Main results and the role of chance: In the 20 semen samples selected by DGC, an increased level of DNA damage was found in 10 subjects when total sDF was considered and in 12 when sDF was evaluated in viable sperm. In the 20 semen samples selected by swim-up, the number of subjects showing an increase of DNA damage was 3 when total sDF was considered and 8 when sDF was measured in viable sperm. Interestingly, the average percentage increase of DNA damage was much greater in the viable than in total sperm population (respectively 388.7 ± 435.5% vs 150.4 ± 231.3% in samples processed by DGC and 61.5 ± 42.3% vs 21.4 ± 27.3% in samples processed by swim up). These results suggest that the measure of sDF in the viable sperm fraction is more sensitive in detecting the eventual induction of the damage by selection. In addition, swim up resulted less invasive than DGC because it provoked an increase of sDF in a lower number of subjects and a lower average percentage increase of DNA damage in their samples.

Limitations, reasons for caution: The comparison between DGC and swim-up was conducted in different semen samples not allowing to compare directly the effect of the two procedures. However, using the same samples would have caused a selection bias (samples with low sperm number could not be included).

Wider implications of the findings: Viable sDF is a more sensitive tool to identify those patients increasing sDF level during selection and, possibly, expecting a lower pregnancy rate after ART. In these patients, swim-up could be recommended for sperm selection in ART lab as it results a less invasive procedure than DGC.

Trial registration number: NA.

P-056 High magnification-sperm morphology and hyaluronic acid-binding assay: is there a correlation?

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Study question: Is there a significant correlation between sperm cells morphology at high magnification (6600 X) and their capacity to bind hyaluronic acid (HA)?

Summary answer: The correlation is significant only for spermatozoa belonging to the first (+) and fifth (-) class of Bartoov-Berkovitz classification.

What is known already: Several studies have shown that sperm selection at high magnification (MSOME) may increase the blastocyst formation rate and implantation rate. HA, the main constituent of the biochemical matrix layer that covers the oocyte, can play an important role in the selection of sperm during fertilization in vivo. Only mature sperms have receptors for HA on their plasmatic membrane. In vitro, it was observed that, by contacting the sperm with HA, if these are mature have a characteristic movement only of the tail while the head remains blocked by the receptors that bind HA molecules.

Study design, size, duration: We evaluated 102 fresh ejaculated samples derived from patients aged 22-60 years, enrolled in the department of PMA of the Clinic Center Hera (NA) in the year 2016.

Were excluded from the study cases of:

- Cryptozoospermia
- Azoospermia
- Teratozoospermia (% of typical forms <4)
- Oncological patients

Participants/materials, setting, methods: We capacitated 102 semen samples by conventional "swim-up" or "density gradient" methods, depending on the sample properties. Following the Bartoov-Berkovitz criteria, we classified the capacitated sperm cells with high magnification microscopy (in five groups) and then we carried out an HA-binding assay (putting sperms in contact with a medium containing HA). So we performed the classification of the same samples only for the portion of sperm cells blocked in the interface with HA.

Main results and the role of chance: By morphological comparisons at high magnification among capacitated sperm samples (A) and their HA-binder fraction (B), we highlighted in group B a decrease (57.51% *vs* 46.03%) in the percentage of spermatozoa belonging to the fifth group of morphological classification according to the criteria of Bartoov-Berkovitz (head with abnormal chromatin, with large vacuole> 4% of the head); the two-tailed P value is <0.0001. For conventional criteria, this difference is considered as highly statistically significant. Similarly we showed an increase (28.27% *vs* 38.95%) in the percentage of sperms of the first group of classification (normal head size, shape and chromatin, with 0 or 1 vacuole <4% of the head) with P value < 0.0001. The statistical analysis was performed using paired *t*-test. Some studies suggested that vacuoles occur during the sperm maturation process even from the early stage. Their size decrease during spermatogenesis and epididymal transit, sometimes correlating with male subfertility, lower mitochondrial membrane potential, increased incidence of chromosomal abnormalities and packaging of sperm chromatin. On the other hand HA allows us to select a mature sperm. So, on the basis of our studies we suggest to select a sperm for injection according to the high-magnification morphology in the HA-binder fraction.

Limitations, reasons for caution: The study should be extended to a larger number of samples for more thorough statistical analysis.

Wider implications of the findings: Our results agree with several studies in which the presence of vacuoles correlate with a lower sperm maturity, evaluated indirectly by means of HA-binding capacity. These data suggest a combined selection method, more rapid than classical IMSI, possibly to improve the outcome of injection procedure mostly in male subfertility cases.

Trial registration number: Not applicable.

P-057 Preliminary results of the FertiSCI protocol: evolution and risk factors of alteration of sperm parameters in spinal cord injured adult males. A prospective study

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Study question:

(1) Is there one or several factors responsive of a degradation of sperm parameters in spinal cord injured (SCI) men

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Summary answer:

(1) Retrospective studies showed no sperm degradation in SCI men and concluded that there is no risk for fertility and no preventive sperm cryopreservation to propose.

What is known already:

(1) Fertility is a main concern of men with SCI. Because of the spinal lesion, they have erection and ejaculatory dysfunction that can be solved for 60% of them by penile vibratory stimulation (PVS). Sperm parameters present normal to diminished sperm volume, normal sperm concentration but a marked decrease in sperm mobility and viability and a rise of round cells principally polynuclear. Several studies reported the presence of ROS and high sperm DNA fragmentation in this population and SCI men are frequently subjected to urinary tract infections. These factors could contribute to sperm degradation over time.

Study design, size, duration:

(1) 35 SCI men were recruited for a prospective study consisting in four sperm retrievals every 6 month for a total study duration of 18 month. Inclusion criteria were age limitation from 18 to 60 years and antegrade ejaculation by masturbation or PVS. Inclusion was realized in a specialized center for SCI reeducation. The duration, level and completeness of the lesion were noticed as well as medical intercurrent events, treatments, bladder management and way of life.

Participants/materials, setting, methods:

(1) On each retrieval, semen parameters and sperm morphology were studied according to WHO recommendations. Inflammation parameters were considered by counting round cells, polynuclear cells evaluated by leucoscreen test and elastase dosage by ELISA. A systematic bacteriological analysis of sperm and urine were realized. Oxidative stress was evaluated by flow cytometer by measuring DNA fragmentation using tunnel assay on total and alive spermatozoa and spermatozoa 8 hydroxy DNA guanosine by oxy DNA Kit.

Main results and the role of chance:

(1) Results presented here are preliminary as they concern uniquely the first sperm retrieval after inclusion. The 35 patients were included in two years, demonstrating the feasibility of the project and the interest of SCI men about their fertility. Mean age was 29.8 ± 6.4 years, mean age at injury was 21.5 ± 8.2 years and time postinjury 8.2 ± 8.7 years. 32 patients had a spinal lesion at T10 or upper. On 35 patients included, 31 came for their first sperm retrieval, 3 had a no ejaculation and 1 presented an azoospermia. 27 semen were analyzed with a mean sperm volume = 1.3 ± 1.2 ml, sperm numeration = 155.8 ± 226.9 million, round cells concentration = 37 ± 37.3 M/ml, a+b motility= 14 ± 12% and viability=23.8 ± 18.8%. Mean polynuclear cells were evaluated to 11.6 ± 15.3 M/ml and elastase (n = 12) to 162.1 ± 212.1 ng/ml. Oxidative stress could be evaluated in 14 sperm. Total DNA fragmentation = 47.4 ± 24.9 % and fragmentation on alive spermatozoa=28.7 ± 27.8%, 8OHdG was measured at 20.2 ± 10.6%. More than half patients had a positive urinary analysis (53.6%) and 1/3 a positive spermoculture. The population was then divided in two groups considering the delay postinjury superior (n = 15) or inferior (n = 13) to 5 years. No significant differences were observed concerning sperm numeration, motility, viability but also DNA fragmentation (total and alive spermatozoa).

Limitations, reasons for caution:

(1) This study is limited by the number of patients included. It constitutes a pilot study demonstrating its feasibility to propose next a multicentric study. Technically, the diminished sperm volume was limitative to realize all planned analyses and the high number of round cells could interfere with flow cytometer analyses.

Wider implications of the findings:

(1) Considering oxidative stress, we confirm increased total DNA fragmentation but a limited fragmentation on alive spermatozoa corroborated by low elastase level in sperm. We also demonstrate that in this population there is no risk of sperm degradation over the time.

Trial registration number:

(1) NCT02144558

P-058 Effects of seminal plasma and semen application during in vitro fertilization treatment - systematic review and meta-analysis

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Study question: Does the intracervical and intravaginal application of seminal plasma (SP) or semen has an effect on clinical pregnancy rate (CPR) in IVF treatment?

Summary answer: The application of SP or semen around the time of oocyte aspiration or embryo transfer is associated with higher CPR.

What is known already: Several studies have demonstrated the potential role of seminal plasma (SP) in non-human and human implantation by modulating endometrial function and the maternal immune system. The effect appears to be due to highly active cytokines and growth factors and is independent from sperm quality. Two pilot studies had suggested but not proven that semen and/or SP might improve CPR by improving endometrial function which seem to be negatively affected in conventional gonadotropin stimulated IVF. Several trials, based on this concept, has been performed since then but a meta-analysis has never been performed so far.

Study design, size, duration: A systematic review and meta-analysis was performed, including all studies identified by searching electronic databases from their inception until October 2016. Trials were considered if women were exposed to any kind of SP or semen (either SP/semen injection or sexual intercourse) around the time of oocyte aspiration and embryo transfer and analysis regarding outcome was performed.

Participants/materials, setting, methods: Eight prospective randomized trials (RCTs) of women undergoing IVF were identified.

Main results and the role of chance: The eight identified RCTs included 2,128 women. The intervention included four studies which analyzed the effect of prepared undiluted and one study of thawed diluted SP after oocyte aspiration, one study which used untreated diluted semen and two studies, which analyzed the effect of sexual intercourse around the time of oocyte aspiration and/or embryo transfer. Women randomized in the intervention group had a significantly higher rate of CPR compared to controls (30.0% vs 25.1%; RR 1.20, 95% CI 1.04 to 1.39). No significant differences were found in the secondary outcomes, including livebirth rate, biochemical pregnancy rate, miscarriage rate, multiple pregnancies and birth weight. The subgroup analysis (4 RCTs, 780 participants) including only those RCTs in which prepared undiluted SP was injected just after oocyte aspiration concurred with the overall analysis for the primary outcome (46.3% vs 37.2%; RR 1.23, 95% CI 1.05 to 1.45).

Limitations, reasons for caution: In the overall meta-analysis only four of the eight studies used placebo as control and were double blind. In the subanalysis three of the four studies included used placebo as control and were double blind. Some of the intervention protocols and the definitions of CPR were different.

Wider implications of the findings: The findings support the hypothesis that first SP does play a role in the regulation of implantation which involves a coordinated modulation of the maternal immune system and second that SP could be used as an implantation supporting therapy in fresh transfer following high dosage gonadotropin stimulation.

Trial registration number: PROSPERO International Prospective Register of Systematic Reviews, CRD42016054354.

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P-059 Differential external morphological changes between laser-assisted immobilized and mechanical immobilized human sperm: by SEM comparison

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Study question: Is the external structure of laser-assisted immobilized sperm different from mechanical immobilized one?

Summary answer: Laser-assisted immobilized sperm showed no external physical damage but distinct morphological changes were observed while mechanical immobilized sperm showed clear external damages.

What is known already: Sperm immobilization can be achieved by both mechanical and laser-assisted methods and showed equivalent fertilization and embryo cleavage rate. However, there is so far no study comparing physical damages between the two methods.

Study design, size, duration: At least 600 human sperm were immobilized by mechanical and laser-assisted methods and then were examined external structure using scanning electronic microscope.

Participants/materials, setting, methods: Ten normospermic samples were collected in our andrology unit for the immobilization-SEM study. ICSI injection needles were used for mechanical immobilization while non-contact infra-red diode laser was used for laser-assisted immobilization. Immobilized sperm were then under SEM for examination. Modified viability test on the two immobilization methods was used to examine differential membrane integrities.

Main results and the role of chance: We found external damages on mechanical kinked sperm, surprisingly, external damage was not found on laser-assisted immobilized sperm. Although no external damage was found on the membrane of laser-assist immobilized sperm, there were three types of morphologic changes were observed. When laser applied on end piece area resulted in 100% immobilization and 94% with immediate coil tail formation was noted; while laser applied on sperm principal piece area resulted in 100% immobilization but only 8% sharp bend of sperm tails were observed. Our modified membrane integrity assay also revealed that majority (91%) of laser-assisted immobilized sperm retained membrane intactness significantly (<0.001) compared to mechanical method that coherent with our SEM observation.

Limitations, reasons for caution: Our study is limited on human sperm with normal WHO semen parameters.

Wider implications of the findings: The intactness of the membrane and external structure on laser-assisted immobilization showed that complete membrane rupture is not necessary for sperm immobilization. This finding is opposite to general concept that both of the immobilization methods can cause physical damages immediately on sperm and losing theirs membrane integrities.

Trial registration number: NA.

P-060 two types of pathologic feature in Klinefelter syndrome result in different sperm retrieval rates during micro-TESE

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Study question: Are there different testis pathologic features between patients with Klinefelter syndrome(KS) who can obtain sperm through micro-TESE and those who can't find the sperm?

Summary answer: Patients with KS can be classified into two types according to the pathologic features, sperm retrieval rate(SRR) and microscopic findings would be different between them.

What is known already: Azoospermic patients with KS could have the chance to find sperm through micro-TESE. The reported SRR of KS ranged from 16% to 78%. Testicular volumes and serum level of FSH, LH, inhibin B and testosterone can't predict the SRR, patient's age is the only prognosis factor found as significant in some studies. The mechanisms of severe spermatogenic failure and focal spermatogenesis are unclear. There are two popular hypotheses on how spermatogenesis occurs in Klinefelter patients: spermatozoa arise from patches of 46,XY spermatogonial stem cells in the testes or 47,XXY germ cells are able to undergo meiosis occasionally.

Study design, size, duration: This is a retrospective study. From Dec.2014 to Dec.2016, 35 NOA patients with KS underwent micro-TESE and were categorized into two groups according to the pathologic features: Leydig cells hyperplasia and most of the seminiferous tubules present severe hyalinization (group A,n = 27), and seminiferous tubules with hyalinization interlace with tubules of maturation arrest (group B,n = 8).

Participants/materials, setting, methods: Micro-TESE was performed at x20 magnification under the operating microscope. Seminiferous tubules were removed and immediately placed within a sperm buffer. Then the tubules were dissected and examined immediately by an embryologist under a phase microscope at x200 magnification. The two groups were compared in age, testicular volumes, FSH, LH, testosterone, SRR and microscopic findings during the operation.

Main results and the role of chance: Leydig cells hyperplasia and most of the seminiferous tubules present severe hyalinization are the frequent pathologic appearances of KS testis(27/35). Within these cases(group A),12 patients (44.4%) showed heterogeneous thickness of seminiferous tubules and 9 of them(75%) found sperms from the focal spermatogenesis area during micro-TESE. The SRR of group A was 33.3%. Meanwhile, in the cases of seminiferous tubules with hyalinization interlacing with tubules of maturation arrest(group B), heterogeneous tubules were easy to find in all of the testes during micro-TESE, nevertheless, none of them had sperm(0/8). Testicular volume in group A was lower than group B (1.8 ± 0.7 ml vs. 2.6 ± 0.9 ml, $p < 0.05$). However, no significant difference was observed in age (29.5 ± 4.2 vs. 29.8 ± 6.4 years), FSH (41.1 ± 12.9 vs. 37.4 ± 7.7 mIU/ml), LH(25.7 ± 8.7 vs. 23.2 ± 7.8 mIU/ml), and testosterone levels (2.1 ± 1.6 vs. 1.4 ± 0.7 ng/ml) between the two groups (All $p > 0.05$).

Limitations, reasons for caution: More cases should be included in the following study to confirm our conclusion that KS testis would have great opportunity to show heterogeneous seminiferous tubules during micro-TESE but the SRR would be depressed if the pathologic appearance is that seminiferous tubules of maturation arrest interlace with tubules presenting severe hyalinization.

Wider implications of the findings: Aneuploidy is the homogeneous etiology of all the KS cases, however, testis pathologic features are extremely different between the patients. Multiple seminiferous tubules present maturation arrest in some cases implies that the major cause of spermatogenic failure might be abnormal testicular environment instead of meiotic failure in 47,XXY germ cells.

Trial registration number: not applicable.

P-061 Omega-3 free fatty acids inhibit sertoli cell apoptosis Induced by palmitic acid

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Study question: What is the effect of elevated free fatty acids(FFA) on the survival of sertoli cells?

Summary answer: The saturated FFA palmitic acid(PA) promoted apoptosis of sertoli cells while omega 3 polyunsaturated FFAs could partly protect sertoi cells from the damage.

What is known already: Nowadays, obesity had become an important risk factor related with male infertility. It had been reported obese men often showed decreased serum Inhibin B and semen parameters but increased serum FFAs. Elevated FFAs including PA had been demonstrated to inhibit the survival of leydig cells in the testis in vitro.

Study design, size, duration: Testis sertoli cells and TM4 sertoli cell line were used to investigate effect of the saturated PA and omega 3 polyunsaturated FFAs on the survival of sertoli cells in vitro. Different treatment time and doses of FFAS were taken into consideration. Viability of sertoli cells was the main outcome measures. Furtherly, we investigated the possible mechanisms by which PA induced apoptosis of sertoli cells.

Participants/materials, setting, methods: MTT assay was used to evaluate the viability of sertoli cells. Apoptosis was detected by flow cytometry

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stained with annexin-V and PI dye solution. The expression of apoptosis-relevabt genes were analysed by qPCR. Western Blot was performed to validate the key pathway. 2', 7' dichlorofluorescin diacetate (DCFH-DA) was used to measure the reactive oxygen species(ROS) generation. The ultrastructure of TM4 sertoli cells after free fatty acids exposure was examined by transmission electron microscopy.

Main results and the role of chance: PA induced the decreased survival of sertoli cells in a time- and dose-dependent manner. However, omega 3 polyunsaturated fatty acid had the potential to protect sertoli cells from the damage from PA while itself had no influence on the viability of sertoli cells in the Physiological concentration range. Flow cytometry demonstrated that the apoptosis of sertoli cells was increased by PA treatment. QPCR and Western blot analysis proved that bcl2/bax might involve in the apoptotic pathway and ROS level was also increased inthe PA treatment group which means mitochondria played an important role in the process. Furtherly, TEM showed the accumulation of lipid in the cytoplasm and the swelled mitochondria. These results indicated that PA caused the accumulation of lipid in the TM4 sertoli cells which furtherly damaged the function of mitochondria leading to the release of ROS. Finally, sertoli cells was attacked by the oxidative stress and apoptosis was induced. Omega 3 polyunsaturated fatty acids decreased the apoptotic rates by increasing nrf2 expression which improve the anti-oxidative stress ability of TM4 sertoli cells.

Limitations, reasons for caution: Obesity was associated with complicated dysregulation oflipid metabolism and changes of reproductive function. In the present study, only PA andomega 3 polyunsaturated fatty acids were chosen to investigate the effects of lipid on survival of sertoli cells.

Wider implications of the findings: Saturated fatty acids damage male reproductive function by inducing the sertoli cells apoptosis, while omega 3 polyunsaturated FFAs protects sertoli cells from the attack of PA, indicating that taking food rich in omega 3 polyunsaturated fatty acids and lower content of saturated fatty acids may be beneficial for male reproduction.

Trial registration number: None.

P-062 Reciprocal translocations – a retrospective study in infertile couples

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Study question: Incidence of reciprocal translocations in infertile couples. Should it be considered?

Summary answer: Although the low incidence of reciprocal translocations detected (1.18%), this analysis permitted a more accurate diagnosis, allowing an adequate genetic counselling for infertile couples.

What is known already: Infertility prevalence is increasing in the developed world affecting 10-15% of couples in reproductive age. It is a complex multifactorial condition with male and female factors involved. The cytogenetic analysis is considered an important approach in this area since numerical and structural alterations can be responsible for infertility. Individuals with reciprocal translocations are fenotipically normal, but due alterations at the meiotic segregation, have an increased risk of producing gametes with chromosomal imbalances leading to infertility, increased risk of pregnancy loss and birth of handicapped children.

Study design, size, duration: Couples who participated in the infertility consultation between November 2009 and December 2016 at the Hospital Center of Trás-os-Montes and Alto Douro conducted a cytogenetic study and analysis of semen in the man. When a cytogenetic anomaly was detected the couple was referred to a genetic consultation. The relationship between the reciprocal translocations found with the semen parameters was evaluated, as well as a bibliographic review of the cytogenetic alterations described in this study.

Participants/materials, setting, methods: This work presents the reciprocal translocations found in 680 cytogenetic studies of 340 couples. Chromosome analysis was performed according to standard techniques and, at least, 20 metaphases were analyzed. In all the men, semen analysis (ejaculate volume, motility, vitality, hypo-osmotic swelling test, sperm concentration and morphology) was performed according to World Health Organization (WHO, 2010) guidelines.

Main results and the role of chance: Eight cases of balanced reciprocal translocations (1.18%) were detected in different couples, 4 in men and 4 in women:

- 46,XY,t(11;22)(q14.2;q13.1) is the most reported translocation, with different break points described, our included.
- 46,XX,t(12;15)(q13.3;q24.3) was also detected in a woman with Family Stickler Syndrome not being associated with infertility.
- 46,XY,t(4;22)(p16.1;q11), 46,XY,t(6;8)(p23;q21.3), 46,XY,t(4;10)(q31.3;p15), 46,XX,t(5;9)(p13;q34), 46,XX,t(10;15)(q11.23;q13) had already been described although with different break points.
- 46,XX,t(2;3;15)(p22;p12.1;q26.2) karyotype patient with a three-break rearrangement, is described for the first time.

Family studies are still ongoing to confirm if these cytogenetic anomalies are *de novo* or inherited.

The semen analysis in the case with a 46,XY,t(6;8)(p23;q21.3) karyotype reveal normal values, in the 46,XY,t(4;22)(p16.1;q11) case an oligoastenozoospermia was detected and in cases 46,XY,t(11;22)(q14.2;q13.1) and 46,XY,t(4;10)(q31.3;p15) an oligoteratozoospermia was found.

Limitations, reasons for caution: Despite the small number of cases, the results of this study strongly point out the importance of peripheral blood karyotype analysis of infertile couples.

Wider implications of the findings: Cytogenetic analysis in infertile couples may allow an accurate diagnosis: a balanced translocation in one of the couple's members may be responsible for early miscarriages before the woman realizes she is pregnant. On the other hand, reciprocal translocations in men may explain the worst semen parameters, namely, motility and morphology.

Trial registration number: not applicable.

P-063 The natural antioxidant Cinnamtannin B-1 could prevent DNA sperm fragmentation

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Study question: Could Cinnamtannin-B1 prevent DNA sperm fragmentation?

Summary answer: Cinnamtannin B-1 is able to prevent sperm DNA fragmentation after exposure to oxidant factors like time and temperature.

What is known already: Oxidative stress is known to interfere with the fertilization capacity of spermatozoa damaging sperm nuclear DNA and affecting the epigenetic profile of these cells. Antioxidants could improve sperm media, protecting spermatozoa from DNA damage. Cinnamtannin B-1 is a naturally occurring A-type proanthocyanidin found in a limited number of plants including *Linderae umbellateae* and *L. nobilis*, which exhibit a large number of cellular actions mostly derived from their antioxidant properties. The objective of this study is to evaluate if Cinnamtannin B-1 could protect the sperm DNA fragmentation after the oxidative stress produced by incubation at 37°C.

Study design, size, duration: Ninety samples were evaluated from fifteen sperm donors. Sperm samples were collected with an abstinence between 48-72 h and were incubated at 37°C for 4 h with 0, 10 and 100 µM of Cinnamtannin B-1. DNA integrity was checked by measuring the index of sperm DNA fragmentation (DFI), for which TUNEL assay (Terminal dUTP Nick-End Labeling) was used. Since Cinnamtannin B-1 was prepared in ethanol, the control tube received an equivalent volume of ethanol.

Participants/materials, setting, methods: A prospective study was carried out to achieve our objective. Sperm donors were selected according to Instituto Bernabeu sperm donation program requirements and ASRM and ESHRE guidelines

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for gamete donation. After liquefaction, we washed the sperm samples with PBS and performed TUNEL assay in different aliquots. This assay consists in measuring the existing breaks in DNA chain incorporating molecules marked with a fluorochrome. For the statistical analysis we used the SPSSv20.0, using the t-Student test.

Main results and the role of chance: We did not observe significant differences in DFI by adding a small volume of ethanol to the fresh sample required for Cinnamtannin-B1 resuspension. After 4 h incubation, we obtained a significant increase of DFI in the sperm sample without antioxidant (10.27 vs 16.47; $p < 0.05$) suggesting that sperm DNA is damaged after incubation at 37°C. However, when we add Cinnamtannin-B1 to the semen sample at different concentrations, we observed a significant decrease in the DFI when we compared the samples incubated with 10 µM (9.47 ± 1.39) and 100 µM Cinnamtannin-B1 (9.73 ± 1.36) versus control (16.47 ± 2.82) ($p < 0.05$). This results suggest that CinnamtanninB-1 supplementation prevents the increase of DNA damage. In addition, this results also showed that antioxidant concentrations higher that 10 µM do not confer a greater protective effect.

Limitations, reasons for caution: We believe that sample size may be a limitation in this study as well as the use of normozoospermic samples. Further studies should be carried out to verify Cinnamtannin B-1 protective effect in samples with low seminal quality or high fresh sperm DNA fragmentation.

Wider implications of the findings: An interesting topic could be evaluating if addition of Cinnamtannin-B1 in sperm crioprotectors could be useful in sperm cryopreservation where spermatozoa are submitted to oxidative stress. Given the beneficial effect of Cinnamtannin B-1 *in vitro*, its use could also be considered as a feeding supplement especially in infertile men.

Trial registration number: not applicable.

P-064 Reduced ejaculatory abstinence period may improve embryo quality for those with high sperm DNA fragmentation

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Study question: Does reduced ejaculatory abstinence period improve embryo quality in ICSI cycles for those with high sperm DNA fragmentation (SDF) and repeated ART failures?

Summary answer: it seems that reduced ejaculatory abstinence period enhance overall embryo quality without compromising other semen parameters when compared to standard abstinence period

What is known already: Accumulating evidence suggests examination of the SDF particularly for those with repeated miscarriages or ART failures through ART. Despite controversies regarding the optimal cut-off point and methodology, several approaches have been suggested to overcome SDF related drawbacks such as oral intake of antioxidants, use of testicular spermatozoa obtained with either testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) or sperm selection techniques.Yet, none of these interventions, alone or combined, have been unequivocally proven to be of clinical value to overcome the potential detrimental effect of high SDF on ART outcomes.

Study design, size, duration: This is an observational study conducted between 2015 and 2016 in a single center including 24 couples with >2 previous ART cycles, All couples were offered to undergo a new ICSI cycle using spermatozoa obtained by reduced abstinence period (1 hour following the first ejaculation on the day of oocyte retrieval) due to high SDF (>20%). Total of 23 cycles (SHORT) were compared with previous 49 standard cycles of couples (STAND), being their own controls.

Participants/materials, setting, methods: The average frequency of intercourse for the men before producing their first semen specimen for study was between once and twice weekly. Exclusion criteria for men were; azoospermia cases, failure to follow the ejaculatory abstinence protocol, current smoking, presence of varicocele, consumption of anti-oxidants or genital infection within 6 months. All female subjects had normal uterine cavity, hormone levels and karyotype. Poor responders, advanced age (>40 years) and cases with other medical disorders were excluded.

Main results and the role of chance: Total of 73 cycles were evaluated. Only 2 cycles were cancelled due to poor ovarian response and were not included. Blastocysts with ICM, A or B and trophectoderm grade A (i.e. AA or BA) were considered to be top quality. Significantly lower spem count was detected in SHORT group, while significantly higher numbers of top quality blastocyst were detected. Accordingly, 25% implantation and 24% live birth rate was detected in SHORT group despite previous unsuccessful cycles.

Table 1 Demographics.

woman age (yrs)	33.5
infertility duration (yrs)	8.4
No of prev. cycles (N)	2.9
mean AMH (ng/ml)	2.5
mean AFC (N)	12

Table 2 Semen parameters.

	STAND (49)	SHORT (24)	
volume (ml)	2.8	1.3	**0.00**
total count	70.2	27.1	**0.00**
concent. (/ml)	29.8	21.9	NS
mean normal morphology %	4.7	5	NS
motility A+B %	48	53	NS
SDF %	33	-	NA

Table 3 Embryology outcomes.

	STAND (49)	SHORT (24)	
No of MII	8	8.5	NS
fertilization%	67	75	0.07
total No of embryos on day 3	4.7	6.3	**0.03**
No of good quality cleavage embryos	1.3	4	**0.00**
No of top quality blastocyts	0.6	3	**0.00**
No of transferred embryos	1.8	1.9	NS

Limitations, reasons for caution: This study is not a randomized controlled one and the main limitation is the lack of SDF data of SHORT group. Moreover, total antioxidant capacity and reactive oxygen species (ROS) levels are also not available.

Wider implications of the findings: Sperms obtained by reduced ejaculatory abstinence has been shown to reveal low SDF, ROS levels and higher antioxidant capacity. This has been validated even for normozoospermic cases. Therefore, performing ICSI with those spermatozoa seems to be effective in terms of top quality embryos particularly for those with repeated ART failure.

Trial registration number: NA.

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P-065 Lower oxytocin mRNA expression levels are present in samples with abnormal sperm parameters

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Study question: Is oxytocin mRNA expression different between normal samples and samples with at least one abnormal sperm parameter?

Summary answer: Compared to samples with normal sperm parameters, mRNA oxytocin expression is significantly lower in samples with at least one abnormal sperm parameter.

What is known already: Oxytocin is synthesized in the penis, testis, epididymis and prostate, whereas its receptor has been detected throughout the male reproductive tract. Furthermore, oxytocin regulates androgen levels by stimulating the conversion of testosterone to DHT by 5-a reductase. During orgasm, a burst of oxytocin is released into the systematic circulation to stimulate contractions of the reproductive tract. Various studies have shown that exogenous administration of oxytocin increases the spermatozoa concentration of ejaculated sperm. Although oxytocin seminal plasma levels have been shown to be lower in fertile men compared to men with oligoasthnoteratozoospermia, mRNA expression levels have never been assessed until now.

Study design, size, duration: A prospective study was performed in 2016, including a total of 41 samples from equal number of men. All samples had >1*10^6 spermatozoa/ml and <1*10^6 leukocytes/ml. Semen analysis was performed according to WHO 2010 criteria and mRNA was extracted to assess the mRNA expression levels of oxytocin. mRNA expression levels were compared between different categories of sperm samples, classified according to their concentration, motility and morphology (oligozoospermia, asthenozoospermia, oligoasthenozoospermia, teratozoospermia and oligoasthenoteratozoospermia).

Participants/materials, setting, methods: All semen samples were subjected to mRNA extraction, cDNA synthesis and quantitative Real-Time PCR (RT-PCR) for the expression of the oxytocin gene. The relative standard curve method was selected as the most appropriate due to the nature of human spermatozoa. Each sample was run in duplicate and normalized to β2-microtubulin. Differences in oxytocin mRNA expression levels were analyzed between different sperm categories using the generalized linear model. Values are expressed as mean±bootstrap standard error.

Main results and the role of chance: Compared to normal samples, samples with at least one abnormal sperm parameter had significantly lower mRNA oxytocin expression levels (14.73 ± 3.61 vs. 4.32 ± 1.63, p = 0.017). Samples with >60% immotile spermatozoa had significantly lower mRNA oxytocin expression levels compared to samples with normal motility, defined as <60% immotile spermatozoa (0.84 ± 0.39 vs. 10.26 ± 2.86, p = 0.000). Furthermore, asthenozoospermic samples with progressive motility <32% had significantly lower mRNA oxytocin expression levels compared to samples with normal progressive motility, defined as >32% (0.85 ± 0.43 vs. 9.96 ± 2.60, p = 0.000). Oligozoospermic samples with concentration <15*10^6spermatozoa/ml had significantly lower mRNA oxytocin expression levels when compared to samples with normal concentration of spermatozoa, defined as >15*10^6/ml (2.18 ± 1.0 vs. 11.30 ± 3.0, p = 0.004). Samples with >10% rapid progressive spermatozoa (spermatozoa with a speed >25μm/sec at 37° C), compared to samples with <10% rapid progressive spermatozoa, had significantly lower mRNA oxytocin expression levels (11.26 ± 2.92 vs. 2.27 ± 1.22, p = 0.006). mRNA oxytocin expression levels was negatively associated both with the proportion of midpiece defects present in spermatozoa (p < 0.011) and the teratozoospermia index, (TZI >1.8) (p < 0.036).

Limitations, reasons for caution: Given that the current study was not conducted in a large sample group, the results should be interpreted with caution.

Wider implications of the findings: We have shown for the first time that mRNA expression levels of oxytocin are lower in semen samples with at least one abnormal sperm parameter. If the results of the current study are confirmed, oxytocin mRNA expression could be used as a novel diagnostic tool of male infertility.

Trial registration number: Not required.

P-066 Male contribution to repeated ICSI failure

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Study question: Is sperm DNA fragmentation index (DFI) more prevalent in cases of repeated ICSI failures and which risk factors are more associated with abnormal DFI.

Summary answer: DFI was found to be high in cases those have done ICSI before or not. Age and forward sperm motility were found to be significant.

What is known already: A substantial number of studies has focused on the effect of female factor in cases of repeated ICSI failures, however much less number of studies has correlated male factor with repeated ICSI failure. Moreover, routine seminal fluid analysis (SFA) is less reliable in distinguishing between infertile and fertile men. Male chromatin damage has been associated with conception problems and repeated pregnancy loss. Sperm DFI has been found to be more correlated than (SFA) concerning fertilization rates, cleavage rates, blastocyst formation, embryo fragmentation and implantation rates.

Study design, size, duration: We have performed a prospective observational study on DFI for those attending infertility clinic from the beginning of May to the end of December 2016. DFI was observed in 1012 male partners.

Participants/materials, setting, methods: New comers to the infertility unit during the study period have been divided into two groups. Group A those who have done ICSI before. Group B those who have not done ICSI before. Risk assessment profile consistent of age, smoking, varicocele, previous and current SFA parameters, previous miscarriages, IUI, and type of infertility. DFI was evaluated in semen by TUNEL assay. Threshold value was considered to be abnormal if equal or above 19.25.

Main results and the role of chance: DFI was prevalent in both groups being 54.7% in group A and 52% in group B with no significant difference in between groups. No correlation between DFI and smoking, varicocele, previous SFA parameters, previous miscarriages, IUI, number of ICSI failures and type of infertility. There was high correlation between age of the male and DFI abnormality. DFI was significantly elevated for men > 35 years old. Relative to men <30 years old, those >45 years were TWO fold more likely to have abnormal DFI. Relative to men <30 years old, those >45 years were FIVE fold more likely to have repeated ICSI failure because of abnormal DFI. Forward sperm motility at the sample where DFI was performed was correlated to the DFI result.

Limitations, reasons for caution: This is a tertiary referral center with a special program for repeated ART failure cases. Also, Cairo is one of the most air polluted capitals, that might explain the high prevalence of abnormal DFI in both study groups. The study results cannot be extrapolated to different communities with different characteristics.

Wider implications of the findings: DFI was abnormal in those who have done ICSI before or not which indicates that the DFI problem is there from the beginning and ignoring it would continuously fail the subsequent ICSIs.

Trial registration number: N/A.

P-067 A new approach to detected copy number variation in Y-chromosome that affects the spermatogenesis

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Study question: Do CNVs in Y-chromosome variations detected by MLPA affect the spermatogenesis?

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Summary answer: MLPA showed a higher sensibility to detect CNVs, suggesting that the presence of duplications in AZF do not have any significant effect on spermatogenesis

What is known already: CNVs in AZF region of Y-chromosome have been associated with the risk of spermatogenic failure. Detect CNVs using multiplex ligation-dependent probe amplification assay (MLPA) has many advantages over multiplex-PCR technique. MLPA allows the detection of microduplications and deletions in a single assay.

Several research groups have investigated the effect of specific gene copies in AZF region, suggesting that duplications present in this region do not have a significant effect on spermatogenesis, whereas the presence of some types of deletions is a risk factor for male infertility. However, the clinical significance of partial duplications on the spermatogenesis are also in controversy.

Study design, size, duration: A retrospective study was performed. A total of 197 patients with abnormal seminogram (azoospermic, criptozoospermic and oligozoospermic) were included in the study. These patients had been previously Y-chromosome microdeletions tested by multiplex PCR method and any alteration in the CNVs were observed.

In control group, we included 41 normozoospermic healthy donors included in the Semen Donation Program of Instituto Berbabeu, selected on the basis of normal semen parameters according to the WHO criteria (2010).

Participants/materials, setting, methods: MLPA was carried out according to the manufacturer's instructions with the P360-B1 Y-Chromosome Microdeletions Probemix, on the infertility patient and control group. MLPA PCR products were analyzed in a AB310 sequencer. The results were analyzed using the Coffalyser v 1.4 software.

Statistical analysis was performed by SPSS 20.0 using a chi-square. P-values <0.05 were considered significant.

Main results and the role of chance: We observed a 4.6% of microdeletions in the patients group, but no deletions were seen in the control group ($p = 0.179$). However, duplications were seen in both groups (control 14.6% vs patients 7.6%). On the other hand, the percentage of duplications in AZF region was higher in control group than in patients ($p = 0.181$).

Five types of CNVs were identified by MLPA. These 5 CNVs consisted in 2 deletions (gr/gr, b1/b3) and 3 duplications (gr/gr, b1/b3 and b2/b4). Regarding to deletions, only gr/gr (3.6%) and b1/b3 (1%) were detected in patients group. However, due to the samples size no significant differences were found. According to duplications, no significant differences were showed for gr/gr duplications (control 4.9% vs patients 6.1%; $p = 0.805$). However, we obtained significant differences in the frequency of b1/b3 (7.3%-1.5%; $p = 0.036$) and b2/b4 (2.4%-0%; $p = 0.028$) duplication among control and patient samples,

To verify how affect the presence or absence of CNVs the spermatogeneis, we analyzed the frequency of duplications and deletions versus control group and azoospermic, criptozoospermic and oligozoospermic patients. Results showed that the highest deletion frequency (7.7%) was found in azoospermic samples ($p < 0.05$). Nevertheless, the frequency between two other patient group were similar ($p>0.05$) when compared with control samples.

Limitations, reasons for caution: The number of control and patient's samples studied should be higher. Moreover, homogeneous groups to perform the study could show a clear effect of CNVs on the spermatogenesis.

Wider implications of the findings: MLPA is a higher sensitive technique that allows to detect the CNVs in the Y-chromosome, than Multiplex PCR. A more accurate diagnosis could be offer to male factor patients. In addition, our results suggest that duplications could be exert a protective role in the spermatogenic process.

Trial registration number: not applicable.

P-068 Oxidation-reduction potential can help distinguish semen samples under oxidative stress

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Study question: Identify if the sORP marker can be used to identify semen that meet the normal reference range of WHO parameters from those that do not.

Summary answer: Over 90% of semen samples with a sORP 1.23 mV/10^6 sperm/mL[S1] were correctly identified as meeting all the WHO parameters.

What is known already: Discrete measures of free radicals, antioxidant activity, and oxidative damage suggest an ambiguous relationship between the redox system and male fertility. Static oxidation-reduction potential (sORP) measures the balance between all oxidants and antioxidants providing a comprehensive status of the redox system. In previous studies, sORP has been tested in semen samples using the MiOXSYS System as an alternative method for measuring oxidative stress and distinguishing normal controls from male factor infertility patients; thus sORP levels may be used to clarify the relationship between the redox system and semen parameters associated with male infertility.

Study design, size, duration: This prospective study was carried out jointly by the Mediland Hospital,Research Center, and Department of Life Science and Bioinformatics of Assam University on 48 subjects. The study was approved by the institutional ethics committee and subjects were consented prior to participation. Subjects were grouped into those that had all normal semen parameters (concentration, total motility, and morphology) according to WHO 2010 guidelines and those who failed to meet one or more criteria.

Participants/materials, setting, methods: Exclusion criteria included azoospermia, presence of STD or chronic disease, use of prescription, OTC medications or antioxidants. Samples were collected and semen parameters assessed using the WHO 2010 guidelines. sORP was measured(mV) using the MiOXSYS system and normalized to concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration >0.999x10^6 sperm/mL were included.

Main results and the role of chance: The measure of sORP reflects the oxidative relationship between the sperm cell and its environment - the expulsion of oxidants, a by-product of cellular metabolism, into the seminal environment and the deactivation of them by extracellular antioxidants. Thirty one samples failed to meet one or more criteria for semen quality; 17 met all WHO criteria. ORP results were significantly negatively correlated with sperm concentration ($p < 0.01$) and morphology ($p < 0.01$). The area under the curve (AUC) was 0.863. The sORP cut-off value 1.23 mV/10^6 sperm/mL), while excellent at differentiating samples with normal concentration and morphology, was less useful in identifying those according to motility.

Limitations, reasons for caution: The current study was performed at a single fertility clinic. Study enrollment was limited and more cases are needed in order to assess the relationship with other semen parameters.

Wider implications of the findings: Measuring sORP can help a clinician understand if comparatively one semen sample is under higher state of oxidative stress than another.

Trial registration number: not applicable.

P-069 Influence of chromosomal structural rearrangement on sperm parameters: study on 305 male carriers

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Study question: Robertsonian translocations have a deleterious effect on spermatogenesis. For reciprocal translocations, this effect is observed when acrocentric, small metacentric or submetacentric chromosomes are involved.

Summary answer: Robertsonian translocations have a deleterious effect on spermatogenesis. For reciprocal translocations, this effect is observed when acrocentric, small metacentric or submetacentric chromosomes are involved.

What is known already: Chromosomal rearrangements are thought to account for 2-8% of male factor infertility. They stop germ cells maturation or lead to the production of defective spermatozoa. Several studies have analyzed the cytogenetic pattern in infertile male population. But only two studies have compared sperm parameters between the most frequent chromosomal structure rearrangements *ie* Robertsonian (RT) and Reciprocal (RCT) translocations. They reported an impact on spermatogenesis especially for RT but not for

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RCT. The present study aimed to analyze sperm parameters according to the chromosome involved in the structural rearrangement.

Study design, size, duration: This study was conducted retrospectively. We included an overall of 305 couples that benefited from a preimplantation genetic diagnosis (PGD) for a male chromosomal translocation (171 for RCT and 134 for RT) between 2006 and 2015. Sperm parameters on initial macroscopic examination (concentration, motility) and after sperm preparation (number of progressive spermatozoa) were analyzed according to the type of translocation and the chromosomes involved in the rearrangement.

Participants/materials, setting, methods: Our study group was compared to a control group of 120 couples with normal karyotype and proven fertility enrolled in PGD for monogenic disease during the same period. Sperm parameters were assessed according to the WHO 2010 references. For each RCT the number of progressive spermatozoa (NPS) obtained after preparation was plotted on a karyogram. The NPS was considered of poor quality when it was $\leq 1x10^6$. Statistical analyses were performed using GraphPad Prism (ver. 5.02).

Main results and the role of chance: Compared to the control group, the mean sperm concentration and mean progressive sperm motility were significantly reduced in RCT and RT male carriers ($P < 0.0001$). Of note, lower sperm characteristics were observed for RT compared to RCT male carriers ($P < 0.0001$). Compared to the WHO references, 32.7% of RCT and 73.2% of RT male carriers had a sperm concentration below $15x10^6$ per ml. 48.8% of RCT and 78.9% of RT had a progressive sperm motility that was below 32%. The mean NPS obtained after preparation was lower in RT compared to RCT male carriers ($1.6x10^6 \pm 0.3$ vs $12.4 \times 10^6 \pm 1.5$ respectively, $P < 0.0001$). For each RCT male carrier, the NPS obtained after sperm preparation was plotted on an original karyogram. This representation permits us to show that acrocentric, small submetacentric and metacentric chromosomes were more often associated with a NPS$\leq 1x10^6$ ($P = 0.02$).

Limitations, reasons for caution: Data about environmental gonad toxicity are missing in the present study. This may constitute a bias in our analysis.

Wider implications of the findings: Structural chromosomal rearrangement involving acrocentric, small submetacentric and metacentric chromosomes, are more prone to alter spermatogenesis. The practice of aCGH systematically for these patients would be of interest to specify the break point and identify gene candidate involved in spermatogenesis.

Trial registration number: NA.

P-070 High levels of seminal oxidation reduction potential (ORP) in infertile men with clinical varicocele

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Study question: Does seminal oxidation reduction potential (ORP) increases in infertile men clinical varicocele?

Summary answer: Seminal ORP is significantly higher in infertile men with clinical varicocele as compared to those with no detectable abnormality in their genital examination (idiopathic infertility).

What is known already: Varicocele is found in approximately 40% of men presenting with primary infertility and up to 80% of secondary infertility. Seminal oxidative stress (OS) plays an important role in the pathogenesis of varicocele-mediated male infertility through different mechanisms. Oxidation reduction potential (ORP) is a measure of the balance between oxidants and antioxidants that provides a comprehensive analysis of the redox system. Measurement of ORP, directly in the neat semen, has been recently shown as a simple, rapid and reliable test seminal OS.

Study design, size, duration: A prospective controlled pilot study was conducted on 36 men seeking medical advice for an infertility problem between October, 2016 and January, 2017. Following clinical examination, and scrotal color Doppler ultrasound, infertile men were divided into two groups. Group1: infertile men with clinical varicocele; and group 2: infertile men with idiopathic infertility (no detectable abnormality in their genital examination.

Participants/materials, setting, methods: Patients were examined in an infertility center by the same Andrologist. Semen analysis was performed according to the WHO manual (2010). Seminal ORP was measured using the MiOXSYS system (Aytu BioScience, Inc., Englewood, CO, USA). Absolute values of ORP, in millivolts (mV), were normalized for sperm concentrations and results were expressed as $mV/10^6$ sperm/mL. Quantitative measures were presented as median and inter-quartile range (25th and 75th centiles). P value less than 0.05 was considered significant.

Main results and the role of chance: The study included 17 patients in group 1 and 19 in group 2. Patient's age and duration of infertility in group 1 [32 (30, 34) years] & [4 (1.5, 5) years] were not significantly different from group2 [33 (30, 36.5) years] & [2 (1.25, 3.5) years] (P values = 0.64 & 0.27; respectively). Patients in group 1 had significantly lower sperm concentrations [20 (5, 30) $\times 10^6$ /mL], and normal forms [3 (2, 4) %] as compared to those in group 2 [30 (20, 72.5 $\times 10^6$ /mL]; and [4 (3, 5) %] (P values = 0.03 & 0.005; respectively). Levels of seminal ORP were significantly higher in group 1 [3.04 (1.94, 7.75) $mV/10^6$ sperm/mL] as compared to group 2 [1.12 (0.42, 2.25) $mV/10^6$ sperm/mL]. A significant inverse correlation was found between ORP and sperm concentration ($r = -0.531$, $p = 0.001$), total sperm count ($r = -0.453$, $p = 0.008$), progressive motility ($r = -0.460$, $p = 0.007$), total motility ($r = -0.526$, $p = 0.002$) and normal forms ($r = -0.597$, $p < 0.001$). A significant positive correlation was found between ORP and seminal leukocytes ($r = 0.476$, $p = 0.003$).

Limitations, reasons for caution: The sample size was relatively small and semen samples from fertile men, with and without varicocele, were not included.

Wider implications of the findings: These findings may have important diagnostic and therapeutic implications. Further studies are warranted to explore the mechanism of increased ORP in a subset of infertile men with clinical varicocele. In addition, future studies may help determine those patients who would benefit from antioxidant therapy and/or surgical repair of varicocele.

Trial registration number: not applicable.

P-071 New phenotype of total Globozoospermia: nuclear sperm quality and molecular investigation

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Study question: *SPATA16* defects could influence the meiosis process besides its crucial role in acrosome formation and lead to a new phenotype of total globozoospermia?

Summary answer: Particular defects of round-headed spermatozoa (double/multiple round-headed and multi-tailed) due to an abnormal meiosis were detected in total globozoospermic patients showing *SPATA16* mutation.

What is known already: Total globozoospermia is a rare but severe form of teratozoospermia. Lack of acrosome and round-headed spermatozoa are its main characteristics. Three genes (*DPY19L2*, *SPATA16* and *PICK1*) are known to be associated to total globozoospermia. Recently a new *SPATA16* mutation was identified in Tunisian patients. It was reported in the literature that alterations in *SPATA16* gene influence the acrosome formation during spermiogenesis, whereas the meiosis was not disturbed. On the other hand, chromatin abnormalities were associated to round-headed spermatozoa, while the studies of sperm aneuploidy and DNA fragmentation were controversial.

Study design, size, duration: This is a case control study carried out on three unrelated Tunisian globozoospermic patients and 20 fertile men with normal semen profiles included as a control group. Patients were recruited between April 2015 and January 2017.

Participants/materials, setting, methods: Chromatin condensation was assessed by aniline-blue Staining. Fluorescence *in situ* hybridization for chromosomes X, Y and 18 was performed to study the chromosomal meiotic segregation. Sperm DNA fragmentation was evaluated by terminal desoxynucleotidyl transferase mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) assay. We performed PCRs and Sanger sequencing for mutations screening of *DPY19L2*, *SPATA16* and *PICK1* associated to globozoospermia.

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Main results and the role of chance: Sperm analysis for the three globozoospermic patients showed a total sperm immobility (akinetozoospermia) and a severe oligozoospermia. Besides the presence of 100% round-headed acrosomeless spermatozoa, a predominance of double or multiple round-headed (between 17% and 42.5%) and multi-tailed (between 14.5% and 53.5%) spermatozoa were observed. To the best of our knowledge, this is the first description of this particular morphological sperm defects frequently observed for these three total globozoospermic Tunisian patients. The mean DNA fragmentation index was significantly higher in patients compared to the control group ($P < 0.001$; 46.67 ± 11.93% VS 10.25 ± 3.83%). The total aneuploidy rate was considerably higher in patients compared to controls ($P < 0.01$) with a significant increase in the frequency of disomy for chromosomes X, Y and XY ($P < 0.05$). The rate of spermatozoa with abnormal nuclear chromatin condensation was significantly higher in patients compared to the control group (43.66 ± 10.67% VS 12.7 ± 6%, $p < 0.05$). For the molecular analysis, two patients were found to be homozygous for the new *SPATA16* exon 2 deletion. Whereas, no mutation was identified for the third one.

Limitations, reasons for caution: A limitation of this study is the low number of patients considering the rarity of this syndrome and the novelty of the phenotype observed.

Wider implications of the findings: We confirm that defects in *SPATA16* could be associated to an abnormal meiosis leading to a new particular morphological sperm defects in total globozoospermia. Phenotype examination, cytogenetic and molecular characterization can orientate the selection of the best treatment options and management of these infertile couples.

Trial registration number: not applicable.

P-072 Possible involvement of interleukin-34 in the development of spermatogenesis

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Study question: Dose testicular cells produce interleukin-34 under normal conditions? Is its affected by age?

Summary answer: Sertoli, Leydig, peritubular cells, macrophages, premeiotic and meiotic cells produced it. Its levels were age-dependent in testicular homogenates (the highest levels in the younger age).

What is known already: Interleukin-34 (IL-34) is a protein produced mainly by neurons and keratinocytes. It is the ligand for colony stimulating factor-1 receptor (CSF-1 R).

IL-34 and CSF-1 are secreted proteins and have great structural resemblance. They compete on binding to the same site on CSF-1 R, but IL-34 binds stronger.

In mice with a mutated CSF-1 R receptor, CSF-1 has been found as the primary factor in development of the male and female reproductive system. In addition, CSF-1 R is expressed on spermatogonial stem cells, and CSF-1 was demonstrated to induce spermatogonial cell proliferation.

Study design, size, duration: Sexually immature ICR mice (1, 2 weeks old) and mature mice (3, 4, 8, 12 weeks old) were sacrificed, and the testes were collected and homogenized to quantify IL-34.

Participants/materials, setting, methods: The expression levels of IL-34 and CSF-1 R were evaluated by qPCR analysis using specific primers. Four micron thick sections from Bouin's fixed, paraffin-embedded testicular tissue and isolated cells of immature and mature mice were used for localization of IL-34 by immunofluorescence staining (IF) using specific antibodies.

Main results and the role of chance: Our results show the localization of IL-34 in different testicular cells such as tubular cells [germ cells (GC), meiotic/postmeiotic cells (MC/PM)], peritubular cells (PC) and interstitial cells (ISC) in all examined ages. IL-34 was also appeared in the branches (Bra) of Sertoli cells in testis of 4-weeks-old mice.

High expression of IL-34 was examined in testicular homogenates of 1-2 – weeks-old mice. This expression was significantly decreased with age starting from 2 weeks-old compared to 1-2 week-old mice ($p< 0.05$). The expression of CSF-1 R (IL-34 binding receptor) was basal in testicular homogenates of 1 and 2 week-old mice but significantly increased in testicular homogenates of 4 week-old mice ($p< 0.05$) and significantly decreased in testicular homogenates of 8 and 12 week-old mice compared to 4 week-old mice ($p< 0.05$) to reach the levels of 1 and 2 week-old mice. Using double IF staining we could show that Leydig cells, VASA (spermatogonial cells) and CREM-1 cells (meiotic cells) and macrophages also produce IL-34. Our results also showed the localization of IL-34 in the cytoplasm of Sertoli cell cultures, and over 2 weeks of age IL-34 began appearing in the nucleus as well. Over 4 weeks of age IL-34 appeared in Sertoli cell branches.

Limitations, reasons for caution: The study was performed in mouse model and the correlation with human should be considered. The specific function of IL-34 in the testis needs to be elucidate.

Wider implications of the findings: This is the first study that shows the expression of IL-34 in testicular tissue and cells, and the possibility to be under hormonal regulation.

Thus, it is possible to suggest that IL-34 could be involved in the regulation of spermatogenesis development, since its receptor was demonstrated in spermatogonial cells.

Trial registration number: NA.

P-073 Intervention to reduce sperm DNA damage prior to fertility treatment

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Study question: Can changing various factors such as diet, exercise, smoking, or intake of antioxidants help to reduce the level of sperm DNA damage?

Summary answer: The average index for sperm DNA damage (DFI) was reduced significantly from 34.1 to 25.7 ($P < 0.0001$) for the 96 men included in this study.

What is known already: Sperm DNA damage negatively affects the outcome of ART, including IVF and ICSI treatments. Factors such as tobacco smoking or metabolic syndrome are among the more common causes of sperm DNA damage. Furthermore, it has been shown that oral intake of antioxidants may reduce sperm DNA damage. However, DNA damage is multifactorial and many factors currently remain unknown. The overall aim of the study was to determine if sperm DNA damage could be reduced and identify the factors leading to such improvement.

Study design, size, duration: Sperm DNA damage was tested in 2287 male patients. The DFI was above 15 for 1178 patients (51.5%), who were then invited to participate. In total, 96 patients were enrolled in the study with interventions individually designed during consultations between physician and patient. The DFI was re-tested after a recommended period of 3 to 9 months. All changes implemented by the patient were recorded using an online self-assessment questionnaire.

Participants/materials, setting, methods: Patients were enrolled at 2 fertility clinics. DFI was determined by a diagnostic laboratory using the sperm DNA integrity test (SDI-test), modified from Evenson & Jost (Current Protocols In Flow Cytometry 2000;7,13:1–27). Data from all 96 men before and after intervention was analysed using a paired t-test as well as the bootstrap method. Changes made by individuals were highlighted using the intra-individual variation of the SDI-test (estimated from control-group data of a previous RCT).

Main results and the role of chance: The overall DFI was reduced significantly from 34.1 to 25.7 (8.4 percentage units, $P < 0.0001$). This reduction in

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DFI was determined by the paired t-test and was confirmed by the bootstrap method. Intra-individual variation (SD_W) of the SDI-test was determined using double-blinded control-group data from a previous RCT. Using the SD_W (=6.36%), 27 men were determined to have improved their DFI significantly (on average from 40.7 to 19.8). One man, however, had increased his DFI significantly from 59.4 to 87.6. The factors implemented by the 27 men were related to dietary changes to reduce possible insulin resistance: Reductions in meat intake (13/27), white bread (13/27), soft drinks (13/27) and alcohol (24/27). In addition, an increased fruit and vegetable intake was reported (15/27), together with increased exercise levels (14/27). Out of the 27 men, 15 reported losing weight. Three men (3/27) reported stopping smoking. Twenty men (20/27) reported starting to take antioxidants. Nine men (9/27) reported taking medication, and one man, who had stopped taking a SSRI medication, achieved the highest reduction in DFI (from 92.0 to 34.1). The man who increased his DFI significantly had taken a high dosage of various antioxidants and was confirmed to have iron intoxication.

Limitations, reasons for caution: This prospective study is based on 96 men with an initial DFI of above 15. For most men, several factors were implemented. It should be noted that some factors, i.e. smoking, may not result in a high DFI unless other factors, i.e. lack of antioxidants, are also involved.

Wider implications of the findings: Reduction of sperm DNA damage before ART is desirable as it may increase treatment success rates. Furthermore, such intervention may also have a positive effect on general male health as DNA damage in sperm is often linked to increased DNA damage in somatic cells.

Trial registration number: None.

P-074 Expression of pigment epithelium derived factor PEDF in mouse testicular tissue; possible involvement in testicular functions

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Study question: Dos PEDF express in testicular cells under physiological conditions? and is it under hormonal regulation?

Summary answer: PEDF expressed by Sertoli, peritubular, Leydig cells and macrophages. The levels of PEDF in testicular homogenates were significantly decreased with age, suggesting hormonal regulation.

What is known already: Pigment epithelium derived factor (PEDF) is released as a soluble, monomeric glycoprotein, encoded by SERPINE-1 gene. The PEDF is known as an anti-angiogenic factor. In endothelial cells, PEDF promotes apoptosis in new vessels and exerts anti-tumor properties. Spermatogenesis occurs in the seminiferous tubules of the testis, which are avascular. However, there are blood vessels in the interstitial tissue close to the seminiferous tubules wall. Recently, it has been shown that cultures of human testicular peritubular cells constitutively secrete PEDF, and suggested that PEDF may prevent vascularization of human seminiferous tubules.

Study design, size, duration: Sexually immature (1, 2 weeks old) and mature (4, 8, 12 weeks old) ICR mice were sacrificed, and their testis were collected for histological examination and/or homogenized to quantify PEDF by qPCR. Some testes were also used to establish Sertoli and peritubular cell cultures and for Leydig cell isolation.

Participants/materials, setting, methods: Testes of mice from different ages were used for RNA extraction, histological evaluation and immunostaining and for establishment of primary cultures for Sertoli and peritubular cells. The expression levels of PEDF was examined by qPCR analysis, and its cellular localization in the testis and Sertoli and peritubulae cultures and in isolated Leydig cells and macrophages was examined by double immunofluorescence staining using specific antibodies for PEDF and specific marker for each examined testicular cell type.

Main results and the role of chance: In testicular sections, PEDF was stained in germ cells, peritubular cells, Sertoli cells, and interstitial cells in both mature and immature mice. In addition, meiotic/postmeiotic cells were also stained in seminiferous tubules of mature mice. High expression levels of PEDF were detected in testicular homogenates of 1-2 weeks-old mice. This expression was significantly decreased with age starting from 4 weeks-old compared to 1 week-old mice. Double immunoflurescence staing showed specific PEDF staining in isolated Leydig cells and macrophages. In addition double immunoflurescence staing showed specific PEDF staining in the cytoplasm of established Sertoli and peritubular primary cell cultures(isolated from mice of different ages).

Limitations, reasons for caution: The study was performed in mouse model and the correlation with human should be considered. The specific role of PEDF in the different cell type of the testis need to be clarified.

Wider implications of the findings: This is the first study to show that mouse peritubular cells, Sertoli cells, Leydig cells and macrophages constitutively produce PEDF; and the possibility to be under hormonal regulation. We suggest that PEDF could be involved in testicular physiological function (spermatogenesis, seminiferous tubule avascularization, testicular immune privilege site)

Trial registration number: NA.

P-075 Biological properties of mouse spermatozoa prepared with the microfluidic sperm sorter

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Study question: The purpose of this study was to investigate the biological properties and the developmental ability of spermatozoa prepared by the microfluidic sperm sorter (MFSS).

Summary answer: In this study, we demonstrated that spermatozoa with high sperm motility and developmental ability were able to separate by using the MFSS.

What is known already: The microfluidic sperm sorter (MFSS) is a novel device that is able to separate spermatozoa with high motility and low DNA fragmentation from raw semen without washing and centrifugation process. However, some biological properties and effect on embryonic development of spermatozoa separated by the MFSS are as yet unexplained.

Study design, size, duration: Spermatozoa obtained from cauda epidetimis of C57Bl/6J mice were divided into two aliquots. One aliquot was processed by the MFSS and separated into high-motility (HM) and low-motility (LM) spermatozoa; the second aliquot was processed by the swim-up procedure (SU). Motility parameters and mitochondrial membrane potential (MMP) were studied. Fertilization and blastocyst rate were compared between embryos with spermatozoa separated by MFSS and those with spermatozoa processed by SU.

Participants/materials, setting, methods: Sperm motility parameters were analyzed with the sperm motility analyzing system (SMAS). MMP was evaluated with the flow cytometer after JC-1 staining. In addition, spermatozoa of each group were injected into ovulated oocytes derived from BDF1 female mice. After 3.5 hours from ICSI, a part of fertilized oocytes derived from each group was stained immunohistochemically with gamma H2AX antibody to detect DNA damage, and the others were cultured until to the blastocyst stage.

Main results and the role of chance: By the SMAS analysis, the motile sperm rate was 90.5%, 47.8%, and 89.5% for HM, LM, and SU, respectively. The average speed of HM was significantly higher than that of LM and SU (HM: 116.8 ± 8.6 μm/sec, LM: 68.2 ± 8.2, SU: 83.1 ± 4.1, $p < 0.05$). MMP of spermatozoa in HM and SU were significantly higher than that of LM. After ICSI, there was no significant difference in the rate of pronuclear formation between each group. By immunohistochemical staining, high positive rate of gamma H2AX was observed in male pronucleus of embryos with LM spermatozoa. In addition, the blastocyst rate of embryos with HM was significantly higher than that of LM and was comparable to that of SU (HM: 84.3%, LM: 56.1%, SU: 79.7%, $p < 0.05$).

Limitations, reasons for caution: This study was conducted using a mouse model, and this finding does not directly represent human infertility.

Wider implications of the findings: This study shows clearly that the MFSS could separate more functional spermatozoa for fertilization and development

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compared with swim-up procedure. In addition, by optimizing the MFSS system, the most appropriate spermatozoa for IVF/ICSI would be provided efficiently.

Trial registration number: Not applicable.

P-076 Effect of ejaculatory abstinence period on sperm DNA fragmentation and fertilization

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Study question: Does ejaculatory abstinence period (EAP) have effect on sperm DNA fragmentation and fertilization?

Summary answer: Longer EAP is correlated with serial increase of sperm DNA fragmentation. EAP above 7 days has negative effect on fertilization rate in conventional IVF cycles.

What is known already: In many clinics, male partners undergoing IVF cycles were advised to sexually abstain for 2 to 7 days prior to semen collection for ART. However, there is currently little evidence to support such advice. This advice may be based on World Health Organization guideline recommending abstinence for 2-7 days before semen collection for evaluation of infertility. Prolonged ejaculation abstinence period maximizes sperm concentration in the ejaculate. However, with long abstinence, sperm could be damaged by oxidative stress. In some study, increased EAP has positive correlation with sperm DNA fragmentation.

Study design, size, duration: This retrospective cohort study included 836 couples undergoing IVF cycles between January 2015 and December 2016.

Participants/materials, setting, methods: On the day of oocyte aspiration, semen collection was done. Sperm DNA fragmentation was examined by sperm chromatin dispersion test (SCD). We divided the couples into three groups by abstinence interval.

GI: interval ≤ 4 days
GII: 5 days ≤ interval ≤ 7 days
GIII: interval ≥ 8 days

Fertilization rates and pregnancy rates of each group were compared in conventional IVF cycles and ICSI cycles.

Main results and the role of chance: In each group, the age of male and female partner and the number of oocytes did not show significant differences. Sperm DNA fragmentation index(DFI) of GI, GII, GIII were 16.6%, 19.3%, 22.1%. If abstinence interval was increased, sperm DNA fragmentation index (DFI) was also significantly increased[GIvs. GII, P = 0.002; GIvs. GIII, P = 0.001; GIIvs. GIII, P = 0.018]. Fertilization rates of GI, GII, GIII in conventional IVF cycles were 53.7%, 58.8%, 47.8%. Fertilization rate of group C was significantly lower than that of group B[P = 0.004]. There were no significant differences among pregnancy rates of three groups in conventional IVF cycles[47.8%(65/136) vs. 51.3%(41/80) vs. 50.0%(20/40), P=NS]. Fertilization rates of GI, GII, GIII in ICSI cycles were 60.2%, 57.2%, 67.3%. They did not show significant differences. Pregnancy rates in ICSI cycles were similar among three groups[45.7%(134/293) vs. 42.6%(69/162) vs. 46.4%(58/125), P=NS].

Limitations, reasons for caution: This is retrospective study. EAP was recorded by memory of male partner. It could cause some recall bias.

Wider implications of the findings: Increased EAP is correlated with high sperm DNA fragmentation. Prolonged EAP above 7 days has negative effect on fertilization rate in conventional IVF cycle. However, in ICSI cycle, fertilization was not affected. Shorter EAP is advisable and if abstinence period is increased, fertilization by ICSI could minimize harmful effect.

Trial registration number: None.

P-077 Does weight loss improve fertility with respect to semen parameters and reproductive hormones? results from a large cohort study

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Study question: Does weight loss improve fertility potential of over weight and obese men?

Summary answer: weight loss has positive impact on major fertility parameters suggestive of improved fertility potential. Weight loss must be considered part of infertility management.

What is known already: while much is known about obesity and its impact on fertility among females, there is little evidence about male obesity and its impact on fertility. Previous studies have shown that normal weight men have better fertility potential compared to obese men. However, there is no data on weight loss by natural means except one. There is a need to understand a better management of fertility issues among obese men.

Study design, size, duration: This study used a prospective interventional design. A total of 105 participants were recruited after initial screening according to our inclusion criteria for the study. Data was prospectively collected between April 2012 and May 2015

Participants/materials, setting, methods: Obese men aged 25 to 40 years (Mean age = 32.5 ± 7.5) with body mass index (BMI) more than 25 kg/m^2 were recruited for the study. The subjects underwent a weight loss intervention and were followed up for one year post intervention. Participants were recruited from infertility center as well as fitness centers around the clinic set up. Participants were advised for weight loss through diet counseling and exercise.

Main results and the role of chance: The subjects underwent a weight loss intervention and were followed up for one year post intervention. We performed blood tests, including the serum sex hormone levels, and conventional and computer-assisted semen analyses. The sperm DNA fragmentation (SDF) was evaluated using the sperm chromatin dispersion test (SCDt) to determine the SDF index (SDFI). The fertility parameters were compared before and after weight loss. The weight loss increased the testosterone to 35.40 ± 20.51 ng/ml compared with 27.16 ± 20.71 ng/ml, and sex hormone binding globulin (SHBG) to 23.72 ± 9.01 µg/dl compared with 19.18 ± 10.44 µg/dl, while as follicle stimulating hormone (FSH) and luteinizing hormone (LH) were non-significant. Weight loss also showed significant changes in progressive motile sperm from 29.35 ± 21.91% to 41.06 ± 24.40%, static sperm from 53.70 ± 24.27% to 39.97 ± 22.50% and no significant change in other semen parameters. Weight loss also decreased the SDFI to 17.49 ± 8.28% compared with 20.21 ± 9.31%.

Limitations, reasons for caution: Only a single measure of semen analysis and hormone assay was performed. Variations in the semen parameters may provide inadequate information. Despite the weight loss many participants still remained obese.

Wider implications of the findings: Considering the well established association between male obesity and altered reproductive hormonal profile, especially testosterone which is required in large concentrations to maintain spermatogenesis, it is reasonable to consider obesity to also affect semen quality. Weight loss should be considered in the management of infertility especially in obese male partner.

Trial registration number: Not applicable.

P-078 Does sperm origin affect embryo morphokinetics parameters?

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Study question: Does sperm origin, testicular or ejaculated, interfere with embryo morphokinetic development?

Summary answer: Embryos derived from testicular spermatozoa showed earlier second polar body (II-PB) extrusion and 4-cells stage while later 9-cells stage, compared to ejaculated normal sperm.

What is known already: It is well known that ejaculated and testicular sperm differ in the degree of nuclear maturation. During spermiogenesis, the transit of spermatozoa in the epididymal tract favors DNA packaging by stabilizing the chromatin structure through protamine dephosphorilation and the formation of molecular bridges. Additional difference in sperm maturity may involve the centriols, structures implicated in embryo cleavage. Based on these observations,

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sperm maturity can affect the timing of fertilization and later cellular divisions during embryo development.

Study design, size, duration: In this retrospective observational study (October 2014-July 2016), we analyzed the developmental kinetics of embryos obtained either with testicular sperm (N = 70 embryos; 21 cycles; TS-group) or with thawed donated sperm (N = 109 embryos; 22 cycles; DS-group). Only correctly fertilized (2PN) oocytes were studied. As expected, the fertilization rate was higher in DS (137/215=63,5%) than TS (239/457=52,3%) groups (p = 0.0060). The developmental markers observed were: II-PB extrusion, pronuclei (2PN) appearance, 2PN fading, divisions at 2 to 9- cells.

Participants/materials, setting, methods: Embryo development was analyzed with time lapse technology. Development timings were recorded for all embryos (TS-group: female mean age=34.47 ± 4.66; DS-group: female mean age=34 ± 4.5). Anova Test was used for statistical analyses. Average hour±SD from ICSI insemination are reported. Donor's age ranged between 18 and 40 years old; sperm parameters for donors recruitment were, on fresh sperm, > 80 million/ml with >50% progressive motility and >4% morphology and, after density gradient, concentration>8 million/ml, progressive motility>50% and morphology>4% (WHO2010).

Main results and the role of chance: In TS versus DS groups respectively, II-PB was extruded at 3.63 ± 1.53 h (N = 239) and 4.04 ± 1.65 h (N = 137) (p = 0.0147); 2PN appeared at 10.17 ± 3 h (N = 239) and 10.29 ± 3.16 h (N = 137) (NS); 2PN disappeared at 25.53 ± 5.75 h (N = 239) and 26.26 ± 4.67 h (N = 137) (NS); cleavage at 2-cells was at 28.85 ± 5.82 h (N = 204) and 29.85 ± 5.68 h (N = 110) (NS); 3-cells at 38.69 ± 8.56 h (N = 139) and 40.3 ± 8.07 h (N = 86) (NS); 4-cells at 41.69 ± 8.81 h (N = 201) and 43.98 ± 10.46 h (N = 106) (p = 0.049); 5-cells at 49.99 ± 10.70 h (N = 160) and 52.19 ± 11.27 h (N = 83) (NS); 6-cells at 54.72 ± 10.85 h (N = 148) and 54.56 ± 8.03 h (N = 71) (NS); 7-cells 57.41 ± 10.61 h (N = 128) and 56.35 ± 7.06 h (N = 64) (NS); 8-cells 62.44 ± 13.12 h (N = 137) and 60.80 ± 6.16 h (N = 55) (NS); 9+-cells at 70.85 ± 14.86 (N = 94) and 65.04 ± 5.55 (N = 28) (p = 0.0005). In brackets numbers of embryos at each specific developmental stage are reported. Data on embryos are relative to cultures until day-3. We observed a significant anticipation in the time of II-PB extrusion in TS-group compared to DS-group. Also the time at which embryos reached the 4-cells stage was anticipated in the TS-group as compared to the DS-group. Conversely a highly significant delay in the timing of the 9 cells-stage was observed in the TS-group.

Limitations, reasons for caution: A limitation of our study is the use of cryopreserved sperm as control, whereas our study group included both cryopreserved or fresh sperm. An additional limitation is the small population size due to the fact that we have started using donor-sperm only after the Constitutional Court Sentence 162/2014 restoring gamete-donation.

Wider implications of the findings: An early paternal effect could be visible after ICSI (II-PB extrusion) and in early cellular divisions (4-cells). A later paternal effect could lead to the retardation seen at 9-cells in the TS-gorup. Morphokinetic parameters might give predictive information for embryo development in relation to the type of sperm used.

Trial registration number: Not applicable.

P-079 Which sperm source and transferred embryo has the best artifical reproductive techniques (ART) outcomes in Klinefelter syndrome (KS)

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Study question: Are there any differences between fresh and thawed testicular spermatozoa, fresh and frozen embryo transfers (FET) over the ART outcomes in KS.

Summary answer: Although fresh testicular sperm usage had high ART success rate than thawed spermatozoa for KS patients, no difference had been observed between fresh and FET.

What is known already: Klinefelter syndrome is the most common sex chromosomal disorder among infertile males. It is now well accepted that new developments in ART have made paternity possible in these cases. Although most of the KS men have azospermia, Mic-TeSe provides high sperm recovery with 35 and 45% success rate. In the literature, while it was reported that fertilization rate was lower in KS cases than other azoospermic male, pregnancy and live birth rates were not different in KS groups than others. However, there was limited knowledge about ART results with cryopreserved–thawed spermatozoa in KS cases.

Study design, size, duration: A total of 133 fresh ET and 24 FET cycles in 206 KS patients were evaluated between 2002- 2016. Fresh or thawed testicular and ejaculated spermatozoa were used for ART. The demographics data, embryological parameters such as total number of retrieved oocytes (COC) and metaphase II oocytes (MII), fertilization (PN), implantation, clinical pregnancy and live birth rates were compared according to the source of spermatozoa. Moreover, fresh and FET cycles were evaluated.

Participants/materials, setting, methods: KS cases were divided into three groups according to sperm source as fresh testicular, ejaculated and cryopreserved-thawed spermatozoa, and all parameters compared among these groups. Additionally, patients' age, clinical pregnancy rate and live birth rates were compared in fresh and frozen-thawed ET cases. Kruskall-Wallis test, Mann-Whitney U test and Pearson Chi-square test were used to determine the independent parameters.

Main results and the role of chance: While 26 (9.4%) of 206 patients had mosaic KS, the rest of them were pure KS cases. 203 out of 206 KS patients underwent to micro-TeSe. Spermatozoa were found in 88 patients (43.3%). Testicular sperm aspiration was performed only in 2 patients. Ejaculated spermatozoa were used in 16 cases, and 13 (81.3%) had mosaic karyotype. Thawed testicular sperms were used in 29 cycles.

Mean age of men displayed statistical difference between groups (p= 0.015). Moreover, mean age of women, duration of infertility, COC, MII and PN rates were similar between groups (p= 0.530, 0.031, 0.03, 0.018, 0.052, respectively). Implantation, clinical pregnancy and live birth rates were significantly different between groups (p= 0.001) and all were at the highest value in favor to ejaculated spermatozoa. This statistical difference is arising from mosaic cases in which ejaculated spermatozoa were used cycles and all were mosaic KS. Clinical pregnancy and live birth rates are significantly higher in fresh testicular spermatozoa compared to thaw testicular spermatozoa cycles among pure KS patients (p= 0.012).

Pregnancy outcomes were evaluated between fresh ET and FET cycles. Demographic data and ART outcomes did not display statistically difference between groups (p>0.05).

Limitations, reasons for caution: The distributions of the participants are not equal in groups. Furthermore, predictive parameters for spermatozoa retrieval rate were not considered in the study.

Wider implications of the findings: We plan using fresh micro-TeSe at the same day with oocyte pick-up and if we are able to retrieve mature spermatozoa for fertilization and generate suitable embryos for transfer, then we can adjust the type of transfer according to the patients situation in KS patients.

Trial registration number: None.

P-080 Effect of Paternal Ethnicity on Live Birth Rates after IVF/ICSI Treatment: Analysis of a National Database

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Study question: What are the potential effects of paternal ethnicity on IVF/ICSI clinical outcome?

Summary answer: Biochemical pregnancy and live birth rates following a single IVF cycle were significantly higher amongst couples with Chinese and white European male partners.

What is known already: In the literature, a limited number of powered studies were published on the relationship between ethnic background and IVF/ICSI treatment outcome, and they are generally focused on the ethnicity of the

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female patient only. Very limited information is known about the paternal patient's ethnicity and its effects on clinical success rates.

Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of IVF and ICSI treatment in the UK. Data from 2000 to 2010 involving 47,500 treatment cycles of White British female patients, of which 28,488 patients were analysed during their first cycle. The data has been analysed retrospectively to determine the differences in IVF outcome amongst patients whose male partner is of different ethnicity.

Participants/materials, setting, methods: Data on all men whose partners is White British and undergoing their first stimulated fresh IVF treatment cycle during the period from 2000 to 2010 were analysed primarily for live birth rate per cycle and cumulative live birth rate. Data analysed after adjusting for age, cause and type of infertility and treatment type (IVF or ICSI) to express results as odds ratio and 95% confidence intervals.

Main results and the role of chance: Partners of White British female patients who are White European (2.06; 0.90 – 4.72) or Chinese (3.56; 0.94 – 13.50) had a significantly higher odds of live birth rate per fresh IVF/ICSI cycle than White British partners, while partner of mixed background (0.55; 0.35 - 0.85) had a significantly lower odds, all other ethnicities had equivalent live birth rates; White Irish (1.24; 0.81 - 1.88), Mediterranean (0.98; 0.48 – 2.01), SA Indian (0.60; 0.26 – 1.41), SA Pakistani (0.71; 0.20 – 2.58), Black African (0.87; 0.36 – 2.1), Black Caribbean (0.59; 0.22 – 1.60), and Black British (0.84; 0.40 – 1.75). The cumulative live birth rates also showed similar pattern across different ethnic groups.

Limitations, reasons for caution: Controlling for confounders like women's BMI, smoking status, treatment protocol and gonadotrophin dose could not be done because these data were not available.

Wider implications of the findings: Ethnicity should be considered while counselling couples about their realistic chances of IVF/ ICSI success. Further research is needed to understand the reasons behind the variation in treatment outcome between ethnic groups which can potentially lead to a more tailored treatment protocols so IVF/ ICSI success rate is maximised.

Trial registration number: Not applicable.

P-081 Combined treatment with inositol and alpha-lipoic acid of dispermic patients significantly improves semen parameters

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Study question: Does combined treatment with inositol and alpha-lipoic acid of dispermic patients improve semen parameters?

Summary answer: For the first time, we show that the combined treatment with inositol and alpha-lipoic acid is effective in treatment of infertile dispermic patients.

What is known already: Reduction in motility and number of spermatozoa and change in their morphology are some of the most relevant causes of infertility in men. One of the factors which may influence male infertility is the production of reactive oxygen species (ROS), since these chemical compounds can affect motility, morphology and DNA stability of spermatozoa. Research over the past decade has demonstrated that the pro-inflammatory state seen in metabolic dysfunction and the subsequent development of oxidative stress may provide a pathophysiologic explanation for the direct effects on normal spermatogenesis and sperm function.

Study design, size, duration: This study included 78 infertile men, 18-55 years aged, no-smokers, with a diagnosis of dispermia due to one or more altered semen parameters, without any testicular pathologies, with a normal endocrinological/metabolic profile (HOMA Index <2.5; testosterone, prolactin, FSH, LH normal serum values), and no concomitant consumption of drugs. Patients signed an informed consent to the enrollment in the study. Semen analysis was performed before (T0) and after 90 days (T90) of treatment and results were compared.

Participants/materials, setting, methods: Diet of patients was supplemented with alpha lipoic acid, myo-inositol, folic acid, vitamin B2, B6, B12, and betaine (2 tablets/day of Sinopol®, Laborest, Italy). At T0 and T90 we evaluated liquefaction, semen viscosity, volume, pH; sperm motility, vitality and numbers; concentration of round cells; sperm morphology. Mann-Whitney test was performed using MedCalc® software (Mariakerke, Belgium), in order to investigate differences between semen parameters before and after the treatment. Results were considered statistically significant at $P < 0.05$.

Main results and the role of chance: Analyses of data showed an improvement of semen quality after 90 days of treatments, in terms of a statistically significant increase of sperm concentration (percentage increase = 50%, $P = 0.0105$), of number of spermatozoa (percentage increase=54%, $P = 0.0054$), of number of motile spermatozoa (percentage increase=56%, $P = 0.0008$), of progressive motility (percentage increase=41%, $P = 0.0006$), and of normal sperm morphology (percentage increase=60%, $P < 0.0001$).

Limitations, reasons for caution: Enrollment is ongoing to validate results in a larger cohort of patients, evaluating also their BMI and metabolic profile at T0 and T90. Moreover, we are collecting data on IVF cycles before and after the treatment, in order to compare fertilization and cleavage rates, quality of embryos, and pregnancy outcome.

Wider implications of the findings: For the first time, we report that the combined treatment with inositol and alpha-lipoic acid is effective in treatment of male infertility. Therefore, our pilot study suggests an important role of such a dietary supplement in clinical management of dispermia by a 'nutraceutical" able to act on the metabolic function.

Trial registration number: Not applicable.

P-082 Do chronic viral infections influence the seminal parameters?

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Study question: To ascertain if viral load therapy and the immunological conditions of patients with chronic viral infections may interfere with sperm function.

Summary answer: Patients with chronic viral infections did not present alteration results in sperm function.

What is known already: The seminal analysis evaluated the fertile potential of man, being divided into the following groups: macroscopic, microscopic and morphological evaluation of the spermatozoa according to the parameters of the World Health Organization (WHO, 2010).Studies have shown that patients infected with hepatitis B and C virus present changes in seminal parameters, as well as decreased testosterone levels, leading to a decrease in total sperm concentration. In HIV-positive patients, sperm production was deficient. One possible reason for this fact would be the influence of viral load therapy and the patients' immunological conditions on sperm function.

Study design, size, duration: A retrospective case-control study using spermogram data from 388 patients from 2010 to 2015.

Participants/materials, setting, methods: The patients were divided to: control (299 seronegative patients), Hepatitis B (22 patients), Hepatitis C (25 patients) And HIV (42 patients) groups. Those who had some other disease besides chronic viral infections, smokers, alcoholics, who made continuous use of any drugs that might cause interference were excluded from the study. The following seminal parameters were evaluated: volume, pH, initial concentration, motility (progressive and non-progressive) and morphology.

Main results and the role of chance: All seminal parameters evaluated in this study did not present significant differences between the studied groups. Only in the Hepatitis B group, 9% of the patients had a pH lower than 7. In control group, 1% of the patients had acid pH and the other groups had normal values. Since frequency is very low, caution is required to establish any causal relationship to such a non-specific event. The results found in this study corroborate with Lorusso et al. (2009) who also verified that the viral infections did not alter the seminal quality of the analyzed patients.

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Limitations, reasons for caution: Some cases, the altered result that was found in the patient's seminal analysis may not have been influenced by the chronic viral disease. This information could not be verified since there was no seminal analysis prior to virus contamination.

Wider implications of the findings: No relation between chronic viral infection and seminal quality was found, making not possible to establish a causal relation between infection and the infertility only by seminal quality.

Trial registration number: None.

P-083 Nutritional factors and sperm motility and progressive motility in oligoasthenoteratozoospermia (OAT) men

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Study question: To evaluate which nutritional factors have impact on sperm motility and progressive motility in Iranian OAT men.

Summary answer: Macro- and micronutrients have structural or protective role in sperm function.Clinicians should be aware about the impact of nutrients on sperm motility and motility progressive.

What is known already: Healthy nutrition is necessary to produce motile and progressive sperm cells. Studies suggest that food dietary intake may play an important role in male reproduction system and pathogenesis of infertility. Dietary intake consisted of macro- and micronutrients have effect on sperm parameters especially motility. Sperm motility has a principal role in male fertility. Little information exists on the optimal fertility diet. Also, the effect of nutritional factors on sperm motility and progression is unclear. The aim of this study was to determine which dietary factors are related to sperm motility and progression in Iranian OAT men.

Study design, size, duration: This cross sectional study was conducted on 300 subfertile men, aged 20-50 years, referring to Royan infertility clinic (Tehran, Iran) from March to September 2014. Participants were categorized into two groups; of 62 normospermic and 238 OAT patients according to World Health Organization 2010 criteria.

Participants/materials, setting, methods: After enrollment and receiving informed consent, all men underwent face-to-face private interviews. Participants completed two questionnaires including FFQ and general data. Dietary data was collected monthly by means of twelve 24 h dietary recalls (24hDR). Subjects completed 168-item semi-quantitative FFQ. Semen analysis was examined after 2–5 days of sexual abstinence based on WHO-recommended methods by CASA system (computer-assisted sperm analysis). Data adjusted for age and BMI. Statistical analysis was conducted by T-test and regression.

Main results and the role of chance: The mean age and BMI were (34.63 ± 5.3 vs 33.71 ± 5.2) and (26.73 ± 4.1 vs 27.05 ± 10.1, P>0.05) in normospermic and OAT men, respectively. Sperm motility was associated to omega-2 PUFA ($\beta = 2.01$, $P < 0.05$), vitamin C ($\beta = 2.02$, $P < 0.05$) and also progressive motility was associated to omega-6 PUFA ($\beta = 1.27$, $P < 0.05$), MUFA ($\beta = 7.05$, $P < 0.05$) in total population. After dividing population into two groups, the reults indicated that some dietary intakes were positively associated with **sperm motility** including Na (β: 0.863, $P < 0.001$), Vitmin A(β: 0.64, $P < 0.01$) and Vitamin C (β: 1.10, $P < 0.0001$) and also other nutrients are relaed to **progressive** motility consisted of protein (β:10.035, $P < 0.0001$), cholestrol (β: 3.92, $P < 0.001$), MUFA (β:4.29, $P < 0.0001$), omega-2 PUFA ($\beta = 7.57$, $P<.0001$), omega-6 PUFA ($\beta = 6.682$,$P<.0001$), Na (β: 2.56, $P < 0.001$), K ($\beta = 6.87$,$P < 0.001$), vitamin A ($\beta = 5.54$,$P < 0.001$) and vitamin B6 ($\beta = 10.53$,$P < 0.001$) in Iranian subfertile men.

Limitations, reasons for caution: It was better to evaluate serum concentration of these factors.

Wider implications of the findings: Our results support of this concept that healthy diet is necessary to improve sperm motility, progressive and function in OAT subjects.

Trial registration number: no.

P-084 Vitrified-thawed mouse oocytes can be efficiently used to determine the oocyte activation potential of human sperm

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Study question: Can vitrified mouse oocytes substitute fresh oocytes to diagnose the activation potential of human sperm in cases of failed or low fertilization after ICSI?

Summary answer: Vitrified mouse oocytes can be reliably used to determine the activation potential of human sperm.

What is known already: The mouse oocyte activation test (MOAT) is a diagnostic test offered to patients experiencing failed or low fertilization following standard ICSI. In this test, mouse oocytes are injected with human sperm to reveal oocyte activation deficiencies in the sperm, whereafter assisted oocyte activation can be applied. Still, the MOAT is only applied by very few IVF laboratories, mainly due to the lack of mouse housing facilities. Vitrified mouse oocytes could provide a solution for this, but oocyte vitrification can affect parameters important in oocyte activation.

Study design, size, duration: Both fresh and vitrified-thawed mouse oocytes were micro-injected on the same day with human spermatozoa from a control with normal fertilization rates after routine ICSI. A total of 3 controls were used. Experiments were repeated 2 times per control sample. Two-cell formation (activation rate) was evaluated 24 hours after injections and compared to a negative (sham injection and medium control) and a positive (strontium chloride, $SrCl_2$) control group.

Participants/materials, setting, methods: MII mouse oocytes (B6D2F1, 7-12 weeks old) were vitrified within 3 hours after collection. Frozen-thawed human sperm from fertility proved donors was used for injections. After microinjections, both fresh and vitrified-thawed mouse oocytes were cultured in KSOM-4%BSA medium until next day to evaluate activation rate. Control oocytes (fresh and vitrified) were either not injected, sham-injected (injection with media without sperm) or exposed to $SrCl_2$ (10 mM, 4 hours).

Main results and the role of chance: A total of 86 fresh and 62 vitrified-thawed mouse oocytes were injected with human sperm from 3 fertility proved controls. Survival rate after ICSI between fresh and vitrified-thawed oocytes was not significantly different, 80.2% (69/86) and 83.9% (52/62) respectively (p>0.5).

All fresh $SrCl_2$-exposed mouse oocytes underwent parthenogenetic activation successfully (23/23), whereas a similar amount vitrified-thawed counterparts were activated (13/15) (p>0.5). No activation was observed in both fresh (0/27) and vitrified-thawed (0/23) sham-injected control groups. None of the fresh medium control oocytes were activated (0/48), whereas only 3.4% of the vitrified-thawed medium control oocytes showed activation (1/29) (p>0.5). The same mean activation rate of 94.2% was observed between fresh (65/69) and vitrified-thawed (49/52) mouse oocytes micro-injected with control spermatozoa (p>0.5).

Limitations, reasons for caution: Until now, only the activation potential of control human sperm was tested after heterologous ICSI using vitrified mouse oocytes. However, vitrification can also affect the calcium pattern during oocyte activation, which requires additional calcium imaging and is currently ongoing.

Wider implications of the findings: Vitrified-thawed mouse oocytes can be efficiently used to perform a MOAT, as an alternative to fresh mouse oocytes. Most importantly, using commercially available vitrified mouse

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oocytes, MOAT can be performed without the requirement of mouse housing facilities.

Trial registration number: not applicable.

P-085 Sperm separation by microfluidic sperm sorter (MFSS): Comparison of DNA fragmentation index and embryo quality with density gradient centrifugation

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Study question: Does Sperm separated using microfluidic sperm sorter (MFSS) have lesser DNA fragmentation index assessed by Sperm chromatin dispersion assay and result in better ICSI outcome in terms of embryo quality?

Summary answer: Sperm separation by MFSS is found to be equally efficient to density gradient centrifugation (DGC) in terms of DNA fragmentation index and embryo quality

What is known already: Conventional sperm separation techniques such as swim up and DGC involves one or more centrifugation steps. There is evidence in the literature on increased DNA damage in samples processed using percoll / density gradient methods, which is attributed to reactive oxygen species generation due to centrifugation process. High DNA fragmentation is implicated in poor outcome in ICSI cycles. MFSS is a polycarbonate device with two chambers into which unprocessed sperm sample is loaded and actively motile sperm are separated by laminar effect after thirty minutes of incubation. Some earlier studies have reported high sperm sorting efficiency using this device.

Study design, size, duration: This prospective sibling oocyte study involved nineteen patients, who were undergoing IVF/ICSI cycles between September 2016 to December 2016 and were also found to have high sperm DNA fragmentation index (DFI) on initial semen analysis

Participants/materials, setting, methods: On the day of ovum pick up, oocytes collected for each patient, involved in the study were equally divided and injected with sperm separated by DGC and MFSS. Total number of oocytes injected in DGC and MFSS groups were 110 and 121 respectively. Sperm DNA fragmentation was assessed in pre-wash and post processed samples using sperm chromatin dispersion test. Fertilization rate and embryo quality on Day 3 were recorded

Main results and the role of chance: DFI on initial semen analysis in these 19 patients was 36 +/- 12.19. On the day of ovum pickup (OPU), the DFI was found to be 37.63+/-12.41. Patients had variable days of antioxidant therapy and repeated ejaculation between the initial semen analysis and the day of ICSI. DFI in density gradient separated sperm on the day of OPU was 15.61+/-11.66 and in MFSS was 9.73 +/- 8.21. Fertilization rates (76% vs 79%) were similar in density gradient (84/110) and MFSS (94/121). Embryo quality on day 3 was also found to be similar (66/84 grade 1 embryos in density gradient group vs 75/94 in MFSS group). Statistical analysis of difference in DFI between two separation techniques by Mann Whitney U test was found to be non significant.

Limitations, reasons for caution: Poor responders and elderly women in whom benefits of using sperm with good DNA integrity might have been more evident were not included in the study. Another potential limitation was, patients with severe oligo astheno teratozoospermia were also not included as MFSS did not yield enough sperm for injection.

Wider implications of the findings: Results obtained provides evidence towards a positive trend in separation of sperm with better DNA quality post MFSS clinically, though not statistically significant. Extrapolation to pregnancy rates, miscarriage rates, live birth rates and involvement of larger number of patients might yield conclusive information on the advantage of this technique.

Trial registration number: not applicable.

P-086 microRNA-155-5p levels in serum and seminal plasma as a biomarker of male reproductive function

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Study question: Do levels of microRNA-155-5p (miR-155-5p) differ between serum and seminal plasma and are they associated with reproductive hormone levels and semen parameters?

Summary answer: miR-155-5p expression was higher in seminal plasma compared to serum. Seminal concentration of miR-155-5p correlated to sperm concentration, FSH, inhibin B and free testosterone.

What is known already: miRNAs are a class of small non-coding RNAs which regulate, mainly at the post-transcriptional level, expression of two-thirds of human genes implicated in a multitude of biological processes. Since different miRNAs are regulated by reproductive hormones and interact with genes controlling spermatogenesis, their potential as functional biomarkers is of great interest. miRNAs are found intracellularly, in the serum and in other biological fluids where they function as paracrine or endocrine messengers. Detection of miRNAs in serum and seminal plasma may serve as biomarkers of male fertility. Earlier studies from our group have associated serum levels of miR-155-5p with male subfertility.

Study design, size, duration: As a part of a study on seasonal variation in male reproductive parameters, 221 Norwegian men from the general population, aged 19-40 years, were enrolled in 2001-2002.

Participants/materials, setting, methods: Semen parameters (total sperm number, concentration, motility, volume, morphology and DNA fragmentation index (DFI)) and reproductive hormones were analyzed. miR-155-5p was determined in seminal plasma (n = 153) and serum samples (n = 199) by quantitative real-time PCR (qPCR) and analyzed for correlation with reproductive parameters.

Main results and the role of chance: qPCR analysis revealed that miR-155-5p had significantly higher expression in seminal plasma in comparison with serum (n = 150 pairs, $p < 0.001$). We observed weak but significant correlations between miR-155-5p levels and sperm concentration (rS=0.165, p = 0.043), DFI (rS=0.172, p = 0.038), FSH (rS=-0.167, p = 0.042), inhibin B (rS=0.191, p = 0.019), and free testosterone (rS=-0.159, p = 0.051). No statistically significant correlation was observed between miR-155-5p levels in serum and seminal plasma which might suggest that other biological processes underlying medical conditions affect expression of this miRNA in serum and disrupt correlations found for hormones at semen level.

Limitations, reasons for caution: The results cannot be generalized to other miRNAs. Lack of information about miR-155-5p levels at tissue/cell level in this group.

Wider implications of the findings: Correlations found between miR-155-5p and selected semen parameters as well as reproductive hormones suggest that its levels in seminal plasma might reflect spermatogenesis related processes with higher probability than miR-155-5p measured in serum.

Trial registration number: Not applicable.

P-087 Improving pregnancy rate in IVF cycles by preparing sperm via microfluidic sperm chips

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Study question: Do microfluidic sperm chips increase pregnancy, implantation, fertilization rates in IVF cycles comparing to conventional gradient centrifugation (Percoll) method?

Summary answer: As one of the novel sperm preparation technologies, microfluidic sperm chips increase pregnancy rate in IVF cycles comparing to conventional gradient centrifugation method.

What is known already: Effect of microfluidic sperm chips to sperm motility and DNA integrity improvement is already known by some studies. Although, microfluidic technologies were introduced in ART field at the beginning of 21st century and are being advanced from day to day by several days, there is still no prevailing utilization in laboratories and theare are few studies presenting their effect on ART outcomes.

Study design, size, duration: This is a retrospective, randomized study evaluating the data of 185 patients between December 2014 and January 2017. Patients were grouped according to sperm preparation technique (Chip or Percoll), each group inclueded 88 and 97 patients respectively. Patients were further grouped by the number of their previous cycles. Previous cycles were

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divieded to 3 groups: 0-1, 2-3, 4 and more. Chip effect was compared in each group in terms of fertilization, pregnancy and implantation rates.

Participants/materials, setting, methods: Sperm was applied into chips according to manufacturers' instructions. %90 density gradient was used in percoll method. Fertilization rate was defined as percentage of 2PN oocytes per total injected MII oocytes. Implantation rate was defined as percentage of 2PN oocytes per total injected MII oocytes. Chi-square test was performed for method/ pregnancy comparitions. Statistically significant results were further analyzed by regression analysis. Mann-Whitney U test was performed to assess fertilization and implantation rates in groups.

Main results and the role of chance: Retrived oocyte number, sperm motility %, previous cycle number were similar between chip and percoll group (p>0,05 with Kolmogorov-Smirnov test) (retrived oocyte number 7,8 ± 3,8-7,6 ± 3,6, sperm motility % 50,1 ± 16,4-51,0 ± 17,2, pervious cycle number 2,1 ± 2,3-1,4 ± 1,9)

Statistically significant result was obtained between chip and percoll group in terms of pregnancy rate (p: 0,03). 1,97 fold increase was found to improve pregnancy rate with chip utilization as sperm preparation method in all patient group. Fertilization and implantation rates were not found statistically significant between two groups (p: 0,06 and p: 0,65) (fertization rate 85,6 ± 15,6 and 84,1 ± 15,8; implantation rate 31,7 ± 42,1 and 21,5 ± 38,3 respectively).

If previous cycle number is 0-1 chip utilization has no effect on pregnancy, fertilization and implantation rates (p: 0,25 p: 0,49, p:0,40). Similar results were obtained for the patient group if previous cycle number is 4 or more (pregnancy p:0,65, fertilization p: 0,81, implantation p: 0,51).

If previous cycle number is 2-3 chip utilization improve pregnancy and implantation rate (p: 0,01, p: 0,02), however has no effect on fertilization rate (p: 0,48). 4,8 fold increase was found to improve pregnancy rate with chip utilization as sperm preparation method in patient group if previous cycle number is 2-3).

Limitations, reasons for caution: Statistically significant difference (p:0,029) at female patients'ages was observed between chip and percoll groups(34,2 ± 4,3 and 32,1 ± 4,7 respectively).Sperm count and morphology was also found statistically different (p:0,019) between groups (39,9 ± 33,1 and 53,1 ± 42,4; 1,4 ± 1,1 and 1,7 ± 1,1 respectively).Statistic tests might be repeated with more patients grouped by similar ages and sperm count.

Wider implications of the findings: Embryo quality was not evaluated in this study. Results might be extended by incluiding embryo quality results.

Trial registration number: not applicable.

P-088 Factors influencing the variability in the morphological assessment of human sperm during external quality control (EQC)

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Study question: What are the sources of variability between laboratories in the assessment of sperm morphology?

Summary answer: Our results confirm the wide variability in the assessment of sperm morphology. The level of experience has an impact on the extent of the variability.

What is known already: Sperm morphology has been shown to have a high predictive value for the outcome of assisted reproductive technologies. However, it is known that the highest variability during external quality control programs is found for sperm morphology assessment.

Study design, size, duration: An external quality control for sperm morphology assessment was conducted in 7 laboratories performing semen analysis in Sfax region (Tunisia). A survey requesting information about experience and methods used to undertake semen analysis was concomitantly distributed.

Participants/materials, setting, methods: Each laboratory received two stained slides (Shorr staining) prepared from two semen samples and was asked to assess sperm morphological abnormalities in the same way *as routine* examination is done.

Main results and the role of chance: All laboratories assessed sperm morphology according to David's criteria. The average inter-laboratory coefficient of variation (CV) was 33 % for normal forms (NF) and 15.5 % for Multiple Abnormalities Index (MAI). The variability was lower in experienced laboratories for NF compared with unexperienced laboratories. The lowest CV were observed for the percentage of post acrosomal abnormalities and bent midpieces (38 % each), of coiled tails (44 %) and of post acrosomal abnormalities (46 %). The highest CV were found for head size abnormalities: thin (176 %) and tapered (160 %).

Limitations, reasons for caution: Other sources of variability in the assessment of sperm morphology, such as different systems of classification and various staining methods, should be considered in future EQC programmes.

Wider implications of the findings: The need for adequate initial and continuing training of the laboratory staff is crutial. The compliance with the guidelines and the use of micrometer eyepiece could be of a great help to minimize the *variation* within and *between laboratories*.

Trial registration number: No trial registration number.

P-089 DMC1 is an encouraging biomarker for non-obstructive azoospermia patients

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Study question: To establish a meiotic biomarker for non-obstructive azoospermia patients enabling us to interprete spermatogenesis status of patient.

Summary answer: DMC1 expression, a protein with a key role in meiotic recombination, shows significant correlation with positive testicular sperm extraction (TESE) outcome.

What is known already: Male-related factors are involved in more than half of infertility cases. Among these patient group 10 % of cases are diagnosed as non-obstructive azoospermia (NOA). Diagnostics tests available for these patient group is limited and standard semen analysis frequently used for male fertility would not provide much infromation for patients. Proteins involed in meiotic recombination may be good candidates for this patient group. PCNA was used for this purpose in a previous study. Another study suggested that interaction between Lim15/Dmc1 and PCNA mediates the recombination-associated DNA synthesis during meiosis which makes Dmc1 a good candidate as a biomarker.

Study design, size, duration: The study took place in Genart IVF center between the years of 2013-2017. 300 NOA patients and 10 sperm positive controls were included in the study. Following testicular sperm extraction patients with negative sperm outcome were involved in patients group and patients with positive sperm outcome were enrolled in control group.

Participants/materials, setting, methods: Testicular sperm extraction tissue of NOA patients was used in the study. RNA was extracted using Zymo-Spin RNA extraction kit. Following RNA extraciton cDNA was prepared using New England Biolabs ProtoScript II First Strand cDNA Synthesis Kit. Real Time PCR was performed using Roche Light Cycler 480 with target probes (PCNA, DMC1) and reference probe (Actin) provided by Roche. Immunohistochemical staining was also performed to compare expression of Pcna and Dmc1 in TESE samples.

Main results and the role of chance: Relative expression of DMC1 and PCNA was calculated relative to reference gene(Actin).Relative expression

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DMC1 gene was significantly higher in control group of 10 samples where positive testicular sperm extraction was observed compared to patients. PCNA (expressed in spermatogonial cells) was used as an additional control. Following reevaluation of control group with seperate real time PCR, similar expression results were achieved.

DMC1 and PCNA expression was also evaluated using immunohistochemical staining with testicular sperm extraction tissue. Results also supported presence of DMC1 and PCNA in both sperm positive and sperm negative patients. Higher Dmc1 expression was observed in sperm positive samples. Expression results can be misleading in samples If Testicular sperm extraction tissue is contaminated with fibrotic tissues which would elevate PCNA expression.

Limitations, reasons for caution: Obtaining good quality RNA from TESE material is cruial to avoid failure in evaluating expression since additional TESE material from patients is not available in most cases.

Wider implications of the findings: These results suggested existence of meiosis and potential of spermatogenesis in NOA patients with negative sperm outcome. Instead of repeated TESE trials elective TESE will be possible with the information about spermatogenic activity. Thus TESE planning will be possible for patients who went through treatment to provide spermatogenesis.

Trial registration number: NA.

P-090 Sperm DNA fragmentation and its role in Assisted Reproductive Technologies: analysis of 1056 cycles

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Study question: Is there a prognostic value of sperm DNA fragmentation (SDF) test on in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) reproductive outcome?

Summary answer: According to our data on 1056 cases, it can be stated that high SDF levels negatively affect both IVF and ICSI reproductive outcome.

What is known already: To date the link between SDF, male infertility and ART outcome has been extensively investigated by many authors. However, in 2015 the Practice Committee of American Society for Reproductive Medicine (ASRM) reported that data published regarding the relationship between SDF and ART outcome are not sufficient to routinely propose the use of SDF test. Moreover, once recognized a high SDF level, are we able to choose the 'healthy" spermatozoa among the entirely population?

Study design, size, duration: Retrospective observational study carried out between January 2004 and December 2015, on 1056 men undergoing to ART cycles in our Fertility Center. Total treatments included, as follow: IVF (N = 265), ICSI (N = 680) and intracytoplasmic morphologically selected sperm injection (IMSI) (N = 111). According to SDF levels, patients were divided into groups and compared taking into consideration the ART outcome of each treatment.

Participants/materials, setting, methods: DNA fragmentation was evaluated by terminal deoxynucleotidyltransferase-mediated fluorescein-dUTP nick end labelling (TUNEL) assay, performed on sperm suspensions after density gradient separation, in 1056 men undergoing to ART cycles within three months from SDF test. The threshold value chosen to differentiate between high and low SDF levels was 10%. The chi-square test was used to compare the ART cycles in terms of main outcome measures. Statistical differences were considered significant at $p < 0.05$ and $p < 0.01$.

Main results and the role of chance: 1)Considering IVF treatments, we observed that the sperm DNA damage exclusively affects the fertilization rate (FR). Our data reported that sperm samples with low SDF (N = 245) displayed a significant higher fertilization rate compared to samples with high SDF levels (N = 20), (69% vs 51%, $p < 0.01$).

2)Taking into account ICSI treatments we obtained a significant higher FR, implantation (IR) and pregnancy (PR) rate in patients with low SDF levels (N = 452) compared to patients with high SDF levels (N = 228); (FR: 77, 6% vs 71, 4%, $p < 0.01$; IR: 14% vs 7,7%, $p < 0,01$; PR: 24, 7% vs 14, 8%, $p<0,01$).

3)Finally, we wondered if the application of IMSI technique, in case of increased SDF, might be more helpful rather than ICSI procedure, in term of ART outcome. Results showed a significantly higher IR and lower pregnancy loss rate (PL) in IMSI treatments with high SDF levels (N = 81) compared to ICSI treatments with high SDF levels (N = 228); (IR: 13, 1% vs 7, 7%, $p < 0.05$; PL: 1, 5% vs 13, 8%, $p < 0.01$).

Limitations, reasons for caution: The study is referred to patients attending our Fertility Center. In many years we performed the TUNEL assay as method for detecting SDF. Nevertheless, this methodology is one of many available in laboratories worldwide. Therefore, our data do not lead to unquestionable results and can not be generally applied

Wider implications of the findings: The worthy aspect of our study is the considerable number of patients analyzed, in comparison to the number in other studies published. We mainly aimed to highlight the SDF role on ART outcome, further to suggest the possibility to perform IMSI as alternative approach in case of high SDF levels.

Trial registration number: none.

P-091 Effect of Seminal ORP Value on Embryo Quality and Clinical Pregnancy Rate

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Study question: Whether ORP in semen of men undergoing ART affects embryo quality during IVF and plays any role in predictive value for clinical pregnancy.

Summary answer: Semen ORP measurement can be used as an indicator of oxidative stress and help determine the successful embryo development during IVF.

What is known already: Excessive amount of oxidative stress can attack biological molecules such as membrane lipids, DNA and proteins affecting sperm function. Morphological abnormalities and poor motility are associated with unsuccessful ART affecting clinical pregnancy and live-birth rate. Although ORP can measure such oxidative stress, it is not known if it plays a role as determinant of successful clinical pregnancy.

Study design, size, duration: This prospective study was carried out in the VKF American Hospital, Assisted Reproduction Unit, Istanbul, Turkey. The study was approved by Koc University the institutional review board and patients signed a consent prior to participation. The 154 male patients who were visiting Andrology laboratory (between May 31st, 2016 and Jan 1st, 2017) were grouped in according to semen ORP values. IVF was performed using the semen samples by our routine established protocol.

Participants/materials, setting, methods: Exclusion criteria included azoospermia, presence of STD or chronic disease, use of any prescription drug or OTC medications or antioxidants. Semen samples were collected and assessed for routine parameters using the WHO-2010 guidelines. sORP was measured (mV) using the MiOXSYS system and normalized to concentration ($mV/10^6$ sperm/mL). Embryo was graded based on the quality (Grade 1 for best quality and Grade 5 is poor).

Main results and the role of chance: All semen samples were grouped as 'High ORP" and 'Low ORP" based upon seminal ORP cut-off value of 1.36 $mV/10^6$ sperm. Mean Grade 1 embryo quality in High ORP group (n = 81) and low ORP group (n = 26) was 2.88 ± 1.76 and 1.33 ± 0.47 respectively. In addition, Mean Grade 2 embryo in High ORP group was 2.47 ± 1.54 and in low group was 4 ± 1.63 accordingly. Clinical pregnancy rate (Mean 0.48; 19/40) was significantly higher ($p = 0.006$) in low ORP group compared to high ORP group (1/8) however there was no significant difference in embryo quality in both high and low ORP groups (Grade 1 embryo $p = 0.67$, Grade II G2 embryo $p = 0.33$). Also, mean mother age in two groups was not significantly different (low ORP = 37.2 years and high ORP = 35.2 years; $p = 0.23$). These

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data suggest that ORP may play an important role in determining the success of clinical pregnancy irrespective of oocyte quality in women >35 yrs. of age.

Limitations, reasons for caution: The sample size was relatively small. Also, ORP is still an investigative tool and further multicenter clinical trials are needed before its establishment as a diagnostic tool in clinical practice.

Wider implications of the findings: These findings may have important diagnostic and prognostic implications for couples experiencing male infertility and undergoing assisted reproductive technique (ART). Further studies are warranted to explore the mechanism of increased ORP in a subset of couples (male factor, no female factor) undergoing ART to corroborate the significance of these findings

Trial registration number: Not applicable.

P-092 Incidence of symptoms associated with testosterone deficiency after TESE: a systematic review

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Study question: Does TESE induce symptoms associated with testosterone deficiency?

Summary answer: Although there is a trend towards decreased testosterone after TESE, there is virtually no data on symptoms related to testosterone deficiency after TESE.

What is known already: Surgical testicular sperm extraction (TESE) is used as a technique to assist azoospermic men to become fathers. Studies on hypogonadism after TESE are limited and show contradictory results. To clarify this issue, we performed a systematic review on the symptoms of hypogonadism being, among other things, sexual dysfunction, decreased testis size, increased fat mass, loss of muscle mass and strength and depression.

Study design, size, duration: In this systematic review we used the databases Pubmed and Embase to search from inception to 12 December 2016. For the search, we combined subject headings with terms in title and/or abstract related to intervention, outcomes and participants and used no restrictions for study types.

Participants/materials, setting, methods: We included all peer-reviewed studies which measured clinical symptoms related to testosterone deficiency before and after any TESE technique in azoospermic men. The measurement before TESE was used as a control. For the analyses we used Review Manager 5.3 software.

Main results and the role of chance: With our search we identified 1525 citations. After independent screening by two individuals, we found 12 studies reporting on potential hypogonadism upon TESE of which 5 studies could be included for data extraction on symptoms of hypogonadism. Cohorts in these studies varied between 26 and 120 participants. Only one study reported on the incidence of erectile dysfunction 6 months after TESE and found that 13 out of 66 men (20%) developed new-onset erectile dysfunction. In these men a decrease of 17.4 nmol/L in testosterone was seen. Four other studies reported on potentially decreased testicular size. In three of these studies no or only a small decrease(<2 ml) was seen in testis size after TESE. One study reported a decrease in testicular size of >2 ml in 12 out of 120 men (10%) with a decrease in testosterone in only two men. No studies reported on any other testosterone deficiency related symptoms such as decreased libido, increased fat mass, and decreased muscle mass and strength. Analyzing all studies reporting on potential hypogonadism, we see an overall decrease in testosterone levels in NOA and OA patients of 3.3 nmol/L six months after TESE, which starts to recover nine months after TESE.

Limitations, reasons for caution: Included studies are very heterogeneous in TESE techniques used (multiple biopsies versus microdissection).

Wider implications of the findings: Although TESE temporarily affects hormone levels in azoospermic patients, limited data is available on whether it leads to a higher incidence of symptoms related to testosterone deficiency. More large-scale monitoring needs to be performed to be able to assess these risks accurately. Only then patients can be counselled appropriately.

Trial registration number: not applicable.

P-093 Inflammasome complex gene expression and its relationship with infertility of male rats after spinal cord injury

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Study question: Evaluation of Inflammasome genes expression and their activity pattern in testis of spinal cord injury (SCI) rat models.

Summary answer: Testis is one of the sources of inflammasome genes which induces pyroptosis and resulted in reduction of sperm concentration and motility in SCI model.

What is known already: Many young men with spinal cord injury (SCI) suffer from infertility. Previous studies showed the low sperm concentration, motility and abnormal morphology in SCI infertile men. Some researchers suggested immunologic reasons for diminished sperm quality in SCI patients. They identified some inflammasome complex productions such as caspase-1 in sperm of these patients, but the class of upstream genes and the time of starting activity in this complex was not determined clearly.

Study design, size, duration: Mature male rats were used in this study. The rats were divided into four groups (n = 4): three surgery groups that SCI at level of T10 performed on them and one intact group as control. We scarified group one, a day after SCI surgery, group two, 3 days after and group 3 a week after surgery. Testes were collected. The most known inflammasome upstream genes expression includes NLRP1a, NLRP3, NLRC4, and AIM2 were assessed.

Participants/materials, setting, methods: Mature male wistar rats with 250-300 gr. body weight were used. The above- mentioned genes were examined by qrt-PCR. The data analyzed by The Kruskal-Wallis Test and Games Howel post hoc test to find the difference between various time points.

Main results and the role of chance: According to the statistical analysis test the gene expression level in NLRP3 (χ^2= 11.373,df=3, P-Value .010) and AIM2 (χ^2= 9.201,df=3, P-Value.027) showed significant difference ($p < 0.05$). No significant difference was detected in NLRP1a and NLRC4 genes expression between the test and control groups in any time points. In group 3 the expression of NLRP3 was significantly different in compare to control group (MD= -2.80, P =0.04) also in group one, the expression of AIM2 (MD= 1.73, P = 0.025) was significantly up regulated in contrast to control group. For the first time we find that expression of NLRP3 as the most known upstream gene of inflammasome complex up-regulates one week after SCI in the testis of rat model. Also we find that expression of AIM2 as another gene of infalmmasome complex up-regulates a day after injury. These up-regulations indicate initiation of inflammasome complex activity. The Inflammasome complex activity can induce pyroptosis (a model of Programmed cell death after formation of inflammasome complex). We suggest that pyroptosis can be the cause of low sperm motility and count in SCI rat models.

Limitations, reasons for caution: The main limitations of this study could be the low number of rats. Also, based on the ethical considerations in this study we only examined the effects of SCI in rat model but not in human.

Wider implications of the findings: Knowing the exact genes of inflammasome complex that express in testis after SCI and the exact time of

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inflammasome up-regulation can be helpful for administration of anti inflammasome drugs in exact time to suppress inflammasome complex formation, excessive pyroptosis and also improve sperm count and motility.

Trial registration number: it is basic science.

P-094 ICSI cycles with cryopreserved or fresh testicular sperm in patients with obstructive (OA) and non-obstructive (NOA) azoospermia: are there differences in outcome?

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Study question: Are there different outcomes between ICSI cycle using cryopreserved or fresh testicular sperm in patients with obstructive (OA) and non-obstructive (NOA) azoospermia?

Summary answer: Live birth, clinical pregnancy, clivage rate: No statistical differences. NOA significantly affects fertilization. Statistical increment in embryo top quality in NOA patients in frozen group.

What is known already: It was proved that cryopreserved testicular spermatozoa in patients with obstructive azoospermia (OA) and non obstructive azoospermia (NOA) maintains adequate characteristics of vitality and reaches excellent fertilization rate (Prins GS, 1999) as well as good pregnancy rate (KUPKER W, 2000)

on the other hand, the use of fresh sperm from tese has resulted in a large number of pregnancy in OA and NOA patients (Vloberghs et al, 2015). The use of fresh or testicular sperms are however still, much discussed (Verheyen G, 1997; Aoki et al, 2004; Mohamed K.M, 2008; Karacan et al, 2013; Ohlander et al, 2014).

Study design, size, duration: Retrospective analysis of 119 ICSI cycles between 2012-2016 in futura P.M.A.

GROUP 1: 31 fresh testicular sample (12 OA; 19 NOA)

GROUP2: 88 frozen-thawed testicular sample (47 OA; 41 NOA)

Inclusion criteria: Normal karyotype, patients undergoing testicular biopsy for OA and NOA, male age <45, female age <38, AMH>1,5 ng/ml

Exclusion criteria: Female age >38, poor ovarian reserve, male age >45

Main outcome: Fertilization rate, embryo quality, clivage rate, pregnancy rate, live birth rate.

Participants/materials, setting, methods: Groups were matched for fresh or frozen testicular sperm sample and for the presence of OA/NOA patients distinguished by histological classification.

Testicular biopsies were retrieved for freezing and later used or for fresh use at the day of pick-up.

Surgery/crioconservation was in-house or transferred from other centers.

Fertilization and embryo development were checked and best quality embryos were transferred.

For statistical analysis: Student's t-distribution, Mann–Whitney U test, analysis of covariance and logistic regression.

Main results and the role of chance: It is not a significant difference between fresh or frozen sperm sample in the clinical pregnancy rate (P=0,7442 in OA fresh vs OA frozen; P=1,000 in NOA fresh vs NOA frozen), in live birth rate (P=0.227 OA fresh vs OA frozen; P=1.029 in NOA fresh vs NOA frozen) but in 8 patients the pregnancys are in progress. The clivage rate doesn't change (P=1 NOA fresh vs NOA frozen and P=0.2330 in OA fresh vs OA frozen).

We find a significant difference in fertilization rate in cryopreserved group: the OA frozen group has an increment respect to the NOA frozen group (P<0,001). So the use of sperm from men with NOA significantly affects fertilization (Nicopoullos et al, 2004).

We find a different fertilization rate in OA fresh respect to OA frozen (P=0.231).

For the quality of embryos (gardner classification) we find a statistical increment in top quality embryo in NOA frozen group vs NOA fresh group (P=0,0347)

Data could be related to a known cryo damage to chromatin but in our study it didn't interfere with embryo quality considering that also tese sperm has less fragmentation respect to ejaculated (Esteves SC, 2015) and there isn't the problem of synchronization ovarian stimulation and biopsy surgery.

Limitations, reasons for caution: This work is a retrospective analysis so some informations are incomplete.

Wider implications of the findings: No differences were found in ICSI outcomes between cryopreserved and fresh testicular sperm.

The problem of synchronization ovarian stimulation and biopsy surgery may be resolved using the cryopreserved sample retrieved inside or transferred from other centers.

Another advantage is reducing the risk of failure spermatozooario recovery (Vehement et al, 2004).

Trial registration number: None.

P-095 Seminal elastase and oxidative-dependent DNA damage of spermatozoa in infertile men

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Study question: Is seminal elastase more reliable than histological determination of hyperleucocytose to detect oxidative-DNA damage? Is it related with modifications of seminal environment?

Summary answer: Dosage of seminal elastase is more sensitive than histological determination of leucocytes for the detection of oxidative DNA damage of spermatozoa.

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What is known already: Leucocytospermia is considered as a major factor to male infertility (decreased sperm motility and normal morphology).

For elastase, a protease secreted by activated polynuclear, the following correlations have been reported:—Positive with the number of leucocytes (but discussed),—Negative with the percentage of motile sperm or with intact DNA (which can explain a poorer blastocyst development rate and a higher number of arrested embryos),—Negative with seminal plasma volume and its citrate content.

There are few studies about the oxidative-dependent damage of DNA spermatozoa in relation with leucocytospermia. According to a recent work, DNA oxydative damage was not associated to leucocytes.

Study design, size, duration: In this retrospective study, elastase concentration (n = 2037) was assessed in 1714 men attending the Cochin hospital center for fertility problems between 2012 and 2015. The correlation of either elastase or leucocytospermia with peroxidase-stained leucocytes, sperm parameters (n = 1921), bacteriology (n = 713), seminal biochemistry (n = 263), oxidative DNA damage (n = 167) results were investigated.

Participants/materials, setting, methods: Sperm parameters were evaluated according WHO standard procedures. Semen leucocyte concentration was determined by peroxidase-stained leucocytes. This test was realized when round cells with 2n content, determined by flow cytometer with propidium iodide, was superior to 1.10^6/mL. Elastase-inhibitor complex was assessed by immunoassay in seminal plasma. Biochemical seminal markers (zinc, acid phosphatase, citrate, fructose, α-glucosidase and carnitine) were assessed by spectrophotometry. DNA-base adducts formation was determined using flow cytometer by OxyDNA test.

Main results and the role of chance: Major significant correlations between polynuclear elastase and semen characteristics have been observed.

Levels of seminal elastase are:

1° positively correlated with peroxidase-stained leucocytes ($p = 1.9 \times 10^{-101}$), negatively correlated with sperm motility ($p = 3 \times 10^{-4}$).

2° positively correlated with 8OHdG ($p = 9 \times 10^{-4}$) in contrast to semen leucocytospermia determined by peroxidase-stained leucocytes.

3° negatively correlated with volume ($p = 1.10^{-3}$), markers of prostate secretion (citrate $p = 4.6 \times 10^{-5}$, zinc $p = 10^{-3}$ and acid phosphatase $p = 4.6 \times 10^{-6}$) and positively with pH ($p = 1.2 \times 10^{-14}$).

A cut-off of 377 ng/mL (ROC curve) allowed to predict leucocytospermia with a sensitivity of 92 % and a specificity of 81%.

The 90^e percentile in the population with « normal sperm parameters » (n = 292), according to the WHO criteria, was 730 ng/mL.

Among the 157 positive bacteriology, only 15,3% had an elastase concentration superior to 377 ng/mL: inflammation and infection are dissociated.

Limitations, reasons for caution: The lifestyle (BMI, tabac, antecedent of uro-genital infection), fertility background, ART outcome of this patients supported by our ART center were not taken into account.

Wider implications of the findings: Patients with increased seminal elastase concentration display reduction of sperm motility, high numbers of oxidized spermatozoa (perhaps due to a loss of the nucleus condensation related to a decreased prostatic zinc secretion?) which might have an impact on techniques of in-vitro fertilization and embryo development.

Trial registration number: Not applicable.

P-096 Relationship between urokinase plasminogen activator (uPA) concentration in seminal plasma and outcome of assisted reproduction treatments

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Study question: Are the concentration of urokinase plasminogen activator (uPA) in seminal plasma related to the outcomes in ART?

Summary answer: Higher values for uPa in seminal plasma were detected in the cases with pregnancy after IUI and IVF-ICSI (p = 0.04), than in failure of pregnancy

What is known already: The plasminogen/plasmin system functions as one of the most important extracellular protease systems in vivo.

Plasminogen-plasmin system has been related to different process of the male reproductive function, as spermatogenesis, sperm capacitation, and fertilization. Some authors have previously detected the presence of plasminogen activators as tPA and uPA in human seminal plasma as well as inhibitors of the plasminogen-plasmin system. uPA activity was correlated significantly with sperm parameters (count, motility and morphology) and fertility.However, the specific role and importance of uPA for the male reproductive function is not well known.

Study design, size, duration: This research was desing as a Case control study and it was carried out in collaboration with department of Physiology of Murcia, University of Murcia. Spain.

55 semen samples were analized in Instituto Valenciano de Infertilidad of Murcia during the period 2013-2014.

Participants/materials, setting, methods: Total uPA in seminal plasma were measured by ELISA test in 55 seminal samples (3o IUI+ 25 IVF/ICSI cases)). Aliquots were centrifuged at 300 g for 10 minutes and seminal plasma and spermatozoa were stored at -80°C until uPA evaluation.

Levels of uPA were correlationed with ART results by Pearson test. Receiver Operating Chacarcteristic curves (ROC) were used to evaluate predictive value of uPA in outcomes of ART

Main results and the role of chance: *IUI treatment.* IUI was used in 30 cases resulting, 20% ongoing pregnancy rate. We detected higher values for total uPA in seminal plasma (26.66 ± 1.61 vs. 20.29 ± 1.47 ng/ mL, p = 0.04,) in the cases with pregnancy than in the cases when pregnancy was not detected. Receiver Operating Characteristic (ROC) curves analysis to evaluate the predictive value of the fertility (pregnancy) of the total uPA in seminal plasma. We found area under the curve was 0.74 ± 0.10, p < 0.05 and the cut off value for predicting pregnancy of 26 ng/ml. the sensitivity was 67% and specificity was 78%.

ICSI Treatment. IVF-ICSI treatment was applied in 25 cases with 56%% pregnancy rate. When we studied the uPA concentration in cases with pregnancy vs. no pregnancy we found a higher difference 22.45 ± 0.82 vs 19.32 ± 1.40, p = 0.04) in total uPA concentration in seminal plasma in pregnancy group. ROC curves analysis to evaluate the predictive value of the fertility (pregnancy) of the uPA concentration in seminal plasma, area under the curve was 0.81 ± 0.09, p < 0.05 and the cut off value of 21,6 ng/ml. Sensitivity was 90% and specificity was 73%.

Limitations, reasons for caution: In next researches we should increased number of patients.

Wider implications of the findings: In this research we have founded total uPA in seminal plasma were higher in ART cycles with pregnancy versus non pregnancy. Total uPA could be a seminal biomarket in ART treatment in especially in ICSI treatment.

Trial registration number: Don't apply.

P-097 The impact of cigarette smoking on sperm nuclear protein and DNA integrity

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Study question: Does cigarette smoking affect histone H2A, H2B, protamine and DNA integrity of human spermatozoa?

Summary answer: Cigarette smoking affects the sperm protein (H2A, H2B and protamine) and increase the DNA damage

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What is known already: Condensation of chromatin during spermatogenesis, and its decondensation at the time of fertilization are essential for successful fertilization. Several mechanisms have been proposed for the presence of DNA damage in ejaculated sperm like excessive production of reactive oxygen species (ROS) in the ejaculate.

Cigarette smoking itself contains high levels of ROS. Smoking metabolites may induce an inflammatory reaction in the male genital tract with a subsequent release of chemical mediation of inflammation that can recruit and active leukocytes, which can generate high levels of ROS in semen that may impair spermatogenesis, resulting in the production of abnormal spermatozoa.

Study design, size, duration: Prospective controlled trail.

Participants/materials, setting, methods: The present study carried out at the department of Obstetrics and Gynaecology, University of Saarland, Germany. 54 patients: 35 smokers and 19 non-smokers were included in this study. Semen samples were prepared according to WHO guideline 2010 and sperm nuclear protein were extracted from each semen sample using acid-urea –PAGE and Western blot. Chromatin condensation was assessed by Chromomycine CMA3 staining and DNA fragmentation by TUNEL assay.

Main results and the role of chance: The sperm concentration, vitality, motility, membrane integrity, chromatin condensation, DNA fragmentation, Histone (H2A) and Histone (H2B), Protamine 1 and Protamine 2 of smokers were (51.87 ± 5.05mill/ml; 41.04 ± 2.49%; 29.67 ± 2.47%; 59.24 ± 1.79%, 36.96 ± 2.33% and 25.29 ± 5.0 %; 219.8 ± 16.35 ng/10^6 sperm; 292.27 ± 58.2435 ng/10^6 sperm; 425.6 ± 14.56 ng/10^6 sperm and 354.9 ± 11.28 ng/10^6 sperm and the corresponding values for non- smoker group were (84.27 ± 6.01mill/mL; 59.00 ± 3.33%; 46.83 ± 4.12%; 71.30 ± 3.16%; 20.95 ± 1.35%, 12.47 ± 1.41%; 156.4 ± 12.6 ng/10^6 sperm; 109.1 ± 43.70 ng/10^6 sperm, 425.8 ± 16.26 ng/10^6 sperm and 412.8 ± 16.24 ng/10^6 sperm respectively). In smoker group (count, vitality, motility, chromatin condensation, and membrane integrity, H2A, H2B and Protamine 2) were significantly lower than non –smoker group. Chromatin condensation and DNA fragmentation were significantly higher in smokers. The value of protamine1 was similar in both groups

Limitations, reasons for caution: The size number of the sample

Wider implications of the findings: The finding of geat clinical value as the P1/P2 ratio could be used as a sensitive parameters.

Trial registration number: Basic Science.

P-098 Selection of spermatozoa with higher chromatin integrity through a microfluidics device

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Study question: We tested a simple method to enrich spermatozoa with higher progressive motility and superior chromatin status in men with normal and abnormal semen parameters.

Summary answer: A microfluidics device was able to isolate spermatozoa with higher progressive motility and the lowest incidence of chromatin fragmentation in oligo- and normo-spermic semen.

What is known already: Semen analysis is currently used as a method to screen for male factor infertility. Standard selection methods provide a cleaner and safer specimen for insemination with enhanced progressive motility albeit without any input on the genomic integrity of the spermatozoon. We learned that sperm DNA fragmentation is linked to motility and therefore a method that is capable of guaranteeing a richer proportion of the most progressive spermatozoa may also provide cells with the highest chromatin integrity.

Study design, size, duration: From October 2016 to January 2017, seminal samples of normozoo- (n = 10) and oligozoospermic men (n = 3) were simultaneously processed by density gradient centrifugation (DGC) and by a new microfluidic sperm sorter (MFSS) chamber to allow selection of the most forwardly progressive motile sperm portion. Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) was carried out on the raw specimen and on the differently selected aliquots to assess sperm chromatin integrity (SCI).

Participants/materials, setting, methods: Consenting men had their ejaculates screened for infertility by standard semen analysis according to WHO 2010 criteria. Standard DGC, centrifugation and a MFSS chamber were used to isolate motile spermatozoa based on cell motility and fluid dynamics. SCI was assessed by TUNEL on at least 500 spermatozoa under fluorescent microscopy considering a ≥15% threshold.

Main results and the role of chance: The mean age for the men was 36.5 ± 8 years. The average semen parameters for the normozoospermic men was: concentration 71.5 ± 25 × 10^6/mL, motility of 44.8 ± 4% and morphology of 2.9 ± 1%. After DGC and MFSS the total motility was 86.4 ± 5% and 98 ± 1%, respectively ($P < 0.001$) while the progressive motility was 84.7 ± 5% and 97.6 ± 2 %, respectively ($P < 0.001$). The original sperm morphology of 2.9% became 4.0% after MFSS. In these men, the average SCI was 14.7 % in the raw sample; however, following DGC, the SCI decreased to 8.8% and after MFSS processing it reduced to 2.4% ($P < 0.001$). The average semen parameters for the oligozoospermic men was: concentration 18.7 ± 28 × 10^6/mL, motility of 6.3 ± 5%, and a morphology 2.0 ± 1%. Following enrichment by DGC the motility became 9.7 ± 5% and following MFSS processing the motility was 98.3 ± 3%. The progressive motility changed from an initial value of 4.0 ± 3% to 7.7 ± 5% with DGC and 98.3 ± 3% with MFSS. The average SCI on these men was 26.5% in the raw sample, 22.6% in the DGC specimen, and following MFSS it decreased to 3.5% ($P < 0.001$).

Limitations, reasons for caution: This analysis is a pilot study on a small number of subjects. However, if confirmed, this microfluidic method, capable of selecting the portion of spermatozoa with the most progressive motility together with the highest level of chromatin integrity, may yield spermatozoa with superior embryo developmental competence for reproductive treatment.

Wider implications of the findings: SCI appears to be related to the kinetic characteristic of the human spermatozoa. MFSS yielded the highest progressively motile spermatozoa characterized by high DNA integrity. Couples with unexplained infertility and unable to achieve a pregnancy due to a concealed male factor may benefit from MFSS to improve their reproductive outcome.

Trial registration number: Not Applicable.

P-099 Sperm progressive motility and strict morphology have a negative correlation with DNA fragmentation

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Study question: Is there any seminal parameter that significantly correlated with high levels of sperm DNA fragmentation?

Summary answer: It was shown that strict morphology, progressive motility and vitality had a negative correlation with DNA fragmentation.

What is known already: In the last decade, the evaluation of sperm DNA fragmentation has become as an important method to assess semen quality. Several authors have reported a negative correlation between high DNA fragmentation levels and assisted reproductive technology (ART) outcomes. Considering a population with male factor, we have previously reported that elevated sperm DNA damage is associated with advance male age and oxidative stress.

Study design, size, duration: This is a retrospective cohort study. The sample size was 684 semen samples (2014-2016). All seminal analysis were performed at the same Andrology laboratory and sperm DNA fragmentation assessment was always blind to the seminal parameters.

Participants/materials, setting, methods: Six hundred and four patients who were undergoing ART were selected. Samples were collected by masturbation after sexual abstinence for 2-5 days. After semen liquefaction, semen analysis was performed (concentration, progressive motility, vitality, strict morphology and polymorphonuclear neutrophils). Motile sperm were selected by swim-up according to the procedures established by the World Health Organization (2010). Sperm DNA fragmentation was assessed over motile sperm by TUNEL assay. For statistical analysis Pearson´s correlation and ANOVA were performed.

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Main results and the role of chance: The correlation between TUNEL sperm concentration was: R = -0.29, sperm motility R= -0.58, strict morphology R= -0.43, vitality R= -0.44 and PMN R= 0.10. All the correlations were statistically significant ($p < 0.05$). When correlations were compared, sperm motility, morphology and vitality were significantly higher than the others, but not were different between them. DNA fragmentation levels were 10.8 ± 8.2 in normozoospermic men (N: 397), 17.3 ± 9.9 in oligozoospermic men (N: 28), 26.8 ± 8.0* in asthenozoospermic men (N:36) and 21.1 ± 10.1* in men with teratozoospermia (N:100) (*$p < 0.05$). When two or more sperm anomalies were found, e.g. asthenoteratozoospermia and oligoasthenoteratozoospermia, DNA fragmentation increased up to 30.2 ± 10.5* and 32.2 ± 8.5*, respectively.

Limitations, reasons for caution: In some Andrology laborartories, sperm DNA fragmentation assay is perfomed in raw samples, we believe that is most important to evaluate DNA damage only in motile (alive) spermatozoa.

Wider implications of the findings: These kind of patients (astheno, terato, asthenoteraro and OAT) had higher levels of DNA damage in compared with normozoospermic and oligozoospermic men, this could be considered as an alert to suspect high levels of DNA damage.

Trial registration number: None.

P-100 Role of Gender Selection in ART

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Study question: We attempt to address an explicit request of infertile couples to select spermatozoa of a specific gender.

Summary answer: Individual selection of spermatozoa post enrichment yielded the desired gender in the extreme majority of cases and this proportion was maintained in the resulting embryos.

What is known already: Several techniques have been developed to successfully select for X- or Y-bearing spermatozoa. Centrifugations methods and layering techniques, as well as the use of electrophoretic devices, have all been previously tested. The most effective method to date was through flow cytometry, which was capable of achieving a selection of 90% X- and 80%. Although successful, this method was expensive and required exposing spermatozoa to a fluorescent dye. We experimented with a method to allow spermatozoa to select themselves and generate embryos in couples being treated with ICSI.

Study design, size, duration: Over a course of 3 years, we processed by density gradient ejaculates from 21 couples being treated by ICSI. Fluorescent in situ hybridization was used to assess the proportion of X- and Y- bearing spermatozoa prior to and following selection. Single spermatozoa from the gender-enriched fraction were selected for injection according to an empirical head morphology. Preimplantation genetic diagnosis (PGD) results and pregnancy outcomes were recorded. Additionally, 26 couples were treated in 48 IUI cycles.

Participants/materials, setting, methods: A total of 47 couples were enrolled in our IRB study. A combination of gradient formulations was used for the ICSI and IUI treatments. During the ICSI procedure, an individual sperm selection was also implemented by carefully assessing spermatozoon. We confirmed the successful selection of the desired gender by performing cytogenetic analysis with 9 chromosome FISH on at least 1000 cells for each specimen.

Main results and the role of chance: A total of 47 couples (sperm concentration 66.7 ± $27x10^6$/ml, 46.4 ± 5% motility, normal morphology). Prior to selection, average total aneuploidy for our subjects was 3.4 ± 2%, compared to a normal value of <1.6%. After selection, our method consistently yielded 81.4% for X- and 78.0% for Y-bearing spermatozoa. In 21 couples treated by ICSI, GS enrichment was followed by pickup of individual spermatozoa selected according to their head morphometry. These couples achieved an average fertilization rate of 79.6% (164/206) and cleavage rate of 98.1%. Of these couples, 11 elected for PGS on the resulting embryos. PGS analysis evidenced that 3/3 (100%) couples selecting for male and 6/8 (75%) couples selecting for female obtained embryos of their desired gender. All embryos were cryopreserved. So far, 5 couples had their embryos thawed (n = 11), 4 for female and 1 for male. Out of the remaining ICSI couples, of those that had an embryo replacement, 7 selected for female and 1 for male. Only 1 couple reported an ongoing pregnancy of female gender. No one else reported a pregnancy. In 26 couples with an average maternal age of 37.7 ± 3yrs undergoing IUI, post sperm gender selection achieved 4 ongoing pregnancies. Thus far, 1 couple delivered the desired baby girl.

Limitations, reasons for caution: Our findings suggest that although it is possible to skew a spermatozoa population towards a specific gender, results are not definite despite taking into consideration sperm head morphometry. In this population with higher incidence of sperm aneuploidy, PGS on the conceptus can help identify euploid embryos of the desired gender.

Wider implications of the findings: Multi-gradient gender selection methods, combined with individual spermatozoa identification by empirical head morphometry, can consistently help identify X- or Y-bearing spermatozoa. If proven reliable, this method to individually select spermatozoa of a particular gender may alleviate sex-linked disorders as well as aid with family planning in a morally accepted manner.

Trial registration number: Not Applicable.

P-101 Sperm nuclear structure in infertile patients with "pinhead sperm" syndrome

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Study question: The impact of a nuclear spermatic quality analysis in patients presenting with "pinhead" sperm defect could assess the chances of success in assisted fertilization?

Summary answer: Infertile men with "Pinhead sperm" have an impaired nuclear sperm which obviously affects the fertilizing sperm power.

What is known already: "Pinhead sperm" or "acephalic sperm" syndrome is a rare but severe form of human teratozoospermia, refers to the condition in which ejaculate contains mostly sperm flagella without heads. The familial incidence and the typical phenotype strongly suggest the genetic origin of the syndrome. Ultrastructral studies of acephalic spermatozoa suggest that this condition results from defects in formation of the connecting piece of spermatozoa during late spermiogenesis. Spontaneous fertilization cannot occur with these sperm and ICSI is the only way in which embryos can be obtained. Otherwise, few data regarding the nuclear integrity of acephalic spermatozoa were reported in the literature.

Study design, size, duration: This is a case control study carried out on 4 Tunisian infertile patients with "Pinhead sperm" syndromeand 20 fertile men with normal semen profiles included as a control group. Patients were recruited between January 2010 and March 2016.

Participants/materials, setting, methods: Semen samples were analyzed according to the World Health Organization criteria (2010). Chromatin condensation was assessed by aniline-blue Staining. Sperm DNA fragmentation was evaluated by terminal desoxynucleotidyl transferase mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) assay and chromosome abnormalities by fluorescence *in situ* hybridization (FISH) for chromosomes X, Y and 18.

Main results and the role of chance: Medical history, hormonal levels, lymphocyte karyotype and molecular analyses of Y chromosome microdeletion were normal. Sperm analysis showed numerous isolated motile tails and fewer isolated heads. The mean DNA fragmentation index was significantly higher in patients compared to the controls (35 ± 6.21 vs 10.41 ± 3.77). The aniline blue-reacted spermatozoa rate was also high in comparison to the controls (63.66 ± 6,027 vs 11.80 ± 5.7). The rate of total sperm aneuploidy was higher in patients compared to the controls (3.74 ± 0.68 vs 1.48 ± 0.22) with a predominance of sex chromosomes disomy (1.86 ± 0.73% vs 1 ± 0.3%).

Limitations, reasons for caution: A limitation of this study is the low number of patients with "Pinhead sperm" considering the rarity of this syndrome.

Wider implications of the findings: Our results confirm the importance of sperm chromosomal aneuploidy, chromatin condensation and DNA fragmentation analysis for patients with "Pinhead sperm" syndrome in order to adopt the best course of treatment for these patients and to predict the chances of success in assisted reproductive technology(ART).

Trial registration number: not applicable' for non-clinical trials.

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P-102 Oral antioxidant therapy improves sperm quality in patients with abnormally high levels of apoptotic spermatozoa

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Study question: To determine whether two months of oral antioxidant treatment could improve sperm quality in neat sperm samples with abnormally high levels of apoptosis.

Summary answer: Oral antioxidant therapy during two months significantly reduces apoptosis levels in sperm samples improving clinical results.

What is known already: Traditional semen analysis is not always sufficient for adequate male infertility diagnosis. In consequence, recent studies have focused on the comprehensive evaluation of sperm features. Apoptosis is a process that induces cellular, morphological and biochemical alterations leading the cell to suicide without an inflammatory response. Despite apoptosis occurs continuously in the testicle, it is known that high apoptosis levels have a negative impact on embryo development and pregnancy outcomes. Several strategies such as magnetic activated cell sorting (MACS) have been proposed to reduce apoptotic sperm levels, however the antioxidant therapy seems to be an easier solution to this problem.

Study design, size, duration: Unicentric and retrospective study including 156 sperm samples from patients undergoing egg donation treatment at our center between January 2014 and September 2015. The influence of oral antioxidants such as vitamins, diclofenac and Seidiferty in human sperm samples with 2 days of sexual abstinence, after two months of therapy is explored in this study.

Participants/materials, setting, methods: Apoptosis was assessed by flow cytometry using Annexin-V-FITC/PI assay in the first visit and two months later at the start of treatment.

When apoptosis level was in normal range (≤20%), patients did not take any medication (control group,n = 34). When altered on the first visit, they initiated the intake of vitamins (group 1,n = 23), vitamins and diclofenac (group 2, n = 73), Seidiferty® (group 3,n = 8) or vitamins, diclofenac and Seidiferty® combined (group 4,n = 17). Apoptosis levels of the groups were statistically compared.Main results and the role of chance:

When assessing the effect of the oral antioxidant treatment on the study groups, a significant reduction of apoptosis levels were found in all groups (group 1: 20.54% to 14.82%, p = 0.025; group 2: 21.42% to 13.45%, p = 0.001; group 3: 25.27 to 8.64%, p = 0.012; group 4: 28.42% to 11.52%, p = 0.002). Results show that the administration of antioxidant treatment to patients producing sperm samples with altered levels of apoptosis improves sperm quality significantly, reducing the number of apoptotic spermatozoa in the ejaculate.

Moreover, in the control group we could find a significant increase in the apoptosis level from 7.82% in the first visit to 13.91% (p = 0.006). Although the level of apoptosis from control group patients remains in the normal range, the absence of medication leads to an increase in the number of apoptotic spermatozoa present in the ejaculate.

Limitations, reasons for caution: The retrospective and unicentric nature of the study, together with the limited number of samples may be reasons for caution. Further studies should be performed to evaluate the effects of oral antioxidant treatment in other sperm quality parameters.

Wider implications of the findings: This study demonstrates that the evaluation and treatment of sperm samples with pathologic levels of apoptotic spermatozoa prior to the completion of the fertility treatment may improve, embryo development and clinical outcomes.

Trial registration number: Non applicable.

P-103 Sperm count upon switch from valproic acid to other antiepileptic drugs of male subfertile patients

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Study question: The aim of the study was to determine whether switching from valproic acid to other antiepileptic drugs improves the sperm counts of male subfertile patients.

Summary answer: There is significant improvement of the sperm count and sperm parameters after switching from valproic acid to other antiepileptic drugs.

What is known already: Apart from epilepsy per se and its role on hypothalamic-pituitary-gonadal axis function, antiepileptic drugs (AEDs) may also have an important impact on hormonal regulation, affecting peripheral endocrine glands, hormones' protein binding and metabolism.Valproic acid (VPA) is a widely used AED, whose adverse reactions in fertility are not limited to women. Effects of VPA on male reproductive health occur early during treatment, with increase in serum testosterone, free androgen index, androstendione and dehydroepiandrosterone sulfate.

Study design, size, duration: Observational study of adult subfertile epilepsy male patients, long term treated with VPA, recruited in a period of three years.

Participants/materials, setting, methods: Seventeen subfertile male patients, followed up at the University Department of Neurology were referred for fertility management at the Assisted Reproduction Unit, Department of Obstetrics and Gynecology. A sperm count was conducted while they were treated with VPA and six months after switching to another AED. Pregnancies, after switching, were also reported.

Main results and the role of chance: A significant improvement of the sperm count was observed in eleven patients (65%) and spontaneous pregnancies were reported in three of the patients.

Limitations, reasons for caution: This is an observational study without blind controls in patients with epilepsy; effective seizures control upon switch is a potential confounding factor of the sperm count improvement

Wider implications of the findings: Epilepsy is a common disorder affecting people with high prevalence in the young age. Epilepsy and AEDs may affect family planning and cause infertility problems. Fertility specialists treating subfertile patients with epilepsy have to be familiarized with issues emerging from certain AEDs and refer their patients for antiepileptic treatment reconsideration.

Trial registration number: None.

P-104 Sperm DNA Fragmentation and Semen Parameters in a cohort of Brazilian Infertility Patients

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Study question: Is there a correlation between sperm DNA fragmentation (SDF) in a cohort of Brazilian infertility patients and their lifestyle and semen parameters, such as sperm morphology, concentration, and motility?

Summary answer: Patients showed high levels of SDF regarding aging, sexual abstinence and lower sperm parameters. No association between alcohol or cigarette consumption and SDF was found

What is known already: Sperm DNA integrity has emerged as an important parameter of sperm quality in the prognosis of infertility and in the assisted reproductive outcomes. There are controversial studies related to an adverse effect of sperm DNA damage on fertility. These studies emphasize that the discrepancy is due to the various methods used for evaluating the cut-off value of SDF, due to different detection methods, studied population, and type of assisted reproductive procedure.

Study design, size, duration: Two hundred and one patients undergoing a private andrology clinic for fertility evaluation were subjected to routine sperm analysis in a cross-sectional study. Personal habits (alcohol consumption and cigarette smoking) were also verified. Patients were stratified into groups regarding age, sexual abstinence, semen parameters, lifestyle habits, and sperm DNA fragmentation.

Participants/materials, setting, methods: Samples were assessed according WHO 2010. SDF was assessed by sperm chromatin dispersion (SCD) test.

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Linear correlation was conducted using Spearman's coefficient. Quantitative variables with normal and asymmetric distribution were respectively compared according to Student's t-test or Mann-Whitney/Kruskal-Wallis test. Results were considered to be statistically significant when $p < 0.05$

Main results and the role of chance: Older men showed higher SDF than younger patients in two different analysis ($p = 0.021$; $p= 0.0221$). Sexual abstinence time was related to SDF ($p = 0.045$), with 3 days of abstinence with the higher index (15.8%). With respect to sperm concentration, oligozoospermic men presented more SDF in distinct analysis ($p= 0.014$; $p = 0.012$). When considering the cut-off value of 30% for SDF, there was an inverse correlation between SDF rate and sperm concentration ($p = 0.0021$). Total sperm motility was significantly related to SDF ($p < 0.0001$). When comparing different patients with rates below or above 40% of total motility, astenospermic group presented a higher SDF ($p = 0.0003$). Comparing patients with DNA fragmentation below or above 30%, higher SFD rates were related to lower motility ($p = 0.0008$). Regarding Kruger sperm morphology, there was a negative correlation with SDF ($p = 0.016$). Patients with ≥ 30% SDF had lower number of morphologically normal sperm ($p = 0.014$). Our data does not support an association between alcohol consumption ($p = 0.82$) or cigarette smoking ($p = 0.93$) and SDF.

Limitations, reasons for caution: Our results do not support that lifestyle habits such as alcohol consumption or cigarette smoking are related to high sperm DNA fragmentation, maybe due to the small number of heavy consumers in the sample analyzed (17.2% and 15.1%, respectively).

Wider implications of the findings: The analyzed cohort of Brazilian infertility patients showed high levels of SDF related to aging, sexual abstinence and lower sperm parameters such as concentration, motility and morphology

Trial registration number: Not applicable, due to the cross-sectional design of the study.

P-105 Curcumin protects human sperm DNA against ROS after freeze-thawing process

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Study question: To examine if curcumin could contrast the ROS-mediated alterations to the motility, viability and DNA integrity of human spermatozoa after a freeze-thawing process.

Summary answer: The addition of curcumin in freezing medium improves the quality and DNA integrity of cryopreserved human semen.

What is known already: The mechanism of reduction of fertilizing capacity of cryopreserved spermatozoa is not still completely understood. ROS influence the sperm function as well as its potential fertility after freeze thawing. Higher production of ROS causes sperm cells immobility and damages DNA. Antioxidant systems are the major defense approach against ROS during semen cryopreservation. Curcumin is one of the natural antioxidants which act as an anti-inflammatory,antitoxic and anticancer agent for medical treatment.With regard to its biological, pharmacological and antioxidant activities, curcumin has been shown to improve frozen-thawed goat, bovine and boar semen qualities. No previous study has been performed on cryopreserved human semen.

Study design, size, duration: Semen samples from 150 men were obtained by masturbation following 3–5 days sexual abstinence from June to December 2016. After liquefaction, the samples were examined for sperm concentration, viability, and motility according to WOH. Semen were divided into 3 groups: one group of frozen-thawed samples without addition of curcumin as negative control and two groups of semen samples with addition of curcumin (10 and 20 μM) in freezing medium.

Participants/materials, setting, methods: The semen freezing was conducted in accord to the manufacturer's instructions (Quinn's Advantage™Sperm Freeze).Sperm parameters, such as progressive motility and concentration were analyzed with Makler camber while sperm viability and morphology were evaluated by Eosin- Nigrosin Test and by Testsimplets® prestained slides respectively.Sperm DNA fragmentation was evaluated by TUNEL Test and the intracellular ROS level was assessed by DFC Assay. The data were analyzed using the unpaired Student's t-test and considered significant if p-value≤0.05.

Main results and the role of chance: None significant differences were found between negative group and group supplemented with 10 μM curcumin. There was a significantly higher percentage of progressive motility in the 20 μM curcumin groups than in the negative group ($p \leq 0.05$). Statistically significant difference in intracellular ROS levels and DNA damage have been observed in 20 μM curcumin group also. In particular, ROS percentage and DNA damage was significantly higher in semen thawing without curcumin compared sperm curcumin supplemented in frozen medium. The supplementation of curcumin at 20 μM in the freezing medium yields better frozen-thawed human semen qualities in terms of progressive motility and DNA fragmentation linked to a ROS reduction when compared with control and other treatment group.

Limitations, reasons for caution: None

Wider implications of the findings: The present study demonstrates the beneficial effect of adding curcumin during cryopreservation on the qualities of frozen-thawed human semen. We suggest that human sperm is able to uptake substances such as antioxidants and utilize them for protecting its plasma membrane and DNA from intracellular ROS during cryopreservation.

Trial registration number: None.

P-106 ART outcome of non-obstructive azoospermia in terms of testicular sperm number by micro-TESE through 16 years experience

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Study question: Is there any difference of ART outcome after ICSI with frozen-thawed testicular spermatozoa of non-obstructive azoospermia in terms of obtained testicular sperm number?

Summary answer: The normal fertilization rate, the implantation rate and the delivery rate per ET in the tiny amount of sperm group were significantly lower.

What is known already: The introduction of ICSI has revolutionized the treatment of severe male infertility. ICSI using testicular spermatozoa has been commonly applied in the treatment of obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Moreover, the ART outcome of NOA is significantly lower than that of OA. However, the number of reports regarding the ART results in terms of obtained testicular sperm number retrieved from testes in NOA patients is still small.

Study design, size, duration: This is a retrospective study of ART outcome in 529 cycles (5,693 oocytes) with ICSI using testicular spermatozoa in NOA patients from 1999 to 2014 in our clinic.

Participants/materials, setting, methods: Sperm number was calculated after sperm preparation before ICSI. Tiny amount of sperm (TAS) was defined less than one sperm in one field of view under 400 magnifications. Meanwhile small amount of sperm (SAS) was defined more than one sperm in one field of view under 400 magnifications. The ART results including the fertilization rate, the implantation rate, the delivery rate and the mean birth weight were compared between TAS and SAS groups.

Main results and the role of chance: The wife's average age, the husband's average age and the number of the oocytes retrieved were comparable between TAS and SAS groups (the wife's average age; 35.3 ± 4.2 vs 35.5 ± 4.6, the husband's average age; 37.9 ± 6.4 vs 39.0 ± 7.9, the number of the oocytes retrieved; 10.2 ± 7.1 vs 10.4 ± 7.8). However, the normal fertilization rate in TAS group (59.5%) was significantly lower than that in SAS group (68.9%). The average number of embryo transferred was comparable between the two groups (1.6 ± 0.6 vs 1.7 ± 0.8). However, the implantation rate, the clinical

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pregnancy rate (FHB) per ET and the delivery rate per ET in TAS group were significantly lower than those in SAS group (the implantation rate; 14.8% vs 23.6%, the clinical pregnancy rate per ET; 19.8% vs 31.2%, the delivery rate per ET; 14.3% vs 26.3%). The mean birth weight, the pregnancy week at term and the congenital abnormality rate were comparable between the two groups (the mean birth weight; 3014.6 ± 334.6 g vs 2703.8 ± 682.0 g, the pregnancy week at term; 38.0 ± 2.4 vs 38.0 ± 2.6, the congenital abnormality rate; 2.6% vs 3.4%).

Limitations, reasons for caution: Genetic counseling and informed consent should be given prior to ICSI with NOA patients.

Wider implications of the findings: The results of this study showed that the normal fertilization rate, the implantation rate the clinical pregnancy rate per ET and the delivery rate per ET were low when the obtained testicular sperm number by micro TESE was very small in NOA patients.

Trial registration number: N/A.

P-107 Temporal trends of semen quality among infertile Greek couples undergoing oocyte donation cycles between 2007 and 2016 in a single IVF centre. A retrospective study

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Study question: Do semen parameters of male partners of infertile Greek couples undergoing oocyte donation cycles in a single IVF centre exhibit statistical significant changes over the last 10 years?

Summary answer: Semen concentration increased but without upward trend. Sperm motility and sperm concentration <20million/mL showed an absence of significant change. Rapid progressive motile sperm deteriorated significantly

What is known already: Temporal global trends of sperm quality remains a matter of debate. Earlier studies suggest a geographical and time related decline in sperm quality parameters while more recent reports failed to reveal a statistical significant change or temporal trend. Such controversies are attributed to the selection bias of the population studied, failure to adjust for confounding factors and different statistical tools employed for analyses. It is widely believed that exposure to endocrine disruptors during fetal life can damage testicular function, leading to testicular cancer and impairment of the reproduction.

Study design, size, duration: This is a retrospective, descriptive study using data registered in a single IVF centre located in Thessaloniki Greece during the period 2007-2016. Data regarding sperm concentration and sperm motility were processed and analyzed from a total of 1113 participants with a mean age of 43.8 (SD, 6.1) years that fulfilled the inclusion criteria.

Participants/materials, setting, methods: The study population included male partners of infertile Greek women living in the greater area of Thessaloniki and undergoing oocyte donation treatment for the first time in a single IVF centre. Semen analyzed was a fresh ejaculate delivered by masturbation. Patients excluded were those with a frozen sperm sample, with obstructive azoospermia, with a history of cryptorchidism and genital cancer or consumption of steroids and procreative drugs.

Main results and the role of chance: Analyzing 1113 semen samples over a period of ten years, the main outcomes were: mean sperm concentration increased significantly between 2007 and 2015 (39.5million/ml versus 59.1million/ml; $P < 0.001$) but without forming a visible upward trend over the years, mean total motility remained statistically unchanged (56% versus 49.8%; $P = 0.239$) as well as the percentage of sperm concentration of less than 20million/mL (23.5% versus 24.4%; $P = 0.249$), whereas mean of rapid progressive motile sperm decreased significantly (41.1% versus 30%; $P < 0.001$), forming a visible downward trend.

Limitations, reasons for caution: This is a retrospective study where the population studied was not adjusted for several confounding factors. Sample size exhibited annual variations and the participants were of advanced age, therefore a degree of selection bias can not be exclude. Prospective, large-scale, population-based studies are needed in order to confirm our results.

Wider implications of the findings: To our knowledge, there is only one study, evaluating possible trends of semen quality in the Greek population published in 1996 and concluding a significant temporal decline in seminal volume and count. The present study ends this long paucity of data, providing evidence that disprove the earlier findings.

Trial registration number: Not applicable for non-clinical trials.

P-108 Do childless sperm donors have a different profile than those who have children? Assessment of the first recruitments

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Université Paris Descartes- Sorbonne Paris Cité- Faculté de Médecine- Assistance Publique– Hôpitaux de Paris AP- HP- Hôpital Universitaire Paris Centre- Centre Hospitalier Universitaire CHU Cochin, Service d'Histologie-Embryologie-Biologie de la Reprod,

Study question: Has the change in donor legislation in France affected the profile and motivations of men wanting to be sperm donors?

Summary answer: Childless donors showed a different profile than donors who have conceived. Altruism was the most important motivation and sperm cryopreservation rarely motivated their donation process.

What is known already: In France, sperm donation proceeds through voluntary, anonymous and unpaid donation. Donors must be under 45 years old, and until 2015, only men who have procreated could donate their sperm. Following a change in the law (Law of Bioethics 2011, Decree 2015), childless men now also have the possibility to donate their gametes, and unlike donors having procreated, they are offered sperm cryopreservation. Studies carried out previously in sperm donors having procreated, showed that most of them were in agreement with anonymity, sensitized by a couple requesting donor insemination, and that altruism most often motivated their approach.

Study design, size, duration: This was a retrospective descriptive study that looked at 82 men who approached the CECOS of Paris Cochin between the October 2015 and August 2016, i.e. just after the change in legislation (October 2015). A total of 82 sperm donors with or without offspring and aged 18-44 were included.

Participants/materials, setting, methods: Two groups were compared: 41 sperm donors with offspring and 41 childless sperm donors. Socio-demographic characteristics, motivations, attitudes toward anonymity and sperm cryopreservation (childless donors only) were analyzed in the two groups. Additionally, sperm parameters were compared between childless sperm donor and father sperm donors.

Main results and the role of chance: Childless sperm donors were significantly younger than donors with offspring (33.4+/-7.0 vs 36.2+/- 5.0 $p = 0.04$) and were less likely to be in a relationship than father-donors (26.8% vs 85.4% respectively; $p < 0.001$). The childless sperm donors were more likely to have a wish for a child than the donors with children. No difference was found between the two groups regarding anonymity and disclosure to their relatives. Childless donors were mostly sensitized by media or promotional campaigns ($p = 0.009$), while donors with children were mostly sensitized by infertile couples requesting sperm donor insemination in their family circle ($p = 0.002$). Altruism was the most important source of motivation among the donors without offspring (78.0%) and was more frequent than the other sources of motivation compared to the donors with offspring (53.6%) ($p = 0.02$). Sperm cryopreservation was a source of motivation in only 4.9% (2/41) of childless donors. In the above mentioned group, 43.9% (18/41) of men were approved for a donation and among them 66.6% (12/18) refused sperm cryopreservation for themselves. The exclusion rate was similar in the two groups (21.9% vs 17.1% respectively) as well as the drop-off rate (21.9% vs 36.6% respectively). No difference was found between the two groups regarding sperm parameters.

Limitations, reasons for caution: As this was a retrospective study, there is a chance for the introduction of bias.

Wider implications of the findings: We have shown that differences exist between sperm donors with and without offspring regarding age, family status, childbearing motivation and recruitment mode. Surprisingly, sperm self-cryopreservation, which is proposed as a reward, is not that successful among

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the childless donors and altruism is always the main motivation for sperm donation.

Trial registration number: Not applicable.

P-109 Testis development in the absence of SRY: chromosomal rearrangements at SOX9

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Study question: to describe and analyze several pathophysiological mechanisms put forward to explain a rare case of a 46 xx male man with male phenotype and azoospermia.

Summary answer: Even in absence of SRY, complete male differentiation may occur, possibly driven by overexpression of SOX9 in the gonadal ridge

What is known already: 46, xx disorders are congenital conditions in which, in the presence of a female karyotype, the development of gonadal and anatomical sex is atypical, ranging from various degrees of ambiguous genitalia to phenotypic males with azoospermia. These conditions are poorly characterized, at least in subjects whose DNA does not contain SRY, the gene triggering testis differentiation in mammals.

Study design, size, duration: A case report, A 34 year old man with complete masculinization who was referred to our institution because of a history of primary infertility.

Participants/materials, setting, methods: Repeated semen analysis, endocrinological data, testicular biopsy, karyotype analysis, Fluorescent in situ hybridization, polymerase chain reaction and Array CGH analysis.

Main results and the role of chance: Physical examination showed normal male secondary sexual characteristics and bilateral gynecomastia. Repeated seminal analysis showed complete azoospermia. Endocrinological data showed lower testosterone levels and testicular biopsy confirmed germinal cell aplasia. Peripheral blood culture for chromosome studies revealed 46xx chromosome complement. Fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) analyses excluded the presence of SRY gene. Array CGH analysis showed partially overlapping 17q24.3 duplications about 500 kb involving the gene desert region upstream of SOX9, inherited from a normal father.

Limitations, reasons for caution: The duplication is inherited by a healthy and fertile father.This suggests that a copy gain of the region does not affect sex development and fertility in 46, xy subjects, where SOX9transcription is anyway activated during gonadal development.

Wider implications of the findings: In the absence of SRY, The Reversal Sex duplication causes increased expression of SOX9 in undifferentiated gonadal cells. The duplication could alter Sox9 expression by increasing the dosage of one or more gonadal specific enhancers located within the minimal duplicated interval defined as reversal sex.

Trial registration number: not applicable.

P-110 The impact of male factor infertility (MFI) on early- and late-morphokinetic parameters: retrospective analysis of 2868 time-lapse (TL) monitored embryos

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Study question: Is there an effect of male factor infertility on either early- and/or late-morphokinetic parameters obtained during embryonic culture to blastocyst stage in a TL-monitored incubator?

Summary answer: Times from either pronuclei fading (tPNf) or 5-cells to early blastulation (periods: PSB and P5SB, respectively), were prolonged in MFI compared to non-MFI embryos.

What is known already: Studies suggest that optimal values of certain morphokinetic parameters correlate with an embryo's ploidy status and potential for blastocyst development and implantation. TL-morphokinetics might be affected not only by inherent embryonic traits but by both external factors (laboratory, culture) and patient-related characteristics, as well. Evidence of paternal influence on embryonic genome activation, early embryonic development, and blastocyst formation in-vitro has been reported. However, the potential impact of paternal factors and male infertility diagnosis, if any, on early- and late-morphokinetic parameters, remains unclear.

Study design, size, duration: Data from 2868 embryos (MFI: 1239, non-MFI: 1629 embryos) derived from 419 IVF cycles (IVF/ICSI: 267, and IVF/conventional insemination: 152) and cultured to the blastocyst stage in a TL-monitored incubator, were retrospectively reviewed. 383 women ≤38 years of age undergoing IVF at a major academic center between 09/2013 and 09/2016 were included.

Participants/materials, setting, methods: MFI embryos were compared to those derived from non-MFI patients, in regards to the following early- and late-morphokinetic parameters: time period from i) tPN*f* to 1st cytokinesis (P1), ii) 2- to 3-cells (P2), iii) 3- to 4-cells (P3), iv) 4- to 5-cells (P4), v) P5SB, and vi) PSB.

Statistics: *t*-test, chi-square were used as appropriately. $P < 0.05$ was considered statistically significant.

Main results and the role of chance: Groups did not differ in baseline characteristics (age, BMI, basal-FSH, AMH, antral follicle counts) but more MFI patients required ICSI (94.3 vs. 40.8%, MFI vs. non-MFI, $p < 0.001$). Time periods PSB and P5SB lasted significantly longer in MFI compared to non-MFI embryos [mean(SD): 75.6(8.7) vs. 73.8(9.2), $p < 0.001$; 51.7(10.9) vs. 49.3 (10.8), $p < 0.001$, respectively]. This difference persisted even when analyzing *usable* (either transferred or frozen blastocysts) separately from *discarded* embryos [*usable*: 72.6(7.0) vs. 71.2(7.7), p: 0.004; and 47.4(8.1) vs. 45.7(8.3) hours, for PSB and P5SB, respectively; and *discarded*: 78.9(9.2) vs. 77.1(9.8), p: 0.006; and 56.5(11.6) vs. 53.5(12.0) hours, $p < 0.001$; for PSB and P5SB, respectively).

All other time periods did not differ significantly between the two groups when either: i) all embryos were included (P1: 2.9(1.6) vs. 2.9(2.0), P2: 9.5(4.8) vs. 9.7(4.7), P3: 2.8(4.4) vs. 2.8(4.4), and P4: 9.2(6.2) vs. 9.9(6.3) hours, for MFI vs. non-MFI, respectively), or ii) *usable* (P1: 2.6(0.6) vs. 2.7(0.6), P2: 10.5(3.3) vs. 10.2(3.2), P3: 1.9(3.4) vs. 1.9(3.3), and P4: 10.6(4.3) vs. 10.7(4.5) hours, for MFI vs. non-MFI, respectively), and iii) *discarded* embryos (P1: 3.0(2.1) vs. 3.1 (2.4), P2: 8.7(5.6) vs. 9.2(5.7), P3: 3.6(4.9) vs. 3.7(5.0), and P4: 8.0(7.1) vs. 9.3 (7.5) hours, for MFI vs. non-MFI, respectively) were analyzed separately.

Limitations, reasons for caution: Limitations include the retrospective design and a potential effect of the fertilization method. To account for the latter and decrease possible bias, times were normalized to a common starting time-point irrespective of fertilization method. Only women ≤38 years were included, so results may not be generalizable to women >38 years.

Wider implications of the findings: The possible effect of MFI on embryonic development was more pronounced from the late cleavage to blastocyst stage, causing a prolongation of both time-periods to early blastulation. No difference was noted in early-morphokinetic parameters, a finding suggestive of a possible late paternal effect visible after paternal genome activation.

Trial registration number: Not applicable.

P-111 Evolutionary-like selection on-a-chip: Using microfluidics to isolate the fittest sperm

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Study question: To develop a microfluidic device for selective sorting of motile, morphologically normal sperm with high DNA integrity for ICSI applications.

Summary answer: Microfluidic chip sorted human sperm showed higher motility, morphologically normal and DNA integrity compared to unsorted or traditionally sorted sperm.

What is known already: The path that sperm take through the female reproductive system has been conserved for millions of years. This suggests that this sperm journey has significant evolutionary importance. In fact, we know very little about how this reproductive journey influences or "filters" sperm quality for fertilization. We hypothesize that evolutionarily important attributes of sperm are improved by this sperm journey. We sought to partially mimic this complex pathway using microfluidics by creating various three-dimensional geometries within microscopic channels. Geometric selection sets were developed based on known hydrodynamic principles that guide the interaction between sperm and surrounding periodic structures.

Study design, size, duration: Sperm sorting analysis was performed using de-identified discarded human semen samples from REI Laboratory, Stanford School of Medicine, Stanford University. All the sperm processing experiments were performed within 1 to 3 hours after the semen samples were collected. The unprocessed semen sample was injected into microfluidic device to sort highly motile sperm. Similarly, unprocessed semen sample was also subjected to traditional swim-up method to compare the sperm sorting efficiency.

Participants/materials, setting, methods: The sperm sorting microfluidic chip consists of inlet, outlet chamber and a middle channel featuring pillars (10 dia.), which are variably spaced to facilitate the transport of the motile sperm into the outlet chamber. Initially, the device was filled with sperm washing media, and the semen sample was injected to the channel inlet. Followed by the incubation at 37 °C for 10-30 min, outlets imaged and sperm trajectories were analyzed using CASA.

Main results and the role of chance: All the sorted sperm samples were analyzed using ImageJ CASA plugin system for sperm motility, trajectory kinetics and percentage of motile sperm. Further, the sorted sperm and unprocessed semen were subjected to morphology and DNA integrity analysis. The results showed that sperm sorted by the microfluidic device have higher motility (98%) as compared to sperm sorted in swim-up approach (65%), and unprocessed semen (43%). Similarly, higher percentage of morphologically normal sperm (51%) was observed for sperm sorted using the microfluidic chip compared with sperm sorted using swim-up (25%), blank channel (37%) and raw semen (11%). Further, DNA integrity of sperm sorted by microfluidic chip was higher compared to sperm sorted using swim-up and raw semen samples. The higher overall sperm quality sorted by the microfluidic chip method is due the unique space constrained pillar geometry in chip, which does not only sort but directionally navigates the motile sperm.

Limitations, reasons for caution: The developed microfluidic sperm sorting device is optimized with specific channel dimension and pillar geometry that are able to isolate the functional sperm with a shorter duration (10 minutes). On the other hand, sperm sorting duration can be increased for handling the sample with lower sperm motility or count, e.g. azoospermia.

Wider implications of the findings: This approach has implications for developing user-friendly, standardized and improved sperm selection methods for assisted reproduction.

Trial registration number: No Trial done in this study.

POSTER VIEWING SESSION
EMBRYOLOGY

P-112 Performing oocyte denudation for ICSI without delay does not compromise cycle outcomes

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Study question: Is there a benefit from delaying oocyte denudation before ICSI in order to achieve better reproductive outcomes?

Summary answer: There is no significant difference in fertilisation and pregnancy rates between early *vs.* delayed denudation of the oocyte in fresh non-donor ICSI cycles.

What is known already: There is increasing pressure on IVF laboratories to work more efficiently in order to meet the rising demands on ART services. Before the ICSI procedure, oocyte denudation of the corona-cumulus-complex (CCC) must be performed. The presence of CCC has been suggested to improve oocyte cytoplasmic maturation and embryonic metabolism. Various studies have been conducted to evaluate the optimal timing of denudation without reaching definitive conclusions.

Study design, size, duration: Prospective cohort observational study of 2052 fresh non-donor ICSI treatment cycles carried out between January 2015 and October 2016.

Participants/materials, setting, methods: ICSI was performed following controlled ovarian stimulation (COH); oocyte retrieval was undertaken 36-hours after maturation trigger injection. Patients were divided into two groups: group I (n = 1064) had the oocytes denudation within 2-hours of retrieval, and group II (n= 988) within 2-5 hours. Timings were recorded on an automated radio-frequency laboratory database. StatView-software for statistical analysis was used to compare fertilisation/ pregnancy rates, and the mean number of embryos transferred/ cryopreserved between the two groups.

Main results and the role of chance: We found no effect of the denudation time on ICSI outcomes. After controlling for confounding variables, groups I and II were comparable in terms of mean patient age (35.5 *vs.* 35.6 years, *P* 0.9), number of previous treatment cycles (1.6 *vs.* 1.5, *P* 0.3) and the total dose of gonadotropins used (2767 *vs.* 2790 IU, *P* 0.7). There was no significant difference with regard to the mean number of oocytes retrieved (11.1 *vs.* 11.2, *P* 0.8), and the number of oocytes injected with sperm (9 *vs.* 8.8, *P* 0.6). The sperm used were ejaculated in 94 *vs.* 93.4% (*P* 0.9), while surgically retrieved in 6 versus 5.2% (*P* 0.9) in groups I and II, respectively. The post sperm preparation motility was 89 *vs.* 88% (*P* 0.24). The mean number normally fertilised oocytes was 6.1 *vs.* 6 in group I and II, respectively (*P* 0.6), while the number of embryos transferred was 1.7 versus 1.6 (*P* 0.5). The number of surplus embryos cryopreserved at the blastocyst stage was 2.3 versus 2.1 (*P* 0.12). The pregnancy, clinical pregnancy and implantation rates were 38 versus 37, 31 versus 30 and 29 versus 27 in groups I and II, respectively, *P* 0.5, 0.4, 0.2).

Limitations, reasons for caution: This is the first study conducted prospectively on a large number of participants. However we evaluated time points up to 2 hours then between 2 and 5 hours post oocyte retrieval. Assessing hourly intervals of retrieval-denudation timings may be more informative.

Wider implications of the findings: The lack of a significant impact of the denudation time on ICSI-outcome has important implications; a delay may present logistical challenges in the laboratory and avoidable financial constrains. Reducing the time over which ICSI is completed can streamline the workload and allow more patients to access the treatment.

Trial registration number: N/A.

P-113 Sperm-specific Phospholipase-C-isoform zeta (PLCζ) is the physiological trigger of calcium oscillation in mammalian eggs

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Study question: What is the physiological agent of calcium oscillation and egg activation in mammals?

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Summary answer: Sperm-specific Phospholipase-C-isoform zeta (PLCζ) is the physiological trigger of calcium oscillations in mammals. However, calcium oscillation-independent fertilisation can still occur, albeit at greatly reduced efficiency.

What is known already: Calcium oscillation is initiated after sperm entry into the oocyte in mammals. A series of calcium spikes triggers cortical reactions to block polyspermy, resumption of the second meiotic division, and metabolic activation of the egg that precedes the first embryonic cell division. This process has been proposed to be caused by a sperm-specific PLCζ. PLCζ acts by catalysing the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) within the membrane of the oocyte to generate inositol 1,4,5-trisphosphate (InsP3). InsP3 binds to its receptor on the endoplasmic reticulum causing Ca^{2+} to be periodically released from the internal stores as Ca^{2+} spikes.

Study design, size, duration: CRISPR/Cas9 gene editing tool was used to knockout the *Plcz1* gene in mice. Two strategies were employed targeting different exons of *Plcz1:* one used $Cas9^{WT}$ endonuclease with single sgRNA, the other used $Cas9^{D10A}$ nickase with two paired sgRNAs. From selected F0 animals showing frameshift-producing deletions, two mutant *Plcz1* mouse lines were produced: $Plcz1^{em1Jparr}$ generated using $Cas9^{D10A}$ nickase (22-nucleotide deletion in exon 3), and $Plcz1^{em2Jparr}$ generated using $Cas9^{WT}$ endonuclease (17-nucleotide deletion in exon 5).

Participants/materials, setting, methods: To study the effect of the mutation on spermatogenesis and other sperm parameters, testicular tissue was examined by sectioning and staining methods, while epididymal sperms were evaluated in terms of viability, motility and hyperactivation, and the ability to induce acrosome reaction. For egg activation and calcium imaging, mouse eggs were incubated with Ca^{2+}-sensitive fluorescent dye, and fertilized by ICSI. Calcium spikes were registered as the 340/380 nm excitation ratio of fluorescence.

Main results and the role of chance: Histological analysis of PLCζ knockout male testes indicated no defects in spermatogenesis. Epididymal sperm showed standard viability, motility and hyperactivity parameters, which reflects their ability to undergo capacitation. Additionally, the ability of PLCζ-null sperm to undergo the acrosome reaction in response to either progesterone or ionomycin was undistinguishable from sperm of wild type animals. Collectively, these results demonstrate that loss of PLCζ has no apparent detrimental effects on spermatogenesis or parameters associated with the sperm's ability to bind and fuse with the egg. However, calcium oscillation studies post ICSI procedure showed that injecting PLCζ-null sperm from either line had failed to induce calcium oscillations ($P < 0.0001$). Such calcium oscillations were however triggered by subsequent injection of $Plcz1^{WT}$ cRNA. Remarkably however, some eggs fertilized by PLCζ-null sperm can develop, albeit at greatly reduced efficiency (two-cell stage: $P < 0.01$; blastocyst: $P < 0.01$), and after a significant time-delay. Suggesting that in PLCζ's absence, eventual, spontaneous egg activation can occur via an alternative route. The study also showed evidences of increased level of polyspermy in oocytes fertilised with PLCζ-null sperm ($P < 0.05$), due to the role of calcium oscillations in egg cortical reaction.

Limitations, reasons for caution: Deciphering whether egg activation is calcium dependent or independent is technically challenging due to the difficulty in extending calcium recordings as there is a reduction in oocyte viability. Nonetheless, we have shown that loss of calcium oscillation reduces the rate of fertilization.

Wider implications of the findings: This is the first demonstration that *in vivo* fertilization without calcium oscillations can result in offspring. PLCζ-null sperm now make it possible to resolve long-standing questions in fertilization biology, and test the efficacy and safety of artificial egg activation stimuli used to treat egg activation deficiency in human infertility.

Trial registration number: N/A.

P-114 Embryonic progesterone receptors: Do they have a role in the implantation and post-implantation development?

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Study question: Do progesterone receptors in the blastocysts have a physiological role in the implantation and post-implantation development?

Summary answer: The implantation and post-implantation development of mouse blastocysts in vitro were adversely affected by blocking progesterone receptors (PR) using PR blockers.

What is known already: The effects of progesterone depend on the existence of their specific receptors. Although PR mRNA was not found in oocytes, fertilized eggs and cleaving embryos, the expression level began to appear at the blastocyst stage. The embryonic expression of PR genes in the blastocyst suggests a possible functional requirement for PR at this stage of development. These results provide a basis for determining the direct role of progesterone in pre-implantation and post-implantation embryo development.

Study design, size, duration: The physiological function of the PR was evaluated by using the PR blockers. Mice blastocysts were cultured for 8 days to evaluate the morphological development. 700 blastocysts were collected from the ICR female mice for the in vitro culture. 100 blastocysts were collected for the immunocytochemical assay to detect the PR in the pre-implantation embryos.

Participants/materials, setting, methods: This was an in vitro experimental study involving the use of mouse blastocysts and the model of in-vitro 8-day culture. Under the RU-486 treatment, implantation and post-implantation development were evaluated daily. The differential staining of blastocysts following the treatment of RU-486 was performed to investigate its effects on the cell proliferation and apoptosis. The PR proteins in the pre-implantation and post-implantation embryos were detected through the immunocytochemical assay.

Main results and the role of chance: The PR proteins were immunostained in the pre-implantation embryos, especially at the early post-implantation stage; the implanted blastocysts and early egg cylinder stage embryos. Following the treatment of RU-486 at the concentration of 10^{-9} M or 10^{-7} M, the development from the implanted blastocysts to early somite stage in vitro 8-day culture was not affected in comparison to control group. However at the concentration of 10^{-5} M of RU-486, the development of later egg cylinder stage and early somite stage was severely affected (39.2 vs 56.4%; 24.5 vs 40.5%; treated vs control). The cell number in the inner cell mass of blastocysts following the 48-hr treatment with RU-486 was adversely inhibited. Conversely, the inhibition of trophectoderm proliferation was mild affected. In conclusion, the dose-dependent adverse effects of PR blocker on the implantation and post-implantation development were demonstrated clearly. Reviewing the literatures, this is the first study to show the physiological role of PR in the blastocysts and post-implantation embryo development.

Limitations, reasons for caution: Considering the ethical and technical limitations inherent to the use of human embryos for implantation studies, the mouse model was used as an approach for exploring the potential role of embryonic PR on the implantation and post-implantation embryo development. Nevertheless, the extrapolation of these results to humans requires further investigation.

Wider implications of the findings: This study presents clear evidence on the potential role of embryonic PR on the implantation and post-implantation embryo development. These new findings could contribute to the physiological knowledge of human embryo culture for the clinical applications in in-vitro –fertilization protocol.

Trial registration number: Not clinical trial.

P-115 Is there any correlation between oocyte polarization microscopy findings with embryo time lapse monitoring in ICSI program?

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Study question: Is there a relationship between the presence of the meiotic spindle (MS) and zona pellucida (ZP) birefringence of MII oocytes with morphokinetic variables of embryos?

Summary answer: High ZP birefringet and visualized MS oocytes have different morphokinetic behavior compared with low ZP birefringet and no visualized MS oocytes.

What is known already: Oocyte quality, especially optimal cytoplasmic and nuclear maturity is one of the most important variables determining the developmental potential of embryos. In an effort to optimize the outcomes of ART treatment, a noninvasive method, polarization microscopy imaging, for evaluating ZP birefringence and MS visualization has been adjudged as predictor factor for oocyte quality. To our knowledge, reports on possible impact of ZP birefringence and MS on early embryo morphokinetic are so limited.

Study design, size, duration: This prospective study on 54 patients performed between November 2015and October 2016. After injection, 478 MII oocytes were cultured in time-lapse incubator. The morphokinetic behavior of each embryo was monitored until day 3.

Participants/materials, setting, methods: Using a polarized microscopy, the ZP birefringence and presence of MS in MII oocytes were evaluated pre ICSI. Also, morphokinetic variables (absolute and relative time points, including time to 2nd PB extrusion (ESPB), pronuclei appearance (PNA), PN fading (PNF), time to 2 cells (t2), t3, t4, t5, t6, t7, t8, S1 (t2-PNF), S2 (t4-t3), CC2 (t3-t2) and CC3 (t5-t3) as well as abnormal cleavage patterns of 368 embryos were analyzed with time lapse monitoring (TLM).

Main results and the role of chance: t5 occurred earlier in high birefringent ZP compared with low birefringent oocytes ($p = 0.001$). In addition, t2 happened later in invisible MS compared to visible MS oocytes ($p = 0.01$). The rates of reverse cleavage as well as reverse and direct cleavages were significantly less in oocytes with high birefringent and visible MS, respectively ($p = 0.005$, $p = 0.001$ and $p = 0.001$).

Limitations, reasons for caution: The limitations of this study were small sample size plus lack of data on the implantation and pregnancy outcomes. Therefore, future studies are warranted to validate findings of the present study.

Wider implications of the findings: The present study focused on the ZP birefringence and MS integrity of the oocytes and its relationship with morphokinetic parameters and atypical cleavage using TLM. It seems that embryo selection based on polarization microscopy imaging can ameliorate good morphokinetc embryos with high implantation potential for single embryo transfer program.

Trial registration number: None.

P-116 Spermatozoa piRNA levels correlate to sperm concentration and fertilization rate after ICSI

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Study question: Is there any relationship between spermatozoa PIWI-interacting RNAs (piRNAs) levels and semen parameters? Does spermatozoa piRNAs play a role in fertilization and embryo development after intracytoplasmic sperm injection (ICSI) treatment?

Summary answer: Spermatozoa piRNA levels correlate with sperm concentration and fertilization rate after ICSI.

What is known already: Sperms contain several small noncoding RNAs, such as microRNAs (miRNAs), piRNAs and endogenous small interfering RNAs. Several transcripts present in human spermatozoa and zygote are absent in unfertilized oocytes, suggesting that spermatozoa deliver these transcripts to oocyte during fertilization and that they may play a role in subsequent zygote and embryonic development. piRNAs are mainly expressed in pachytene spermatocytes and spermatids, suggesting direct roles in spermatogenesis. The concentration of seminal plasma piRNAs are altered in infertile patients with azoospermia and asthenozoospermia compared with fertile control. Next generation sequencing revealed that 20121 piRNAs are present in human testis.

Study design, size, duration: Retrospective observational study was performed in 186 patients undergoing ICSI from April 2014 to May 2015.

Participants/materials, setting, methods: Participants includes couples who underwent first ICSI cycles in our University affiliated IVF unit with the diagnosis of male factor infertility. The levels of three piRNAs (hsa-piR-31704, hsa-piR-39888 and has-piR-40349) in spermatozoa were measured by real time RT-PCR. The relationship between semen parameters and spermatozoa piRNA levels was investigated. And the effect of piRNA expressions on fertilization and embryo development after ICSI treatment was assessed.

Main results and the role of chance: Spermatozoa levels of piR-31704 and piR-39888, but not piR-40349 were decreased in male factor infertility group as compared with control group (piR-31704, $P = 0.027$; piR-39888, $P = 0.041$). And these two piRNAs were expressed at higher levels in patients with normal sperm concentration compared with subnormal sperm concentration group (piR-31704, $P = 0.042$; piR-39888, $P = 0.047$), while there were no correlation between the three piRNAs levels in spermatozoa and the rates of sperm progressive motility and normal sperm morphology. There were significant increases of spermatozoa piRNAs levels from the group with higher 2PN rates (piR-31704, $P = 0.002$; piR-39888, $P < 0.001$; piR-40349, $P < 0.001$), but there was no correlation between spermatozoa levels of these three piRNAs and the rates of embryo early cleavage, Day-3 good-quality embryo and pregnancy.

Limitations, reasons for caution: Only ICSI cycles using ejaculated sperms were included in our study. And we only evaluated the levels of three piRNAs which were most abundantly expressed in human testis.

Wider implications of the findings: The present study demonstrated a paternal effect of piRNA on fertilization, which has not been reported before. After prospective confirmatory studies are performed, piRNAs may be used as indicator of fertilization to provide male infertility patients proper treatment before they undergo ICSI cycles.

Trial registration number: no.

P-117 Dehydroepiandrosterone sulphate (DHEAs) supplementation: A promising non-invasive futuristic strategy for oocyte activation in IVF cycles

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Study question: To investigate if baseline Day3 serum DHEAs levels in PCOS/Non-PCOS women undergoing IVF could be indicative of oocyte activation and its competence to undergo fertilization.

Summary answer: DHEAs levels in Baseline serum strongly correlate with number of MII oocytes and fertilization rates thus implicating its significant role in augmenting oocyte activation

What is known already: The sulfonated steroid DHEAs is considered to be a storage reservoir for DHEA and an inactive component in follicular steroidogenesis. However recently, DHEAs has been implicated in generation of calcium oscillations necessary for oocyte activation and its impending fertilization. Due to its unique chemical properties, this 'androgen' behaves so much like an antiandrogen that it has been proposed to be an important, potentially major endogenous estrogen in the body. Lower fertilization rates have been reported in elderly/poor responder women as well as PCOS women. Therefore, we hypothesized that DHEAs may play a significantly major role in oocyte activation and fertilization.

Study design, size, duration: In lieu of our previous study with FF DHEAs, we prospectively estimated baseline D3 serum DHEAs levels in all women (PCOS/non-PCOS, poor/average/high responders; n = 680) undergoing antagonist stimulation protocol from August 2014- December 2016 at our private infertility centre. Out of these, n = 46 cycles had total fertilization failure (TFF). So, n = 634 cycles were evaluated for fertilization rates and embryo development to blastocyst stage. DHEAs level in day3 serum sample was measured by Radio-immuno assay (RIA) method.

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Participants/materials, setting, methods: Cycles were initially classified on the basis of ovarian response into poor (<5 eggs retrieved), average (5-15 eggs retrieved) and hyper-responders (>15 eggs retrieved). Women were then classified into PCOS (n = 102) and Non-PCOS (n = 532) groups and each of these groups were subsequently divided into Low, Medium and High Baseline DHEAs Groups respectively (PCOS: n = 26, <100 μg/dL; n = 50, 100-191 μg/dL; n = 26, > 191 μg/dL. Non-PCOS: n = 140, <98 μg/dL; n = 256, 98-179 μg/dL; n = 136, > 179 μg/dL). Statistical analysis was done using Prism VI software.

Main results and the role of chance: Low and Medium groups of Non-PCOS women differed significantly in mean number of MII oocytes (6.7 and 7.0 vs. 8.8,p = 0.03), Fertilization (63 and 67 vs. 81%,p < 0.0001), Cleavage (61.5 and 64 vs. 80,p < 0.0001) and Blastocyst formation (25 and 29 vs. 39%, p = 0.01) rates compared to High day3 DHEAs group, whereas there was no such statistical difference in these parameters between low vs. medium groups (p > 0.05). Among the PCOS group, number of MII oocytes (22.07 and 22.15 vs. 25.1, p = 0.02), Fertilization (59 and 55 vs. 69%,p = 0.0003, 0.03), Cleavage (59 and 54 vs. 68%,p = 0.0006, 0.03) and Blastocyst formation rates differed significantly when compared between low and high vs. medium but not between low vs. high day3 DHEAs groups. Fertilization rates correlated with day3 DHEAs levels (Pearson r = 0.22, 95%CI=0.14-0.29). After correcting for very high levels of DHEAs in PCOS women at which also low fertilization rates were observed, Pearson r = 0.52. Lower threshold of day3 DHEAs for fertilization was >95 μg/dL whereas upper threshold limit was <270 μg/dL (ROC_{AUC} = 87.48, p < 0.0001, Sensitivity 71.92%, Specificity 78.08%). These results suggest that while high baseline DHEAs levels (95-270 μg/dL: PCOS/Non-PCOS) are conducive, very low (<95 μg/dL:PCOS/Non-PCOS) and very high levels (>270 μg/dL:PCOS), are detrimental for oocyte activation, fertilization and embryonic development.

Limitations, reasons for caution: Larger multicentric studies are required to establish the exact metabolic role of DHEAs in the process of generation of calcium oscillation and oocyte activation. Extensive research is necessary to arrive at a threshold physiologic, ovarian (not adrenal) dose of DHEAs that will be sufficient for oocyte activation in IVF cycles.

Wider implications of the findings: Mechanical, chemical, electrical methods of oocyte activation with calcium ionophores, recombinant PLCζ, mitochondrial insertion have been employed for oocyte activation. However, these are invasive or may cause unknown epigenetic effects. Therefore, understanding oocyte physiology, metabolism and endocrine pathways may prove to be more effective for oocyte activation with DHEAs supplementation.

Trial registration number: Not applicable.

P-118 Clinical outcomes of day 5 morula transfer are acceptable when no blastocysts are available after prolonged embryo culture

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Study question: How do morula and blastocyst transfers on day 5 differ in terms of pregnancy rate and outcome in the in vitro fertilization programme?

Summary answer: On day 5, the outcome of morula transfer is poorer than that of blastocyst transfer but is acceptable in terms of pregnancy rates and outcomes.

What is known already: It is not evident what is the clinical outcome of morula transfers, when no blastocysts are available. Rare studies have shown that day 5 morulae may result in an acceptable pregnancy rate (Wilson et al., 2004), although no pregnancy outcome has been reported. A significant proportion of morulae on day 5 are euploid, and the majority of them progress to the blastocyst stage by day 6 (Kort et al., 2015). Compacted morula transfer on day 5 may be a good alternative to day 6-7 blastocyst transfer (Su et al., 2016) in delayed embryos not to miss the implantation window.

Study design, size, duration: In this retrospective study we compared the clinical outcomes of early and expanding/expanded blastocyst and compacted morula transfers (if no blastocysts were available) after prolonged embryo culture to estimate the clinical outcomes of delayed embryos. The study included 694 compacted morula and 3007 blastocyst (246 early and 2761 expanding/expanded blastocysts) transfers performed between January 2011 and September 2015; the transfers were compared in terms of clinical pregnancy outcome, and newborn characteristics at birth.

Participants/materials, setting, methods: In women aged 24-43 years one or two optimal embryos were transferred into the uterus on day 5: blastocysts (early or expanding/expanded) or compacted morulae (if no blastocysts were available) based on Gardner and Schoolcraft scoring system. The factors affecting the developmental stage of transferred embryos and clinical outcomes (pregnancy and birth characteristics) of morula and blastocyst transfers were compared using the statistical tests (Chi-Square, Mann-Whitney U test, logistic regression) with significance set at p < 0.05.

Main results and the role of chance: The compacted morulae were transferred in 18.7%, early blastocysts in 6.6%, and expanding/expanded blastocysts in 74.7% of transfers. The developmental stage of transferred embryos was connected to female age (p < 0.001; OR 0.960; 95%CI 0.940-0.980) and endocrine disturbances (p = 0.012; OR 0.765; 95%CI 0.621-0.942), number of oocytes (p < 0.001; OR 1.072; 95%CI 1.051-1.092), ICSI (p < 0.001; OR 0.666; 95%CI 0.540-0.822), and sperm motility (p = 0.003; OR 1.007; 95%CI 1.002-1.011). The clinical pregnancy rate increased with increasing developmental stage of embryos (13.5%, 28.0%, and 44.1%); after morula transfer the pregnancy rate was significantly lower than after early and expanding/expanded blastocyst transfer (p < 0.01) but acceptable. There were no significant differences in ectopic pregnancy (1.0%, 1.4%, and 0.7%), miscarriage (25.5%, 31.9%, and 22.8%), and twin pregnancy (18.0%, 16.3%, and 14.1%) rates regarding the developmental stage of transferred embryos. The monozygotic twin pregnancy occurred after the transfer of a single expanding/expanded blastocyst (0.8% of pregnancies) but not in earlier stages. At birth, there were no differences in gestational age. The mean birth weight of singleton newborns (n = 797) did not differ significantly: 3203.4 g (range: 760-4470) after morula transfer (n = 55), 3201.5 g (range: 1050–4260) after early blastocyst transfer (n = 36), and 3202.8 g (range: 620–5310) after expanding/expanded blastocyst transfer (n = 706).

Limitations, reasons for caution: The results of this study show acceptable pregnancy and birth rates after transfer of compacted morulae on day 5, if no blastocysts are available. The analysis of the health status of babies born after morula transfer has been undertaken in our centre to make relevant conclusions.

Wider implications of the findings: In spite of several advantages, there are some negative aspects of prolonged embryo culture: a relatively low proportion of embryos developing to the blastocyst stage and a higher embryo transfer cancellation rate. This may be improved by morula transfer in couples without blastocysts on day 5 after prolonged embryo culture.

Trial registration number: This retrospective study was approved by the National Medical Ethical Committee; the clinical trial registration number is not needed.

P-119 Live birth delivery predictors of ICSI cycles with ejaculated sperm

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Study question: To assess whether some critical factors in intracytoplasmic sperm injection (ICSI) cycles can be used to predict the likelihood of live birth delivery (LBD).

Summary answer: In ICSI with ejaculated sperm, several cut-offs of demographic, stimulation, embryological and clinical characteristics were found associated with an increased likelihood of live birth.

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What is known already: Although numerous studies have tried to establish predictive clinical factors associated with a higher likelihood of attaining a live birth, there is still no consensus.

Study design, size, duration: Retrospective evaluation of 3844 treatment cycles using homologous ejaculated sperm and intracytoplasmic sperm injection, conducted at the Centre for Reproductive Genetics A. Barros between 2003 and 2014 (11 years).

Participants/materials, setting, methods: The 3844 ICSI cycles were divided in two groups according with their outcomes and compared regarding demographic, stimulation, embryologic, clinical and newborn (NB) characteristics: 993 cycles (25.8%) with NB and 2851 cycles (74.2%) without NB. Seropositive, anejaculation, retrograde ejaculation, preimplantation genetic diagnosis and Y chromosome microdeletions cases were excluded.

Main results and the role of chance: Higher LBD rate was associated to the following factors. Demographic: lower age and lower bFSH levels. Stimulation: lower total dose of gonadotropins, time of stimulation and dose of HCG, higher number of follicles and estradiol levels. Embryologic: higher number of aspirated oocytes and mature oocytes, and higher rates of embryo cleavage, high quality embryos and blastocysts.

Based on the present results and on those of the literature it is possible to suggest the following cut-off associated with a higher likelihood of LBD. Female age ≤ 35y; time of infertility ≤ 5y (ideal: < 2y); bFSH ≤ 10 IU/L; both antagonist and agonist protocols, as well as rFSH and HMG, gave similar outcomes, and thus the decision for one or another should be patient personalized; estradiol levels 2000-4000 pg/ml (3000-4000 pg/ml if women <38y; 2000-3000 pg/ml if women > 38y); number of follicles ≥ 4 (maximum: 14); number of aspirated oocytes ≥ 4 (maximum: 15); number of mature oocytes ≥ 4 (maximum: 15); number of high quality embryos ≥ 3; day 3 single embryo transfer when women <35y; day 3 double embryo transfer when women > 35y; single embryo transfer of blastocysts; no relation between spermiogram values and LBD.

Limitations, reasons for caution: This study included only ICSI cycles with ejaculated sperm. Additionally, seropositive, anejaculation, retrograde ejaculation, preimplantation genetic diagnosis and Y chromosome microdeletions cases were excluded. Thus, findings should remain restricted to this kind of patients.

Wider implications of the findings: From the analysis of a large series of 3844 ICSI cycles, data revealed critical demographic, stimulation and embryological factors, and cut-offs for live birth delivery are suggested. These findings are considered relevant for clinical guidance and patient information regarding the likelihood of achieving a live birth delivery.

Trial registration number: None.

P-120 Fresh embryos versus all-freezing embryos transfers strategies: nuances of a meta-analysis

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Study question: Can cryopreservation of all embryos with subsequent embryo transfers (ALL/FREEZE-ET) promote improvements in clinical ART outcomes compared to fresh embryo transfers (FRESH-ET)?

Summary answer: The all-frozen embryo transfer strategy does not have an advantage compared to fresh embryo transfer if the mean number of oocytes collected was <15.

What is known already: Meta-analysis is a powerful tool and considered the highest in the evidence-based pyramid, but their strength depends on the quality of randomized controlled trails (RCTs) analyzed. Recently, a RCT including high-responders patients, and favorable to the use of the All/Freeze-ET was retracted of the literature access (Aflatoonian,2010). This retracted RCT composes a relatively recent meta-analysis (Roque,2013) concerning of the beneficial effects of cryopreservation and subsequent frozen-embryo transfer, but with the removal of the previously mentioned study, the prior meta-analysis (Roque,2013) loses its power to assist in medical decision when the All/Freeze-ET should be used or not in clinical practice.

Study design, size, duration: A systematic review based on electronic searches in databases (PubMed, EMBASE, Web of Science, SCOPUS, and Cochrane Central Register of Controlled Trials; Keywords: all-freezing; poor-/normal-/high-responder; clinical outcomes; collected oocytes) up to December/2016 were conducted to identify RCTs that compare the ART outcomes of fresh versus elective frozen embryo transfer. The primary outcome was ongoing pregnancy rate (per woman randomized). Secondary outcomes included clinical pregnancy rate (per patient randomized) and miscarriage rates (from clinical pregnancy).

Participants/materials, setting, methods: Four RCTs (Shapiro et al.,2011a;Shapiro et al.,2011b;Chen et al.,2016;Vuong et al.,2016) were included as targets for data extraction and meta-analysis involving 2,549 women. Data were combined for meta-analysis using the StatsDirect statistical software. Dichotomous data were expressed as relative risk (RR) with a 95% confidence interval (CI). The measure of heterogeneity was evaluated using the Cochran's Q and I^2 tests. Study data were combined using a random-effects model. P-values<0.05 were considered statistically significant.

Main results and the role of chance: The results of this meta-analysis were divided into two parts: Part I- All trials where the mean of the collected oocytes was > 12 and <21; and Part II- Three studies where the mean number of oocytes retrieved was > 12 and <15.

Part I:

(1) Ongoing pregnancy rates: FRESH-ET: 44.3% (568/1282) versus ALL/FREEZE-ET: 48.3% (612/1267) with statistically significant differences (RR=1.09; 95%CI=1.01-1.19; P = 0.03). Heterogeneity: I^2=0%; Cochran Q=2.0, P = 0.57.
(2) Clinical pregnancy rates: FRESH-ET: 55.1%, (491/891) versus ALL/FREEZE-ET: 59.2% (519/876) with no significant differences (RR=1.12; 95%CI=0.97-1.31; P = 0.13). Heterogeneity: I^2=34.5%; Cochran Q=3.05, P = 0.22.
(3) Miscarriage rates: FRESH-ET: 13.1% (68/519) versus ALL/FREEZE-ET: 10% (52/519) with no significant differences (RR=0.71; 95%CI=0.51-1.01; P = 0.05). Heterogeneity: I^2=0%; Cochran Q=0.16, P = 0.92.

Part II:

(1) Ongoing pregnancy rates: FRESH-ET: 43.8% (534/1220) versus ALL/FREEZE-ET: 47.6% (574/1207) with no significant differences (RR=1.09; 95%CI=1.00-1.19; P = 0.052). Heterogeneity: I^2=0%; Cochran Q=1.90, P = 0.39.
(2) Clinical pregnancy rates: FRESH-ET: 55.1% (457/829) versus ALL/FREEZE-ET: 58.8% (480/816) with no significant differences (RR=1.15; 95%CI=0.88-1.49; P = 0.30). Heterogeneity: Cochran Q=2.6, P = 0.11.
(3) Miscarriage rates: FRESH-ET: 13.8% (63/457) versus ALL/FREEZE-ET: 10.0% (48/480) with no significant differences (RR=0.71; 95%CI=0.50-1.01; P = 0.06). Heterogeneity: Cochran Q=0.16, P = 0.69.

Limitations, reasons for caution: Data regarding clinical/ongoing pregnancy rates were included without data on live births. In addition, all trials included patients who were considered normal/high-responders; therefore, these results may not be held for the poor-responders. More RCTs are required to evaluate whether the all-freezing strategy can influence the clinical outcomes.

Wider implications of the findings: The all-freezing strategy could be favourable when high numbers of oocytes were collected, signalizing one association between higher COS and the consequent impairment of endometrial receptivity. However, when the number of oocytes collected was <15, the all-freezing strategy does not appear to be advantageous.

Trial registration number: Not applicable. The local Research Ethics Committee approved the study.

P-121 Glycan profile of blastocyst spent culture medium varies in implantation success vs failure: a potential non-invasive viability assay

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Study question: Is there any difference of glycan profile in blastocyst spent culture medium between implantation success and failure group?

Summary answer: The gycopattern and expression levels in blastocyst spent culture medium showed significant statistical difference between successful implantation and failure implantation.

What is known already: Current methods of pre-implantation selection of embryo mainly are subjective and inefficient. Glycan profile may reflect health condition and interaction capability of embryo with uterus, however currently there has not been any report on glycan profiling for prediction of embryo viability and implantation rate. Glycan profile can be assessed in spent culture medium non-invasively.

Study design, size, duration: This was a case-control study to compare the difference of gycopattern and expression levels in the blastocyst spent culture medium grouped according to the implantation results. A total of 78 cases (success vs failure, 39 samples each) of blastocyst spent culture medium were analyzed by lectin array (37 lectins). The lectins with difference were chosen to further verify by sample microarray stained with specific lectin and lectin blot.

Participants/materials, setting, methods: The patients were enrolled underwent single blastocyst transfer on day 5 in fresh IVF/ICSI cycles. A total of 78 cases of blastocyst spent culture medium were analyzed. The half of one sample in each group was pooled and spotted on the lectin microarray. Another half of one sample was produced as sample microarray to further verify by sample microarray stained with specific lectin.

Main results and the role of chance: The blastocyst spent culture medium (success vs failure) was detected by lectin microarray (37 lectins) (Fig 2). 10 lecins (AAL, NPA, UEA-I, MAL-I, LCA, PHA-E+L, GNA, DBA, BPL, LTL) showed the significant difference of NFLs fold change (>1.5-fold or <0.67-fold) (Table 1). In the blastocyst culture medium with successful implantation, 6 specific glycans (NPA, UEA-I, MAL-I, LCA, PHA-E+L, GNA) showed up-regulation and 4 (AAL, LTL, DBA, BPL) showed down-regulation. LCA specific glycan structure showed the highest change fold of up-regulation (ratio=3.900). LTL failed to be detected in blastocyst culture medium microarray. The other 9 lectins were followed by blastocyst culture medium microarray analysis and lectin blot analysis (Fig 3). All 9 lectins showed the significant statistical difference in blastocyst spent culture medium between successful implantation and failure implantation. Of these, the difference of UEA-I, LCA and GNA were the most significant with $p < 0.001$.

Limitations, reasons for caution: Successful implantation is a complex process involves reciprocal interactions between blastocyst and uterus. The current study does not assess the uterus factors such as endometrial receptivity. In addition, the results are affected by numerous risk factors which may cause bias in the results in spite of exclusion of special cases.

Wider implications of the findings: The results suggest that the change of glycan profile in spent culture medium may led to a novel non-invasive assessment assay of embryo viability. These results may be helpful to understand about glycobiology of embryo implantation.

Trial registration number: ChiCTR-DOD-15006743

P-122 the relationship between embryo gender and morphokinetics parameters analyzed by time-lapse imaging in human embryo

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Study question: The gender-related differences in embryo morphokinetics are still not well known. Does the gender difference of human embryo affect embryo morphokinetics parameters?

Summary answer: In early stage embryo, the male showed significantly earlier development and shorter cell cycles than the female. These results suggested that embryo gender affects morphokinetics.

What is known already: Many studies have reported that there might be gender-specific differences in human embryo development. Most studies have indicated that male embryos showed earlier development than female embryos. However, there are few studies that confirmed the gender-related differences in embryo development analyzed by TL imaging.

Study design, size, duration: This study is a retrospective observational study, conducted at Kyono ART Clinic in Japan from June 2013 to January 2016. It was approved by the Ladies Clinic Kyono Ethics Committee. A total of 65 euploid embryos from 53 patients were included in this study.

Participants/materials, setting, methods: Following ICSI, all embryos were monitored by TL system and morphokinetics parameters were analyzed. The data from 34 infants conceived by TL monitored embryo transfer and 31 discarded blastocysts that were confirmed normal karyotype by next-generation sequencing were analyzed. Chi-squared and Mann-Whitney U tests were used for statistical analysis.

Main results and the role of chance: There was no significant difference in parental age between embryo genders. The gender ratio in the infants and the embryos were 41.1% and 48.4% male respectively, and there were no differences in the gender ratio of both groups. Male embryos showed significantly faster morphokinetics parameters such as time of cleavage to 5-cell (t5) (t5: 48.7 ± 6.6 vs. 51.8 ± 5.1, P = 0.045) and the third cell cycles (cc3=t5-t3) (cc3: 13.2 ± 2.8 vs. 14.6 ± 2.1, P = 0.023) than female embryos. Although male embryos showed faster morphokinetics parameters from time of cleavage to 6-cell (t6) to time of expanded blastocyst (tExpand) than female embryos, yet the results did not reach statistical significance.

Limitations, reasons for caution: Unlike previous studies, our study could not show gender-specific development patterns such as faster blastocyst formation in male embryo. There is a possibility that our results have been influenced by small sample size of our study.

Wider implications of the findings: We found a strong relationship between embryo gender and development speed in early stage embryo. At the 4 to 8-cell stage, genome impression pattern is drastically changed because the embryo produces its own genome. Our results indicate that this phenomenon called zygotic gene activation has an effect on sex chromosomes.

Trial registration number: None.

P-123 Evaluation of efficacy of GM-CSF in embryo culture for frozen embryos on embryo development and pregnancy outcomes

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Study question: Does presence of GM-CSF in the embryo culture medium influence a thawed embryo's ability to grow and implant?

Summary answer: It is suggested that presence of GM-CSF in embryo culture of thawed embryos, has not effects on blastolation and implantation

What is known already: Naturally, there are GM-CSF (granulocyte macrophage-colony stimulation factor) and another growth factors in the female reproductive tract and they play a physiologic role in normal blastocyst development and embryo implantation. Ziebe showed that the incidence of blastulation in human embryos is increased when GM-CSF is present in the culture medium and was seen to significantly increase in implantation and survival of transferred embryos to week 12 gestation and live birth rate(2013). Herein, we attempted to evaluate the effect of adding GM-CSF in thawed embryo's media culture to determine the blastulation rate, implantation and clinical pregnancy rates

Study design, size, duration: The present randomized clinical trial was performed in Assisted Reproduction and Infertility Clinic affiliated to Avicenna Research Institute, Tehran, Iran, from February 2015 to January 2017, with a total of 90 patients. 10 patients were excluded from the final analysis due to different reasons.Blastocyst formation rate, Implantation and clinical pregnancy rates were the primary outcome measures

Participants/materials, setting, methods: Randomly, frozen embryos were thawed and cultured in control medium (n = 40,Vitrolife,USA) or test medium (n = 40, BlastGen,Denmark) containing 2 ng/mL GM-CSF. Pregnancy was tested by measuring serum beta-hCG levels 16 days after embryo transfer. The implantation rate and Clinical pregnancy was defined as the presence of

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gestational sac and fetus with a heartbeat by vaginal ultrasonography at 7 weeks of pregnancy

Main results and the role of chance: Although the age of patients, the mean number (P = 0.155) and the quality of thawed embryos were similar in the two groups, there was not significantly differences in blastocyst (28.9% vs. 30.9%, P = 0.932), early blastocyst (9.2% vs. 8.9%, P = 0.716) and compact embryos (18.5% vs. 24.4%, P = 0.405) formation between two groups. Pregnancy (40% vs.32.5%, P = 0.642) and implantation (32.5% vs. 30%, P=1.000) rate did not have significant differences

Limitations, reasons for caution: Culture mediums that were used in this study were from different companies. It is better to design another studies with more patients and adding GM-CSF for another development stages of embryos

Wider implications of the findings: The present findings suggested that the presence of GM-CSF in culture medium after 3th day of development has not effect on blastulation in thawed embryos

Trial registration number: IRCT 2014021611430 N4.

P-124 Identification of a novel long intergenic noncoding RNA gene palying vital roles during porcine early embryonic development through genome-wide analysis

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Study question: How does long intergenic noncoding RNA (lincRNA) regulate the gene expression network, and its subsequent effects on embryonic development in pig.

Summary answer: After genome-wide analysis, we identified a novel lincRNA palying vital roles during porcine early embryonic development.

What is known already: Many events are crucial for the whole embryonic development during the cleavage stage, including nuclear reprogramming, zygote genome activation (ZGA), and the first cell fate specification. Although a few have been mechanistically characterized, lincRNAs have emerged as a new field in biology, playing significant roles in many biological functions, including X chromosome inactivation, imprinting, cell apoptosis and cell cycle control, immune response, and mitochondria regulation. However, the underlying molecular mechanisms related to lincRNAs remain unclear. Overall, lincRNAs and/or their transcripts program various biological functions via epigenetic and nonepigenetic, mechanisms at the pre-transcriptional, transcriptional, and post-transcriptional levels.

Study design, size, duration: Five RNA-seq data sets from the NCBI SRA database were analyzed for predicting potential lincRNAs. The zygote microinjected with a novel linc-RNA locked nucleic acid (linc-RNA-LNA), control-LNA (negative control) and none (control) were used to evaluate the pronucleus formation, cleavage rate and blastocyst formation rate.

Participants/materials, setting, methods: RNA-seq data were analyzed by TopHat version 2.0.9, CNCI and CPAT were used to predict the coding potential. The expression pattern of these novel transcripts were analyzed by real-time quantitative PCR (qPCR). LincRNA depletion were performed through LNA microinjection at 3 h after ICSI. Nucleus stage, DNA damage and cell death were evaluated by EdU, γ-H2AX and Annexin-V assay. Pronucleus formation and pronucleus fusion were assessed at 12 h and 21-24 h after ICSI by Immunofluorescence staining.

Main results and the role of chance: Here we identified 7,618 novel lncRNAs from 4,776 loci based on published RNA-seq data. These lincRNAs show low exon number, short length, low expression level, tissue-specific expression and cis-acting, which is consistent with previous reports in other species. By weighted co-expression network analysis, we identified 5 developmental stages specific co-expression modules. A novel lincRNA depletion caused the developmental arrest at 1-cell stage. Then, we compared the nucleus stage among the three groups. Major of zygotes have entered or passed mitotic stage at 21 h after ICSI in control and negative control groups. Zygotes divided and entered the 2-cell stage at about 24 h after 24 h after ICSI. However, the male and female pronuclei begin to migrate toward each other, but fusion did not occur in linc-RNA-LNA group. Meanwhile, we detected more DNA damage and cell death signals in the linc-RNA-LNA group. Futhermore, depletion of the lincRNA in one blastomere of the 2-cell embryo have no effect on the following cleavage, indicating that the lincRNA might only function in the first cell-cycle during embryonic development.

Limitations, reasons for caution: Findings in porcine may not be fully representative of human. Further functional studies are needed to explain the molecular mechanism.and determine the clinical significance.

Wider implications of the findings: Our study provide the first lincRNA profiles of procine early embryonic development. In particular, it is the first study reporting the function of lincRNA during porcine early embryonic development.

Trial registration number: NO.

P-125 Effects of direct cleavage on embryo development and clinical outcomes

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Study question: How often does direct cleavage (DC) of embryo occur? Does the timing of DC of embryo affect blastocyst formation rate and pregnancy rate?

Summary answer: The frequency of DC was 20%. Blastocyst rate of DC at the first-cleavage was low. Pregnancy rate was the same regardless of the DC timing.

What is known already: Recently, time-lapse (TL) imaging has revealed the relationship between morphokinetic parameters of human preimplantation embryo and clinical outcomes. DC, defined as one blastomere into three daughter blastomeres at cell cleavage, was observed with large variations in frequency, ranging from 8.3% to 26%. It has also been reported that embryos with DC showed lower implantation rate than embryos with normal cleavage pattern.

Study design, size, duration: This study is a retrospective observational study, which was conducted at Kyono ART clinic in Japan from August 2013 to March 2016. It was approved by the Ladies Clinic Kyono Ethics Committee. A total of 494 human embryos observed by TL imaging system were included in this study.

Participants/materials, setting, methods: Ninety-nine embryos which underwent DC were divided into three groups by timing of DC (first cleavage, second cleavage, and third cleavage or later). Blastocyst formation rate and pregnancy rate of each group were analyzed.

Main results and the role of chance: The total incidence of DC per embryo was 20.0% (99/494). With regard to timing of direct cleavage, the first cleavage was 46.5% (46/99), the second cleavage was 39.4% (39/99) and the third cleavage or later was 14.1% (14/99). Blastocyst formation rate of embryos with DC at the first cleavage, the second cleavage and the third cleavage or later were 10.9% (5/46), 43.6% (17/39) and 50.0% (7/14) respectively. The embryos with DC at the first cleavage showed significantly lower blastocyst formation rate than embryos with DC at other timings ($p < 0.01$). Fifteen blastocysts which underwent DC were transferred, and 6 blastocysts (40%) implanted. There was no significant difference in pregnancy rate among the three groups. Pregnancy outcomes were 2 live births, 2 ongoing pregnancies and 2 miscarriages.

Limitations, reasons for caution: Our results might be influenced by the small sample size of our study. To clarify the safety and efficacy of DC embryo transfer, euploidy of DC embryos should be assessed.

Wider implications of the findings: Our findings have shown that DC embryos have a potential to develop to blastocyst, although blastocyst formation rate was low, especially at the first cleavage. If DC embryo develops to blastocyst, DC embryos could be selected as an option for transferred embryos.

Trial registration number: None.

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P-126 The role of amino acid metabolism in bovine inner cell mass development and pluripotency

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Study question: To investigate if the metabolism of candidate amino acids (AAs) is crucial for bovine inner cell mass (ICM) development and pluripotency

Summary answer: Bovine ICM develops without several AAs but requires threonine dehydrogenase (TDH) activity.Its inhibition stimulates autophagy and increases histone 3 lysine 4 trimethylation (H3K4me3) in blastocysts.

What is known already: Mouse ICM and ICM-derived pluripotent stem cells (PSCs) specifically need threonine and ICM-restricted TDH to produce the methyl donor S-adenosylmethionine (SAM). Depleting threonine (ΔT) or TDH decreases SAM levels, limits H3K4me3 and induces autophagy in PSCs. By contrast, human PSCs do not survive without lysine (ΔK), leucine (ΔL), tryptophan (ΔW) or methionine (ΔM). In cattle, bona fide embryonic PSCs have not been isolated.

Study design, size, duration: Experiments were conducted on *in vitro* fertilised preimplantation bovine embryos, individually cultured in chemically defined, protein-and glutamin-free synthetic oviduct fluid (gSOF) with essential (E) and non-essential (NE) AAs. Selective AA depletion or pharmacological TDH inhibition, using the specific QC1 inhibitor, was applied from the morula to the blastocyst stage (day 5 to 8) in single embryo culture. SOF with and without E/NE_AAs served as positive and negative controls, respectively.

Participants/materials, setting, methods: For AA drop-out experiments, 2509 embryos were scored for embryo development according to the International Embryo Transfer Society morphological grading guidelines. For TDH inhibition experiments, 658 embryos were analysed for embryo development, cell numbers (Hoechst/ Propidium iodide staining), lineage-specific marker expression (quantitative PCR), histone trimethylation (immunofluorescence quantification) and autophagy (Autophagy Detection Kit, Abcam #139484). Statistical significance was determined using Fisher's exact test for development and t-tests for cell numbers, gene/protein expression and autophagy.

Main results and the role of chance: Depleting one (ΔT, ΔM), two (ΔMT, ΔCM, ΔCT; ΔIL, ΔIK, ΔKL), three (ΔCMT, ΔIKL) or six (ΔHPRVWY) EAAs did not affect ICM formation, even when NEAAs were also removed for ΔT and ΔM groups. However, depleting another six (ΔCIKLMT), nine (+CMT, +IKL), eleven EAAs (+T, +M) or all twelve EAAs impaired blastocyst development ($P < 0.05$). As no clear EAA candidate emerged from this targeted screen, we focused on TDH. *TDH* mRNA was present at similar levels in trophectoderm (TE) and ICM. Exposure to the TDH inhibitor QC1 reduced ICM and TE cell numbers ~2-fold ($P < 0.005$). This cell loss was due to increased autophagy ($P < 0.05$). Hypoblast (*PDGRFα*), pluripotency-related epiblast (*NANOG, FGF4* and *SOX2*) and TE (*CDX2*) markers were not altered in the QC1 group compared to DMSO controls. QC1-treated ICM and TE nuclei were specifically hypermethylated at H3K4me3 ($P < 0.05$), while H3K9me3 and H3K27me3 were not affected.

Limitations, reasons for caution: Conclusions drawn from pharmacological inhibition rely on specificity of the inhibitor for TDH enzyme and need to be complemented by genetic evidence, using both gain- and loss-of-function approaches. Furthermore, isolated bovine ICM cells require complex culture conditions and do not robustly propagate in chemically defined, protein-free medium.

Wider implications of the findings: Our findings highlight differences in amino acid dependency between bovine, mouse and human ICM cells. Such species-specific connections between metabolism, epigenetics and pluripotency regulation may help understand why bona fide bovine embryonic PSCs have not been isolated yet.

Trial registration number: NA.

P-127 Rapid warming method can improve the survival rate of slow freezing with cryovial carriers

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Study question: The survival rates for slow freezing are lower, especially when cryovials are used as carriers. Can rapid warming improve the survival rate for slow freezing with cryovials?

Summary answer: The rapid warming can improve the survival and intact survival rates for slow freezing even if cryovials are used as carriers.

What is known already: The rapid warming can improve the survival rate when cryostraws are used as carriers.

Study design, size, duration: The first part presents a retrospective design. This part involves 90 cycles which underwent frozen embryo transfer between January 2015 and October 2016. The second part is a prospective design which includes 79 embryos from three pronuclear (3PN).

Participants/materials, setting, methods: Cycles underwent slow freezing and frozen embryo transfer from January 2015 to October 2016 were defined as traditional group (90 cycles, 288 embryos). Cryovials with 2 ml volume was the carriers. The thawing method followed the traditional protocol. Embryos derived from 3PN were included in modified group, which were frozen by slow freezing and thawed using the rapid warming, based on the method described by Parmegiani (Parmegiani et al., 2014).

Main results and the role of chance: The survival and intact survival rates in modified group were 88.61% (70/79) and 59.49% (47/79), respectively, which were significantly higher than that in traditional group (64.58% (186/288) and 25% (72/288), respectively, $P = 0.000$). Different temperature of water bath was used in modified group, there were no significant differences in terms of the survival rates for different temperature (88.24%, 88.89%, 88.89% for 37°C, 38°C, 39°C, respectively).

Eight cycles in traditional group were canceled for embryo transfer because of the failure of embryo survival. Eighty two cycles received embryo transfer. The implantation and clinical pregnancy rates were 36.59% and 28.95%, respectively. From November to December in 2016, there were 11 cycles in which the rapid warming method was used, five of them were clinical pregnancy.

Limitations, reasons for caution: The limitation of this study was the small sample size. We should notice that embryos in modified group derive from 3PN. Larger, clinical trials are needed.

Wider implications of the findings: Slow freezing using cryovials is a hard challenge compared with cryostraws because of the high volume of cryovials. This study proves the efficiency of rapid warming method on slow freezing even if cryovials are used.

Trial registration number: no.

P-128 Effect of trophectoderm projection on blastocyst development in relation to hatching, pregnancy, and miscarriage rates

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Study question: Whether frequencies of trophectoderm projections are greater with conventional IVF or ICSI is unclear. Relationships between the TPs site and pregnancy, miscarriage rates remain unclear.

Summary answer: Incidence of TPs was higher in ICSI than conventional IVF (c-IVF). However, similar pregnancy and miscarriage rates occurred in vivo irrespective of c-IVF or ICSI.

What is known already: We believe TPs represent a response to a spontaneous trigger from inside the blastocyst to its exterior, which initiates hatching. These projections display amoeboid-like movement, suggesting that they actively seek an implantation site. However, only morphological features of TPs have been characterized, and biological actions remain unclear. Relationships to pregnancy and miscarriage also remain unclear.

Study design, size, duration: Study 1 This study compared the incidence of TPs in relation to hatching in c-IVF and ICSI cycles. Experimental vitrified-warmed

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blastocysts (n = 112) were allocated to two groups (TPs present or absent), and were retrospectively compared between groups.

Study 2 This was a retrospective clinical study of 944 embryo transfer cycles in 712 clinic patients who received a single vitrified-warmed blastocyst. Finally, pregnancy and miscarriage rates were compared during January 2014 and July 2016.

Participants/materials, setting, methods: Experimental vitrified-warmed blastocysts were surplus embryos. Time-lapse cinematography was used to observe the presence of TPs in them. All embryos were graded using three 3AA or 4AA per person according to the Gardner criteria.

In the clinical retrospective study, blastocysts were categorized according to the presence of TPs (TPs(+) group: 213 embryos, TPs present; TPs(-) group: 731 embryos, TPs absent). Pregnancy and miscarriage rates were retrospectively compared from time-lapse cinematography. Statistical analysis was the $\chi 2$ test.

Main results and the role of chance: The incidence of TPs was significantly higher in embryos inseminated by ICSI compared to c-IVF, (ICSI: 51/56, 91%; c-IVF: 25/56, 45%; $p < 0.01$). The successful hatching rate was significantly lower with ICSI than with c-IVF (ICSI: 11/56, 20%; c-IVF: 29/56, 52%; $p < 0.01$). In addition, the hatching rate was significantly lower when TPs were present (14/76, 18%) than in non-TPs embryos (26/36, 72%; $p < 0.01$).

With regard to the clinical study results, no significant differences were found between groups in terms of the pregnancy rate (TPs(+) group: 125/213, 58.7%; TPs(-) group: 402/731, 55.0%) or miscarriage rate (TPs(+) group: 23/125, 18.4%; TPs(-) group: 66/402, 16.4%). We hypothesized that in vivo hatching would occur naturally by assisting protease activity in the uterus. These results suggest that the presence of TPs has no effect on pregnancy rates in clinical settings.

However, the TPs site position around the inner cell mass(ICM) was associated with an increased miscarriage rate (6/12, 50.0%) compared to the from-elsewhere group (17/113, 15.0%; $p < 0.01$) and non-TPs (66/402, 16.4%; $p < 0.01$).

Limitations, reasons for caution: It is more necessary to study the mechanism producing of TPs, and relation between TPs site and implantation and miscarriage.

Wider implications of the findings: We report here a new finding of TPs action that it is no direct relation between projection and implantation. This appears to be the first discussion reported worldwide.

Trial registration number: Not applicable.

P-129 Mitotic pulses and cell apoptosis are the two major factors contributing to collapse of blastocoel cavity

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Study question: What are the causes of collapses occurring during blastocyst development and should they be always regarded as a negative phenomenon?

Summary answer: Collapses resulting from physiological cell changes during mitosis should not be considered as negative marker. However, the negative impact of apoptosis-induced collapses can be expected.

What is known already: Studies have found that collapse-expansion cycles may decrease implantation potential of blastocysts. Embryos exhibiting weak contractions should be preferred for transfer since blastocysts with strong collapses usually fail to hatch. Undergoing collapses are accompanied by an efflux of blastocoel fluid but the cause(s) and exact mechanism of blastocoel contraction and recovery is still not known. It can be speculated that collapse is a consequence of compromised embryo viability arising from poor oocyte/sperm quality or it may be an artefact of extended in vitro cultivation.

Study design, size, duration: Retrospective cohort study conducted at private fertility clinic from May 2016 to January 2017. The blastocoel collapses were analyzed in time-lapse images of 308 blastocysts (118 hatching and 190 expanded non-hatching blastocysts) resulting from ICSI fertilization of own/donated oocytes.

Participants/materials, setting, methods: A total of 59 ICSI patients were included in this study. Time–lapse images (Primovision) were taken every 30 min. for 115 hrs. **CATI** software (**C**ognitive **A**utomation of **T**ime-lapse **I**mages) was used to detect cleavages up to 9 cells and blastocoel expansion/collapse(s)/reexpansion. The mitotic cycles beyond 9 cells and the abnormal cleavages were annotated manually. Apoptotic collapses were confirmed by the presence of apoptotic cells underoing lysis in blastocoel. Trophectoderm was evaluated according to Gardner scoring.

Main results and the role of chance: The mean values (hrs) of mitotic cycle timing observed during 115 hrs of cultivation period (in 308 embryos) were: t2:25, t3:36, t4:37, t5:50, t8:56, t9:72, t17:85, t33:96, t65:105, t129:112. 76% (no. 235) of embryos cleaved normally and in 24% (no. 73) some kinds of abnormalities were detected.The collapses were detected in 63% of embryos (no. 194). 49 % (no. 96) of these collapses were considered as mitotic pulses and 51 % (no. 98) as apoptotic collapses. 26% (no. 80) of analyzed embryos were evaluated as low cell number trophectoderm (TE) group (Gardner TE: B, C).

Significantly more embryos with abnormal cleavages (34 vs. 1%) were detected in a group of embryos with collapses when compared to no-collapse group.Significantly more embryos with abnormal cleavages (59 vs. 11%, total no. 308) were detected in low cell number TE group (Gardner TE: B, C) when compared to normal TE group (Gardner TE: A).Significantly more embryos with apoptotic collapses (75 vs. 37%, no. 194) were detected in low cell number TE group when compared to normal TE group.Significantly more embryos with apoptotic collapses (69 vs. 41%, no. 194) were detected in abnormally cleaved embryos when compared to correctly cleaved group.

Limitations, reasons for caution: Inexact apoptotic cell identification: limited value of subjective noninvasive detection of apoptotic cells. Not sufficient time-lapse data: just one focal plane was captured and analyzed. Limited period of embryo observation: some apoptotic cells may undergo lysis after finishing of cultivation.

Wider implications of the findings: The presence of apoptotic cells in TE was proven in many studies. We have described the impact of apoptotic cells on collapse occurrence and reduction of TE cells. Apoptosis-induced collapses can be considered as negative marker unlike the collapses described here as mitotic pulses induced by cell changes during mitosis.

Trial registration number: Not applicable.

P-130 Age and oocytes retrieved as predictors of blastocyst formation

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Study question: The objetive of the study is to calculate the probability of having at least 1 expanded blastocyst on day 5 in women undergoing ICSI relative to age and injected oocytes

Summary answer: Probability of obtaining 1 expanded blastocyst on day 5 is about 80% in women under 35 years in contrast of 40% for women over 40

What is known already: Blastocyst-stage transfer has more known advantages than cleavage-stage transfer. According to a recent Cochrane Review, blastocyst-stage transfer is associated with higher live birth rates compared with cleavage-stage transfer with no reduction in clinical pregnancy rate and miscarriage rate. However, the major disadvantage of extended culture is the risk that no blastocysts will be available for transfer on day 5.

Study design, size, duration: This study was a retrospective cohort study analysis of 329 ICSI cycles with blastocyst transfer completed between January 2014 and December 2015

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Participants/materials, setting, methods: Patients were classified in 3 groups according to age. Group1: < 35 years, Group2: 35–39 years, Group3: ≥40 years. Each group was divided in 3 subgroups according to the number of injected oocytes, a: ≤4 M2, b: 5–9 M2, c: ≥10 M2. The probability of obtaining at least 1 expanded blastocyst was calculated for each age group. Data analyses were performed using Fisher test. A P-value of < 0.05 was considered to be statistically significant.

Main results and the role of chance: The probability of obtaining at least 1 expanded blastocyst was 82% (IC95% 0.73–0.88), 65% (IC95% 0.57–0.72) and 46% (IC95% 0.33–0.60) for group 1(n = 109), 2 (n = 166) and 3 (n = 54) respectively. When each group was divided according to the injected oocytes the results were:

G1a(n = 9): **67%** (IC95% 0.30–0.93),G1b(n = 46): **78%** (IC95% 0.64–0.89), G1c(n = 54): **87%** (IC95% 0.75–0.95)

G2a(n = 43): **40%** (IC95% 0.25–0.56),G2b(n = 68): **65%** (IC95% 0.52–0.76),G2c(n = 55): **86%** (IC95% 0.73–0.94)

G3a(n = 19): **42%** (IC95% 0.20–0.67),G3b(n = 26): **39%** (IC95% 0.20–0.59),G3c(n = 55): **78%** (IC95% 0.40–0.97)

Overall, blastocyst formation increase 25% and 76% when comparing G1 vs G2 (RR:1.25 IC95% 1.09–1.45) and G1 vs G3 (RR:1.76 IC95% 1.31–2.38) respectively.

Not statistically differences could be found when comparing different quantities of injected oocytes among the youngest group G1c vs G1b RR 1.11 (IC95% 0.92–1.34) G1c vs G1a RR 1.31(IC95% 0.81–2.10).However, in the middle age group the probability of quality blastocysts increase when more oocytes are available to inject G2c vs G2b RR 1.32(IC95% 1.07–1.62) G2c vs G2a RR 2.16(IC95% 1.47–3.18). In the oldest group the probability increase as the injected oocytes increase but not statistically differences could be found when comparing more than 10 vs less than 4 G3c vs G3b RR 2.02(IC95% 1.11–3.68) G3c vs G3a RR 1.85(IC95% 0.98–3.48).

Limitations, reasons for caution: The main strength of this study is the sizable sample of patients over 40 years with more than 10 oocytes and less than 4. For this reason the results in this group are in the limit of the statistical significance. Clinical outcomes after transfer of these blastocysts were not calculated

Wider implications of the findings: Blastocyst formation is associated with maternal age being 80% for patients under 35 and 40% for patients over 40. Total number of quality blastocysts increased as injected oocytes rose for all groups, however this finding seems to be not relevant for young patients but regain importance as the age rose.

Trial registration number: Not applicable.

P-131 High reliability of morphokinetic annotations among embryologists

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Study question: Are morphokinetic measurements of time lapse videos comparable among operators?

Summary answer: There is little variation among morphokinetic measurements taken by different operators when analyzing the same time lapse videos.

What is known already: Morphokinetic analysis of preimplantation embryo development is a complementary method of embryo assessment increasingly used in IVF laboratories. Time-lapse videos of embryo development are usually viewed by trained embryologists and the times at which certain cellular events occur manually annotated. Such annotations form the basis for embryo selection algorithms, whish are used to rank the embryos for transfer. The reliability of morphokinetic annotations is dependent on both the quality of the video, and the training of the embryologists. Only one study so far reported the reliability of morphokinetic annotations among 3 embryologists; larger studies are needed to address this further.

Study design, size, duration: Prospective study carried out between October 2015 and June 2016. Six senior embryologists with an experience in classic morphology-based evaluation of several years individually annotated the same 93 videos of preimplantation development, corresponding to 18 IVF/ICSI cycles, recorded by a Primo Vision® System.

Participants/materials, setting, methods: Times of second polar body extrusion, appearance and disappearance of pronuclei, and cleavages of embryo development (times from 2-cell to 5-cell stage: t2, t3, t4, t5) were annotated. Each embryologist was blinded to the annotations of the others. Intra- and inter-observer agreement was evaluated by computing intra-class correlation coefficients (ICCs).

Main results and the role of chance: In the inter-observer analysis, most ICCs obtained were higher than 0.80, indicating a high degree of concordance: t2: 0.93; t3: 0.80; t4: 0.89; t5: 0.90; disappearance of two pronuclei: 0.98. However, the ICCs obtained from second polar body extrusion and the appearance of two pronuclei annotations was lower: 0.49 and 0.56 respectively, indicating an average degree of concordance. The ICCs obtained from the intra-observer analysis were mostly higher than 0.80 (t2: 0.96; t3: 0.89; t4: 0.88; t5: 0.86; disappearance of two pronuclei: 0.96). Similiarly to the interobserver's, the intra-observer ICCs obtained for the second polar body extrusion and the appearance of two pronuclei annotations were 0,77 and 0,66 respectively. These results indicate that the measures of embryonic development times annotated by analyzing time-lapse videos are highly reliable both intra observer and among observers.

Limitations, reasons for caution: The developmental stages from 6-cells to blastocyst were not evaluated; since some morphokinetic algorithms use times past the 6 cell stage in their calculations, further studies should be carried out to understand the variations among operators in these cases.

Wider implications of the findings: Time-lapse measurement should be as objective as possible, especially for the first cleavages of embryo development, because they are often measured to define algorithms to assess the embryonic implantation potential. Our results show that measurements using Primo Vision® System are consistent and reliable within and among different operators.

Trial registration number: NA.

P-132 The implantation chance of an euploid blastocyst may not correlate with its morphological grading at all

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Study question: Do better morphology scores of blastocysts mean a better chance of implantation when euploid blastocyst are being transferred, or vice versa?

Summary answer: The implantation and ongoing pregnancy chance of an euploid blastocyt may not correlate with its morphological grading at all.

What is known already: The ploidy status of the blastocysts is known to affect their implantation potential. It is not known weather the implantation and ongoing pregnancy chance of an euploid blastocyst is affected by its morphological grading.

Study design, size, duration: This is a retrospective study of 88 blastocysts transferred between March 2013 and November 2016, that have been tested as euploid by comprehensive chromosome screening (CCS) on aCGH or NGS genetic platforms. The cycles included were either single or double embryo transfer cycles in which case, either both or none of the embryos had implanted. The blastocyst morphology was evaluated according to standard Gardner's classification system.

Participants/materials, setting, methods: 88 euploid blastocysts were from 74 patients undergoing preimplantation genetic screening for recurrent IVF failures, habitual abortion and advanced maternal age. The implantation and ongoing pregnancy rates of each embryo were analysed with respect to the genetic platform, weather the transfer was a fresh or frozen ET, and the expansion status, inner cell mass (ICM) and trophectoderm morphology of the blastocyst at the time of the transfer, by univariate analysis.

Main results and the role of chance: The implantation rate (IR) of euploid blastocysts did not differ between aCGH and NGS platforms (60.7 % vs 40.7 %,

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respectively; p = 0.134). It also did not differ if the transfer had been a fresh or frozen one (55.8 % vs 52.8 %, respectively; p = 0.953).

The IR did not differ significantly with regard to the degree of blastocyst expansion; being 0 % for 1, 25 % for 2, 50 % for 3, 62.5 % for 4 and 5 and 25 % for 6 (p = 0.375). IR of euploid blastocysts also did not differ in different inner cell mass (ICM) and trophectoderm cathegories (53.9 % for A, 54.5 % for B, 100 % for C, p = 1,000 and 56.2 % for A, 42.9% for B and 100 % for C, p = 0.471; respectively). The lack of correlation persisted for all comparions when the ongoing pregnancy rate was chosen as the outcome parameter instead of IR (aCGH vs NGS, p = 0.743; fresh vs frozen, p = 0.854; different expansion classes, p = 0.383; ICM morphology, p = 0.697; trophectoderm morphology, p = 0.720)

The ongoing pregnancy chance of the euploid blastocysts did not differ among different blastocyst expansion scores, inner cell mass and trophectoderm morhology cathegories.

Limitations, reasons for caution: Low occurence number in certain subgroups of blastocyst morphology may introduce a statistical bias, therefore continuation of such a study with a greater number of cases is warranted for a stronger conclusion statement.

Wider implications of the findings: CCS may be the ultimate route of embryo selection and once an embryo is found to be euploid, blastocyst morphology may not be so influential on the outcomes.

Trial registration number: -.

P-133 Comparison of blastocyst expansion morphokinetics in euploid versus aneuploid embryos from infertility patients

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Study question: Can the morphokinetics of blastocyst expansion distinguish euploid from aneuploid embryos and provide a basis for embryo ranking and selection for transfer?

Summary answer: Of several morphokinetic parameters obtained from time-lapse imaging, blastocyst expansion dynamics best distinguished euploid from aneuploid embryos.

What is known already: Without preimplantation genetic screening (PGS), the laboratory must rank embryos for transfer based on morphology. Compared to traditional grading, time-lapse imaging offers new possibilities, but applying this approach to embryo selection remains controversial. Blastocoel expansion rate is a new metric recently described using donor egg embryos having known positive implantation data (Huang et al., 2016); however, there is no information describing expansion in autologous embryos from infertility patients or how expansion correlates to karyotype after PGS. Results reported here describe these features and suggest how expansion metrics can be applied to embryo selection.

Study design, size, duration: This was a retrospective observational study initially involving 188 (non-donor) blastocysts biopsied from 34 infertility patients having preimplantation genetic screening for aneuploidy. The study period involved sequential biopsy cases from June 2014- June 2016 using a single laboratory (Genesis Genetics).

Participants/materials, setting, methods: The median age of study patients was 35.0 yrs (sd = 5.7). Retrievals averaged 9.6 mature and 7.1 fertilized eggs. After ICSI, embryos were cultured in an Embryoscope (Vitrolife) with laser hatching on D3 and biopsy on D5-D6. Blastocoel expansion was defined as the cross-sectional area of trophectoderm enclosed space both within and herniated outside of the ZP (in u^2) and measured hourly for 10 hours beginning at blastocyst formation (Tb).

Main results and the role of chance: Of 188 blastocysts from 34 PGS cycles analyzed, 89 (47.3%) were euploid and 99 (52.6%) aneuploid. The euploids' expansion slope (756.3) was statistically higher than for aneuploids (503.3) beginning 6 hours from Tb (p < 0.004). A scatter plot correlating Tb with expansion cross sectional area (CSA) at 8 hours from Tb showed a similar distribution of euploids and aneuploids at an intermediate expansion CSA of 15,000–20,000u^2 (euploid = 58%; aneuploid = 54%). In contrast, a greater percentage of all euploids (29%) showed more robust expansion > 20,000u^2 versus aneuploids (12%). Conversely, a higher proportion of all aneuploids (32.3%) expanded more slowely (< 15,000 u^2) versus euploids (12.3%). Based on these findings, individual blastocyst expansion slopes were used to rank embryos for hypothetical transfer within each patient's cohort. The percent euploid was highest in embryos ranked 1 or 2 (61.7% and 62.5%). Euploidy decreased in embryos ranked 3 or 4 (34.4% and 30.7%). Notably, 27/34 (79.4%) patients had at least one euploid embryo ranked 1 or 2, and in 14/34 (41.1%) patients, both embryos ranked 1 and 2 were euploid. No clear differences between euploid and aneuploid embryos were apparent in several earlier morphokinetic parameters (t2, t3, t4, t5, t6, Tsb, Tb).

Limitations, reasons for caution: This was a retrospective observational study. It is also limited by the sample size of both patients and blastocysts. In addition, this is the first application of a new metric of blastocyst expansion to biopsied embryos which limits wider comparison of results and power analysis.

Wider implications of the findings: Morphological selection of single embryos for transfer remains challenging. Results are the first to describe a new metric of blastocyst expansion rate in embryos from infertility patients undergoing PGS. Results suggest that expansion rate correlates with euploidy and describe its application to embryo ranking for transfer with or without PGS.

Trial registration number: No trial registration.

P-134 Relationship between early division in embryo and their ploidy status. A retrospective review

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Study question: Is there any correlation between early cleavage (EC) and ploidy status of embryo in different female age groups?

Summary answer: Embryos that showed early division had significantly lower risk of being aneuploid compared to non-early cleavage (NEC) or non-syngamy (NS) embryos, regardless patient age.

What is known already: The aneuploidy formation in human embryos can be meiotic or mitotic origin. The relationship between timings of early mitotic divisions and ploidy status of embryo was reported in previous studies, where delay in cleavage division were correlated with aneuploidy. Some time-lapse studies showed that the timing of first cleavage division is not age-related.

Study design, size, duration: This is a retrospective study from January 2015 until November 2016, with 165 ICSI+PGD cases were included during this period of study. EC embryos were compared to NEC and NS embryos respectively, subdivided into two age groups (≤35 year-old and > 35 year-old).

Participants/materials, setting, methods: EC assessment was performed on normally fertilized embryos at 25–27 hours post-injection. EC (2 cells), NEC (no sign of cleavage) and NS embryos (remained 2-pronuclei) were cultured separately. Biopsy was performed at either cleavage or blastocyst stage. Biopsy criteria: ≥6cells embryo scored grade 1–3 quality (in-house grading system, grade 1 = best, grade 5 = worst); full blastocyst scored AA to BB quality (Gardner's grading system). Array CGH for chromosome screening. Fisher's exact for statistical analyses (significant level at < 0.05).

Main results and the role of chance: A total of 755 biopsied embryos (327 Day 3 embryos and 428 blastocysts) were included in this study: 314 were EC embryos, 441 were NEC embryos at which 213 were NS embryos. The proportion of EC embryos in patients ≤35 year-old (35.8%) was slightly higher compared to patient > 35 year-old (31.0%) but this was not significant, p = 0.0567. In general, the proportion of euploid embryos from EC group (57.3%) was significantly higher than NEC group (45.4%), regardless of their age group; p = 0.0015. In patient ≤35 year-old, the percentage of euploid embryos from EC group was significantly higher than NEC group, 61.9% vs 51.5%, similar event was found in patient > 35 year-old, 44.6% vs 29.7%; p = 0.023 & p = 0.0389,

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respectively. Meanwhile, the ploidy status of NS embryos were also investigated. The proportion of NS embryos from patients > 35 year-old was slightly higher than patient ≤35 year-old, 39.5% vs 35.9%, p = 0.1643. Our study showed that the percentage of aneuploid embryos found in NS group was statistically higher compared to EC group (60.5% vs 42.7%, p = 0.0001); this was most significant in patient ≤35 year-old (56.4% vs 38.1%, p = 0.0005) in comparison to patient > 35 year-old (70.5% vs 55.4%, p = 0.0829).

Limitations, reasons for caution: The retrospective nature of the study may be a source of undetected bias. There patients have various indications requiring PGS and may not reflect the general subfertile population. Besides, inconsistency during early cleavage assessment and the subjectivity in selecting embryos that fit our criteria for biopsy is our limitation.

Wider implications of the findings: Our observation shows that EC embryos are related to lower aneuploidy rate and should be given the priority for transfer or freezing. By performing routine EC checking, we believe it can optimize the selection of potential euploid embryos for patients as an alternative to PGS.

Trial registration number: None.

P-135 Magnetic-activated cell sorting of non-apoptotic spermatozoa improves the good quality blastocyst rate in women over 30 years old: a prospective study on sibling oocytes

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Study question: Does magnetic-activated cell sorting (MACS) of non-apoptotic spermatozoa improve the outcome of intracytoplasmic sperm injection (ICSI) in couples treated due to teratozoospermia?

Summary answer: If MACS-selected non-apoptotic spermatozoa are used for ICSI, the rate of good quality blastocyst is improved, but only in women older than 30 years.

What is known already: MACS selection of non-apoptotic spermatozoa enables the selection of more quality and functional spermatozoa for ICSI. Besides the selection of only non-apoptotic spermatozoa, these spermatozoa also have a lower level of DNA fragmentation. Previous studies have indicated that using such spermatozoa for an in vitro fertilization procedure improves the fertilization rate, embryo quality and pregnancy rates. Despite these improvements, there is still no general consensus about using MACS selection of non-apoptotic spermatozoa in IVF or indications for it.

Study design, size, duration: Our prospective sibling-oocyte study included 26 couples who were treated in 2016 with ICSI due to male infertility, more precisely due to teratozoospermia (defined by strict Kruger criteria). The women in the couple included in the study were not older than 36 years (mean age 31.3 ± 2.4 years) and were required to have a normal ovarian response after controlled ovarian hyperstimulation (at least 8 mature MII oocytes).

Participants/materials, setting, methods: The semen sample preparation was performed using two-layer density gradient (40%/100%) centrifugation method. Then the 100% fraction was divided into two parts, where the first part was washed and swim-up performed and the second part was washed, processed with MACS® ART Annexin V System (Miltenyi), washed again and swim-up performed. With each part of prepared spermatozoa, half of mature MII oocytes were fertilized. To determine the differences between these two groups, multivariate analysis was performed.

Main results and the role of chance: The comparison of MACS-selected and non-selected spermatozoa showed that the percentage of morphologically normal spermatozoa was statistically lower in selected non-apoptotic spermatozoa (P = 0.025). When looking closer into morphologically abnormal spermatozoa, there were significantly more spermatozoa with abnormal tails (P = 0.013) and a trend towards more spermatozoa with an abnormal neck and midpiece in MACS-selected non-apoptotic spermatozoa (P = 0.057). On the contrary, there were significantly more spermatozoa with abnormal heads in unselected population (P = 0.015), which indicates MACS selection could have positive effect on the population of spermatozoa used for ICSI procedure. This positive effect was further observed when these spermatozoa were used for fertilization with ICSI. More precisely, the good quality blastocyst rate was improved with MACS-ICSI in women over 30 years old (33.3% vs. 75.0%, P = 0.028), but in patients aged 30 years or less, there was no difference (69.2% vs. 50.0%, P = 0.336). Despite the good quality blastocyst rate was improved with MACS-ICSI, the overall blastocyst rate was comparable (ICSI vs. MACS-ICSI; 44.8% vs. 41.3%). No significant difference was observed in terms of the good quality day 3 embryo rate (58.4% vs. 62.4%), and pregnancy rate (33.3% vs. 31.3%).

Limitations, reasons for caution: The limitations of the study are in the relatively low number of included couples, but the study is still in progress. Achieved pregnancies must also be followed in order to evaluate the miscarriage and birth rate.

Wider implications of the findings: Non-apoptotic sperm selection could be of benefit to infertile couples treated due to teratozoospermia having previously blastocysts of poor quality, in order to improve the quality of embryos, but only when women are older than 30 years of age.

Trial registration number: The study was approved by the Slovenian Medical Ethical Committee.

P-136 Blastocyst Morphology (ICM and TE) stipulates endometrial receptivity during implantation window period and strongly correlates with 17-hydroxyprogesterone (17-α-OHP) as well as pregnancy outcome

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Study question: To investigate if blastocyst morphology correlates with the hormone 17- α-hydroxyprogesterone and whether this correlationship has any bearing on pregnancy outcome in IVF cycles.

Summary answer: Blastocyst morphology (Inner cell mass/trophectoderm) correlates with 17-α-hydroxyprogesterone (17-OHP) formation as well as pregnancy outcome and dictates endometrial receptivity during implantation window in IVF cycles.

What is known already: The endometrium prepares itself for implantation in every menstrual cycle. The hormones estrogen and progesterone have been known to enhance endometrial receptivity. The endometrium becomes receptive strictly during a window period, outside of which frame, it remains non-receptive for this highly dynamic and multifactorial phenomenon. We therefore speculated that blastocyst entry in uterus must be coinciding with a sequence of reactions including conversion of progesterone to its hydroxylated metabolite, 17- α-hydroxyprogesterone. Since same dose of progesterone is supplemented to all women before transfer in IVF cycles, its conversion to 17-OHP must hold the key for successful implantation and ensuing pregnancy.

Study design, size, duration: Prospective analysis of 17-Hydroxyprogesterone levels on day of embryo transfer (dET) in 181 women undergoing antagonist stimulation protocol at our private infertility centre from April 2015-April 2016. All women received elective single blastocyst transfers on day5 after OPU. 17-α-OHP was measured in serum by RIA method. Blastocyst stage and morphology: Inner cell mass (ICM) and Trophectoderm (TE) were graded as per Gardner's Blastocyst gradation system. Statistical analysis was performed using Graph pad prism V software.

Participants/materials, setting, methods: Women were classified into 3 groups (top, average, poor) respectively on the basis of ICM (grade 3: n = 92, grade 2: n = 76, grade1: n = 13) and TE cells (Grade 3: n = 78, grade 2: n = 88, grade 1: n = 15). Hormones estrogen (E2), progesterone (P), hydroxyprogesterone (17-α-OHP) and pregnancy outcome were intercompared between the groups. Endometrial thickness (ER) and echopattern (ER grade) were measure ultrasonographically on dET. Clinical Pregnancy rate (CPR) and Live birth rate (LBR) were main outcome measures.

Main results and the role of chance: Women with ICM grade 3, 2, 1 differed significantly (One way ANOVA) w.r.t following parameters: TE (p < 0.0001); E2/dET (p < 0.0001); OHP/dET (p = 0.0046); E2/d14ET (p = 0.04); OHP/d14ET

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(p = 0.0007); CPR (p = 0.0008) and LBR (p = 0.0015). However there was no statistically significant difference in P/dET (0.4); P/d14ET (p = 0.49) as well as Endometrial thickness on dET (p = 0.53); endometrial echopattern dET (p = 0.52). ICM correlated significantly with OHP/dET (Pearson r = 0.24, p = 0.0011); CPR (Pearson r = 0.27, p = 0.0002) and LBR (Pearson r = 0.24, p = 0.0005).

Women with TE grades 3, 2, 1 showed significant differences w.r.t ICM (p < 0.0001); E2/dET (p = 0.0081); OHP/dET (p < 0.0001); E2/d14ET (p = 0.04); OHP/d14ET (p = 0.03). However there was no significant difference between the groups in endometrial thickness (p = 0.57); endometrial echopattern (p = 0.82); P/dET (p = 0.94); P/d14ET (p = 0.39). Although CPR and LBR were higher in the TE grade3 group compared to others, the difference was not statistically significant (p = 0.1, 0.13 respectively). TE correlated strongly with OHP dET (Pearson r = 0.42, p < 0.0001) but weakly with CPR (Pearson r = 0.14, p = 0.05) and LBR (Pearson r = 0.15, p = 0.05).

These results indicate that morphological and hormonal modifications including formation of 17-α-OHP from progesterone occur simultaneously during implantation window period. These parameters together influence pregnancy outcome irrespective of the endometrial thickness and echopattern. ICM correlates more than TE with pregnancy outcome.

Limitations, reasons for caution: Not all morphologically top embryos lead to pregnancy. On the other hand, some morphologically poor embryos do successfully implant. Therefore, a deeper understanding into the finer aspects of morphological assessment, hormonal investigations and their correlationship is required. More detailed, multicentric studies are required to comprehend this complicated human implantation process.

Wider implications of the findings: Rather than relying on ultrasound finding of endometrial thickness and echopattern on dET, it is better to assess hormonal parameters before transfer. This may give us a better indication of endometrial receptivity and therefore to decide whether to transfer embryo in same or next natural cycle and increase pregnancy rates.

Trial registration number: Not applicable.

P-137 The regulatory roles of polyamines/let-7 signaling in embryo dormancy and activation in mice

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Study question: Does the balance of polyamines and let-7 determine whether blastocysts should implant or become dormant?

Summary answer: The blastocysts implant when polyamine is high but become dormant when that of let-7 is high.

What is known already: Polyamines are required for embryonic development as embryos lack of Ornithine decarboxylase (ODC1), the rate-limiting enzyme in the biosynthesis of polyamines, cannot survival after E7.5. Deprivation of polyamines significantly decreases implantation sites in mice and mink. Let-7 is up-regulated in dormant blastocyst and its forced-expression inhibits embryo implantation in vivo.

Study design, size, duration: The expression of ODC1 in dormant and estrogen-activated embryos was detected. The effects of polyamines on the expression of let-7 in dormant embryos, uterine primary epithelial and stromal cells were determined. The possible involved molecular mechanisms between them were explored. The influence of polyamines in activating dormant embryos in vitro was studied. The Kaplan-Maier survival curves of electroporated blastocysts with or without pre-let-7a during ex vivo culture was depicted.

Participants/materials, setting, methods: ICR female mice aged 6–8 weeks were used in this research. The expression of let-7 was detected by qPCR and the expression of Odc1, eIF5A, Lin28 and c-Myc was detected by immunostaining/western blotting. D4 blastocysts were electroporated with pre-let-7a and dormant blastocysts were cultured in the presence/absence of polyamines. All experiments were repeated more than 3 times and at least 5 embryos used in each group.

Main results and the role of chance: The expression of Odc1, Spermine Synthase, eIF5A and c-Myc in both embryos and the uterine epithelial cells was up-regulated after estrogen injection. While let-7a expression in primary epithelial and stromal cells was downregulated. Polyamines positively regulated the expression of eIF5A, Lin28 and c-Myc, but negatively regulated let-7 expression in both blastocysts and JEG3 cells. However, let-7 overexpression has little effect on the expression of ODC1 in embryos, primary epithelial and stromal cells. These results implied that polyamines may regulate the expression of let-7 via eIF5A/Lin28/let-7 axis and this regulation is unidimensional. Polyamines increased implantation rates and outgrowth of blastocysts in vitro. Proliferation (EdU) and nascent translation (HPG), markers of dormant embryo activation, were elevated by polyamines in dormant embryos. Blastocysts with let-7a overexpression can survive up to Day 13 and still have the ability to implant and give rise to live-born mice.

Limitations, reasons for caution: The balance between polyamines and let-7 has an important regulatory role in embryo implantation and dormancy. The molecular mechanisms involved in the balance needs to be explored. The applicability of this result in other animals and the utility of manipulations of this balance in assisted reproduction warrant further investigation.

Wider implications of the findings: Our data shows that polyamines activate dormant blastocysts in vitro and let-7 induces embryo dormancy. The balance between polyamines and let-7 is important for embryo implantation and dormancy. This study will shed light on the understanding of embryo dormancy in other animals and provide possible explanations for human implantation failure.

Trial registration number: No.

P-138 MicroRNAs in day three embryo culture media as non-invasive biomarkers of implantation and live birth

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Study question: Are microRNAs (miRNAs) detected in the culture media of individual *in vitro* produced embryos potential biomarkers of embryo implantation and live birth?

Summary answer: The miRNA signature may be a tool to predict the embryo implantation and live birth.

What is known already: In spite of remarkable improvements in *in vitro* fertilization (IVF) since it was established, its efficiency, measured as the live birth rate, is usually low. Successful improvements to IVF depend on the ability to select the most viable embryos for transfer, which remains a challenge. MicroRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression through mRNA degradation or inhibition of translation. MicroRNAs can be detected in the extracellular environment, such as biological fluids. Circulating miRNAs are already used as diagnostic and prognostic markers of several diseases; however, its utility as a biomarker of embryo implantation is still unknown.

Study design, size, duration: This case-control study included spent culture media from 36 blastocysts transferred on day five. Samples, collected on day three, were split into groups according to the implantation: Positive-Implantation-Group (n = 18) or Negative-Implantation-Group (n = 18). For the first analysis, samples were pooled in three sets for each group and the expression of microRNAs was compared between the groups. To check if miRNAs could be detected in individual samples, in a second analysis, ten more samples were tested.

Participants/materials, setting, methods: Samples from culture media were derived from patients undergoing intracytoplasmic sperm injection (ICSI), in a private university-affiliated IVF center. Samples were collected between January/2015 and November/2015 and were included exclusively samples

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from high-quality blastocysts. The Positive-Implantation-Group included exclusively samples from patients with 100% implantation diagnosis. Seven miRNAs (*miR-21, miR-142-3p, miR-19b, miR-92a, miR-20b, miR-125a, and miR148a*) selected according with the literature, was tested. For the second analysis, 10 more samples were tested for *miR-21* and *miR-142-3p*.

Main results and the role of chance: All blastocysts from the Positive-Implantation-Group lead to live births. From the seven tested miRNAs (*miR-21, miR-142-3p, miR-19b, miR-92a, miR-20b*, and *miR-125a*), expression of four (*miR-21, miR-142-3p, miR-19b,* and *miR-92a*) could be detected in spent media from pooled samples. Three miRNAs (*miR-21, miR-142-3p,* and *miR-92a*) were differently expressed between the groups. A significant increased expression of *miR-142-3p* could be noted in the Negative-Implantation-Group ($p < 0.001$). For other two miRNAs *(miR-21* and *miR-92a)* a difference in expression was observed, however it did not reach a statistical significance. In addition, when ten non-redundant samples were tested to check if miRNAs could be detected in individual samples of culture media, highly specific amplification of mature miRNAs, including *miR-142-3p*, could be noted.

Limitations, reasons for caution: Besides proper embryo development, embryo implantation also depends on the acquisition of a receptive endometrium and appropriate dialogue between the embryo and endometrium. Therefore, the identification of the embryo with the best implantation potential may not guarantee the positive implantation and live birth outcome.

Wider implications of the findings: *MiR-142-3p* may be a biomarker of implantation failure. The identification of specific miRNAs on the culture media offers opportunities for early, fast and cheap diagnosis of implantation, and live birth. This may reduce emotional and financial costs. Moreover, it favors the single embryo transfer, avoiding multiple pregnancies and its consequences.

Trial registration number: None.

P-139 Managing supernumerary embryos at the day of embryo vitrification

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Study question: Benefits of culturing day-2 supernumerary embryos whatever their quality and evaluation of predictive factors of ART outcomes in frozen-thawed blastocysts cycles originating from these embryos.

Summary answer: Poor quality embryos (PQE) are able to give healthy babies. Women under 35 years old and day-5 blastocyst expansion are predictive of live birth.

What is known already: The management of supernumerary day-2 PQE is extremely different from an IVF laboratory to another between culturing these embryos until the blastocyst stage and destroying or including them in research programs. The few available data on this topic don't seem to be sufficient to make conclusions concerning the possible benefits of culturing these embryos. Some authors proffer that embryo quality at cleavage stage is correlated with implantation potential, whereas others have stipulated that poor morphological parameters do not preclude successful implantation and that day-2 PQE should be given a second chance by performing extended culture.

Study design, size, duration: Cohort study from November 2012 to February 2015 in the IVF Laboratory Unit of Cochin University Hospital (Paris, France). A total of 3108 supernumerary embryos resulting from 1237 IVF/ICSI cycles were included. Exclusion criteria were cycles with gamete donation and freeze-all and deferred embryo transfer. This study was approved by the National Data Protection Authority.

Participants/materials, setting, methods: At day-2, embryo quality was assessed according to the ESHRE consensus (Istanbul, 2011) distinguishing good-quality embryo (GQE), fair and PQE. After fresh embryo transfer, supernumerary embryos were cultured. Rates of blastulation, good-quality blastocysts, pregnancy,live birth and neonatal outcomes were compared between PQE and GQE. At the blastocyst stage, top, good and fair-quality blastocysts were selected for vitrification. We evaluated factors associated with ART success when transferring frozen-thawed blastocysts originating from PQE and GQE.

Main results and the role of chance: The blastulation rate was 48.7% in PQE group and 67.2% in GQE group. The percentage of good-quality blastocysts was 47.9% and 60.7% respectively including 7.3% and 14.7% top-quality blastocysts. A total of 150 blastocysts originating from GQE and 729 from PQE were frozen. From the frozen blastocysts, 37 and 161 were thawed and transferred respectively resulting in 19 (51.4%) and 61 (37.9%) clinical pregnancies. These pregnancies gave 13 (35.1%) deliveries in GQE group and 32 (19.9%) in PQE group ($p = 0.046$). Interestingly, the quality of blastocysts that resulted in live birth was similar in the two groups. We didn't observe any statistically significant difference in neonatal outcomes between the groups. Regarding age and time of blastocyst expansion at day-5 or 6, women under 35 years old and day-5 blastocyst expansion were predictive of ongoing pregnancy and live birth.

Limitations, reasons for caution: The poor predictive value of the morphological approach in embryo selection could constitute a limitation in this study. This aspect is illustrated with the possible contribution of the trophectoderm (TE) to implantation and delivery success but not to the live-birth. More studies on larger series are needed to evaluate it.

Wider implications of the findings: Our study clearly demonstrated the merits of keeping PQE in extended culture, as these gave rise to pregnancies and live births. These data should encourage embryologists to vitrify surplus good and fair-blastocysts quality originating from poor-quality cleavage stage embryos in order to optimize the chances for infertile couples to conceive.

Trial registration number: Not applicable.

P-140 The choice of the best embryo originating from spermatozoa of men with severe male factor infertility: morphokinetics and next-generation sequencing (NGS) results

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Study question: To examine the influence of poor sperm quality on embryo morphokinetics and aneuploidy rate; to evaluate implantation potential of euploid embryos obtained from poor sperm.

Summary answer: Embryos of men with severe oligoasthenoteratozoospermia have slower morphokinetics and lower implantation potential, however their aneuploidy rate does not differ from embryos of normospermic men.

What is known already: Time-lapse (TL) technology provides more informative evaluation criteria of embryo development that can increase the chance of identifying the embryos with higher implantation potential and improve embryo selection. However, the data concerning the association between morphokinetic parameters and ploidy of embryos after trophectoderm biopsy are controversial. It is known that not all high quality embryos determined according to TL-test, become blastocysts, and among the blastocysts almost 50% embryos with good morphology are aneuploid. Moreover, there are no consistent results regarding possible influence of poor sperm quality on early embryo development and the risk of aneuploidies.

Study design, size, duration: Retrospective study (2015–2016) of morphokinetics and ploidy of 188 blastocysts obtained after ICSI procedure was conducted. 148 blastocysts were formed from spermatozoa of men with severe oligoasthenoteratozoospermia (OAT) and 40 blastocysts were formed from spermatozoa of men with normozoospermia using donor oocytes (female mean age 26 ± 1,8 years). Only the good and top quality hatching blastocysts were biopsied and vitrified on 5–7 day followed by NGS PGD.

Participants/materials, setting, methods: Two groups of patients were compared: 36 men with OAT (< 5 million/ml, motility ≤5%, normal sperm morphology ≤2%) and 9 men with normozoospermia (≥20 million/ml, motility > 50%, normal morphology > 35%). Embryo morphokinetic parameters (2pn fading, cell divisions, intervals between divisions, direct and reverse cleavages, blastocyst hatching) and implantation potential based on the TL-test results (TL-high vs.TL-low) were analyzed using TL monitoring system (PrimoVision). The NGS test was performed using low coverage whole-genome sequencing platform (Illumina).

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Main results and the role of chance: Among 148 biopsed blastocysts from the group of men with OAT 92 embryos were euploid (62.2%). Almost the same result (65.0%) was obtained in the group of men with normospermia (the control): 26 euploid embryos of 40 biopsied blastocysts. The times of 2pn faiding, division to 2, 3 and 5 cells, duration of t3-t2, t3-2pn faiding, t5-t2 and t5-t3 intervals in the euploid embryos were similar in the both groups, but the times of division to 8 cells, blastocyst hatching beginning and synchrony in division from 3 to 4 cells (s2) were significantly longer in the euploid embryos of OAT men comparing with the control ($p < 0.05$). Direct and reverse cleavages were observed more often in the embryos of OAT men (respectively, 26.0 and 21.7%) vs. control (10.0 and 10.0%, $p < 0.05$). The implantation potential of euploid blastocysts was lower in the OAT group vs. control (respectively, 34.8 vs.77.5% of TL-high embryos, $p < 0.05$). The morphokinetics of abnormal embryos of the both groups were similar to the morphokinetics of euploid embryos in the OAT group and significantly differed from the euploid control. The complex embryo aneuploidy was found in 53.6 and 42.9% of cases in the OAT group and in the control respectively.

Limitations, reasons for caution: The study included only biopsied blastocysts obtained from donor oocytes and the best spermatozoa of men with very poor or high sperm quality. The embryos, which have not become blastocysts, or blastocysts of low morphological quality were not biopsied and not included in the study.

Wider implications of the findings: The complex applying of embryo TL monitoring and comprehensive, cost-effective NGS for PGD of blastocysts aneuploidies allows to select the healthy embryos with high implantation potential. Besides the severe male factor infertility, it is recommended in cases of advanced female age and the high miscarriage rate.

Trial registration number: No registration number.

P-141 Intraindividual embryo morphokinetics are not affected by a switch of the ovarian stimulation protocol between GnRH agonist versus antagonist regimens in consecutive cycles

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Study question: Are intraindividual embryo morphokinetics affected by switching the type of GnRH analogue used (agonist versus antagonist) in consecutive treatment cycles of controlled ovarian stimulation (COS)?

Summary answer: Embryo morphokinetics are not affected by a switch in the type of GnRH analogue used for down-regulation in consecutive cycles of COS.

What is known already: Murber et al. (2009) described differences between oocytes/embryos from patients treated with gonadotrophin-releasing hormone (GnRH) agonists versus antagonists and found a higher number of blastomeres in day 2 embryos after antagonist treatment. Muñoz et al. (2013) found that embryos cleaved faster when they were generated in cycles with GnRH antagonist plus GnRH agonist-trigger versus GnRH agonist plus hCG-trigger. However, the group did not detect differences in embryo quality based on morphokinetics. A recent intervention review found no difference between GnRH antagonist and the long protocol of GnRH agonists for COS regarding live birth and miscarriage rates (Al-Inany et al., 2016).

Study design, size, duration: This retrospective cohort study analyzed morphokinetics of embryos from patients (n = 49) undergoing a switch in COS protocols between GnRH agonists (n = 49 cycles, n = 175 embryos) and GnRH antagonists (n = 49 cycles, n = 164 embryos) after culture in a time-lapse incubator (EmbryoScope®, Vitrolife) in our clinic between 06/2011 and 11/2016. Included were all embryos of the two consecutive cycles before and after a switch in the type of COS in the same patient.

Participants/materials, setting, methods: IVF or ICSI was performed on oocytes from agonist cycles (Nafarelin, Triptorelin or Leuprorelin), or antagonist cycles (Cetrorelix or Ganirelix). Embryos were time-lapse imaged up to day 5. Morphokinetics were annotated as described by Ciray et al. (2014) and presented in hours (h) as median (quartiles 25–75). Data were analyzed by pairwise exclusion using Mann-Whitney-test or Fisher's exact test (SPSS Version 22). The significance level is $p = 0.05$. Patients with preimplantation genetic screening cycles were excluded.

Main results and the role of chance: The mean age (years ± standard deviation) of patients at the time of treatment was 35.65 ± 4.28 (agonist) and 35.84 ± 3.96 (antagonist) ($p = 0.94$). The number of oocytes collected was 9.03 ± 4.52 (agonist) and 9.53 ± 4.08 (antagonist) ($p = 0.54$). The fertilization rate was 47.2% (239/506; agonist) and 44.8% (209/467; antagonist) ($p = 0.441$). We found no statistically significant difference between the analyzed morphokinetic parameters between the study groups. Time to extrusion of polar body 2 (tPB2) was tPB2(agonist) = 3.52 h (2.77–4.22; n = 126) or tPB2 (antagonist) = 3.44 h (2.84–4.16; n = 93) ($p = 0.63$). The pronuclei had faded (tPNf) at 24.43 h (22.68–27.46; n = 163) (agonist) or 25.13 h (22.89–27.74; n = 152) (antagonist) ($p = 0.17$). The time to 2-cell stage (t2) was at t2 (agonist) = 27.38 h (25.18–30.60; n = 172) or t2(antagonist) = 27.60 h (25.56–30.68; n = 159) ($p = 0.72$). There was no significant difference in the times to 3- to 9-cell and morula stage. Early blastocyst stage was reached at tSB (agonist) = 99.74 h (91.37–102.93; n = 65) or tSB(antagonist) = 100.56 h (91.40–104.54; n = 66) ($p = 0.41$). There was no significant difference in the times to blastocyst, expanding blastocyst, the duration of embryonic cell cycles (ECC) 1 (= t2-tPB2), ECC2 (= t4-t2) and ECC3 (= t8-t4), as well as the synchronicity of cell divisions s2 (=t4-t3) and s3 (=t8-t5). There was no significant difference in the rate of direct cleavage (DC; t3-t2 < 5 h) between the study groups (DC(agonist) = 10/174 (5.75%) vs. DC(antagonist) = 7/158 (4.43%) ($p = 0.627$)).

Limitations, reasons for caution: We did not test for differences in embryo quality based on standard morphology assessment. Larger confirmation studies need to be performed, as our sample size is small.

Wider implications of the findings: In contrast to another study (Muñoz et al., 2013) we did not find differences in embryo morphokinetics after switching the type of GnRH analogue used for COS. This finding supports the flexible use of GnRH analogues to optimize patient treatment for COS without affecting embryo morphokinetics.

Trial registration number: A trial registration number was not required due to the retrospective study design.

P-142 Extended culture and Day-5 transfer of frozen-thawed cleavage stage embryos is associated with higher implantation rate when compared with embryos frozen-thawed-transferred at the cleavage stage

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Study question: Do implantation rates differ of Day-3 frozen-thawed-transferred cleavage-stage embryos versus Day-3 frozen-thawed-extended cultured and Day-5 transferred blastocysts?

Summary answer: Extended culture of thawed cleavage-stage embryos to Day-5 and transfer enhances implantation and minimizes multiple rates when compared thawing and transferring at the cleavage-stage.

What is known already: When compared to cleavage stage, live birth rate is higher with fresh blastocyst-stage transfer despite lower embryo freezing rate. With improvements in in-vitro culture systems and vitrification techniques, there is, currently, a worldwide trend of more fresh blastocyst-stage transfers with surplus blastocysts frozen on Day 5/6. Progression to blastocyst formation following thawing of a cleavage-stage embryo may reflect competency and viability, and hence may be employed as a selection criterion. There is paucity of data whether thawing cleavage-stage embryos and transferring on Day-5 following extended culture enhances implantation rate when compared with transfer at the cleavage-stage.

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Study design, size, duration: Retrospective cohort study of frozen embryo replacement (FER) cycles between April-2013 and November-2016. As a policy, surplus embryos have been frozen exclusively at blastocyst-stage since June-2014; before that date cryopreservation was done at cleavage-stage. At cleavage-state, when number of frozen embryos exceeded planned number of embryos to be transferred (1 or 2), extended culture of two additional days was undertaken. Only, first FER cycle was included per patient. Implantation rate was primary outcome.

Participants/materials, setting, methods: Three groups of patients were generated according to the day of freezing-thawing and embryo transfer. Group A constituted 91 cycles frozen-thawed and transferred at cleavage-stage. Group B constituted 232 cycles frozen (Day 5/6)-thawed and transferred at blastocyst-stage. Group C constituted 41 cycles frozen-thawed at cleavage-stage, extended cultured and transferred at blastocyst-stage (Day 5). Slow freezing was more commonly employed in Groups A (64.8%) and C (65.9%), whereas vitrification was used exclusively in Group B.

Main results and the role of chance: The mean female age, body-mass index, duration of infertility, number of previous in-vitro fertilization attempts was comparable among the three groups. As expected, the number of surplus embryos to be frozen was significantly less in Group B when compared with Groups A and C (2.7 ± 1.7 vs. 6.7 ± 2.8 and 6.7 ± 3.7, respectively, $p < 0.001$). The survival rates of cleavage-stage and blastocyst-stage embryos were 76.4% and 95.4% ($p < 0.001$), respectively. The mean number of embryos transferred in Groups A, B and C was all statistically different from each other (1.9 ± 0.3, 1.4 ± 0.5 and 1.3 ± 0.5, respectively, $p < 0.001$). The respective figures for ongoing pregnancy were 26.4%, 41.8% and 34.2% (Groups A and B, $p = 0.01$). Implantation rate in Group A was significantly less when compared with Groups B and C (21.6% vs. 49.1% and 40.7%, $p < 0.001$). Ongoing pregnancy and implantation rates of Groups B and C were comparable. Miscarriage rates were comparable among the three groups. Multiple pregnancy rates were 15.2% and 19.9% in Groups A and B, respectively ($p > 0.05$); there was no case of multiple gestation in Group C.

Limitations, reasons for caution: Despite comparable baseline demographic features of the three groups, retrospective study design is a drawback. The lack of use of a single freezing method is another drawback despite comparable rates of use of slow-freezing and vitrification methods in Groups A and C.

Wider implications of the findings: If frozen at the cleavage-stage and number of frozen embryos exceeds planned number of embryos to be transferred (1-2 in current series), extended culture of two additional days might enhance selection of the most competent embryo. Such a policy is associated with higher implantation rate and minimizes risk of multiples.

Trial registration number: None.

P-143 Does vitrification of day3 embryos impose methylation errors in in vitro produced human blastocysts?

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Study question: Regarding the possibility of epigenetic risks induced by vitrification, this study assessed the effect of vitrification on DNA methylation pattern of ICR1 in human blastocyst.

Summary answer: Vitrification of Day3 embryo does not appear to alter the DNA methylation pattern of ICR1 in high - quality blastocyst.

What is known already: 'Freeze-all' strategy has been proposed in ART clinics due to prevention of interference between superovulation and endometrial receptivity. Superovulation may decrease endometrial receptivity results in an imbalance synchronized between endometrium and embryo. In addition of benefits of embryo vitrification, there are concerns about possible neonatal complications in children born after freezing ET such as large baby syndrome. Also, vitrification of Day 3 embryo coincides with genome-wide epigenetic reprogramming and zygotic genome activation (ZGA) which may prone embryos to alterations of imprinting control region (ICR). Therefore, we compared methylation status of ICR1 in human blastocyst-derived from vitrified with non-vitrified embryos.

Study design, size, duration: A total 30 embryos were donated from 11 healthy couples with at least two children of same-sex referring for family balancing. Assessment of the embryos was performed in two non-vitrified and vitrified groups. Day3 embryos derived from ICSI were either biopsied or vitrified/warmed and subsequently biopsied. Following biopsy, embryos were cultured to day 5. Day5 blastocysts with sex desired were transferred or vitrified for future use. Un-desired blastocysts were donated for our study.

Participants/materials, setting, methods: The methylation pattern of ICR1, also known IGF2/H19 DMR was determined by bisulfite conversion and sequencing, on 30 blastocysts and one million peripheral blood lymphocytes as quality control to obtain the pattern of imprinted gene methylation in somatic cells. This region involved in imprinting disorders such as BWS and SRS which are linked to ART. We assessed the methylation of 18 CpGs in a 220 bp fragment of ICR1 contains the CTCF binding site.

Main results and the role of chance: To identify whether embryo vitrification could alter the DNA methylation degree of

ICR1 in the blastocyst, the region including 18 CpGs was analysed. In addition, more than 15 clones were sequenced per replicate. The average methylation pattern of CpGs in the non-vitrified and vitrified groups were 38.27 ± 4.1% and 35.3% ± 3.6, respectively. Thus, this level of overall methylation between the two groups was not significantly different ($P > 0.05$). The methylation status of lymphocyte was 49.52 ± 1.86%. The percentage of hyper-methylated clones of ICR1 in non-vitrified and vitrified groups were 43.75 ± 5.1% and 38.7% ± 4.9, respectively. The difference of proportion of hyper- methylated clones between two groups was not significantly ($P > 0.05$). This value for the lymphocyte was 50%. Therefore, the data revealed that vitrification could not affect DNA methylation pattern of ICR1 in the blastocyst. However, the methylation status of ICR1 in both groups is lower than the expected methylation level of somatic cells. One likely possibility for the observed methylation level in vitrified and non-vitrified embryos would be that methylation level of control regions in imprinting genes are not rigid during pre-implantation development embryo and can be affected at least in part by the genome-wide demethylation.

Limitations, reasons for caution: Possibility the preferential amplification of maternal or paternal allele due to small starting amounts. Also, further studies are needed to make sure that expression of H19 and IGF2 is not changed.

Wider implications of the findings: 1) This finding may confirm the policy of 'freeze all embryo" regarding the adverse effects of superovulation which may perturb endometrial receptivity. 2) Despite a general consensus that imprinted genes are highly protected from genome-wide demethylation during pre-implantation development embryo, but these can be affected at least in part.

Trial registration number: No = 24088.

P-144 The effect of changes in embryo culture environment on the rate of pregnancy and miscarriage

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Study question: Do changes in embryo culture environment effect the rate of clinical pregnancy and miscarriage?

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Summary answer: In this study, changing the culture environment resulted in a significant increase in the rate of Day5 blastocyst transfer whilst the miscarriage rate significantly decreased.

What is known already: Previously, we reported an improvement in blastocyst development and number of good quality embryos by changing from sequential medium to single culture medium (Shimomura et al ESHRE 2014). In addition, we have reported that the blastocyst development rate was improved by changing from standard culture dish to WOW culture dish (consists of 25 microwells and allows group culture under a single drop of medium) (Watanabe et al ASRM 2015).

Study design, size, duration: This retrospective cohort study involved 557 cases (33.5 ± 3.6 years old) of classic culture method (sequential medium & single culture using standard dish: 2010 to 2012) and 550 cases (33.5 ± 3.5 years old) of the current method (single culture medium & group culture using WOW dish: June 2014 to May 2016). The subjects were all undergoing a first cycle of vitrified-warmed single blastocyst transfer with hormone replacement treatment.

Participants/materials, setting, methods: In the classic method, embryos were cultured in 30 µl of sequential Medium(SAGE) with medium changes on Day3/5 using a standard dish. In the current method, embryos were cultured in 60 µl of single culture medium(NakaMedical) using WOW dish(DNP) without medium change. In both methods, embryos were cultured up to Day7. The resulting blastocysts (≥Blast3) were transferred following the vitrified-warmed with Cryotop procedure (KitazatoBiopharma), and the rate of pregnancy and miscarriage was compared by chi-square test.

Main results and the role of chance: There was no significant difference in the pregnancy rate between the two methods (57.2% vs 56.0%). However, the pregnancy rate of the current method was significantly lower in Day6/7 blastocysts compared with the classic method (53.7% vs 36.6%; 42.9% vs 0%). There was no significant difference in the pregnancy rate on Day5/6/7 blastocysts following the classic culture method (59.6% vs 53.7% vs 42.9%). Yet using the current method, the pregnancy rate of Day5 blastocysts was significantly higher (61.0% vs 36.6% vs 0%) between Day5/6/7 blastocysts. C Comparing the miscarriage rates of classic vs current methods, the latter was significantly lower (23.6% vs 13.3%). Also, the miscarriage rate of Day5 blastocysts between the two methods, the current method was significantly lower (20.9% vs 12.6%). There was no significant difference in the miscarriage rate between Day5/6/7 blastocysts of the classic method. Further, there was no significant difference in the miscarriage rate between Day5/6 blastocysts of the current method (20.9% vs 29.3% vs 0%; 12.6% vs 20 .0%). Furthermore, the percentage of Day5 blastocysts was significantly increased (82.9% vs. 61.4%) as compared with the classic method.

Limitations, reasons for caution: This analysis compares methodologies carried out during two time periods.

Wider implications of the findings: The current method resulted in Day5 blastocysts with a significantly higher the pregnancy rate than Day6/7 blastocysts of the same method. Also, the miscarriage rate was significantly lower than in the classic method. Therefore, it is suggested that embryos of current method are improving quality.

Trial registration number: Not applicable.

P-145 Which is the best culture system? Comparison of biological outcomes obtained in single vs group culture performed on 204 sibling zygotesin PGS cycles

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Study question: It is possible to obtain an improved blastocyst formation rate and/or to increase the number of euploid embryos performing group instead of single culture?

Summary answer: Both culture systems perform similarly; anyway in group culture, increasing the number of embryos cultured simultaneously could be a promising strategy

What is known already: Embryo culture is a critical aspect in IVF. Both single and group cultures are currently applied for human embryos although it is not known which one performs better. Animal studies revealed that culturing embryos in smaller volumes and/or groups significantly increases blastocyst cell number and elevates embryo viability. Several studies highlighted the importance of autocrine and paracrine growth factors influencing embryo development. Culture volume and/or density of co-cultured embryos could be really important in determining the right equilibrium between the final concentration of these embryo beneficial autocrine factors and the embryo-toxic metabolic products which progressively accumulate during embryo culture.

Study design, size, duration: This prospective randomized study started in June 2016 and is still ongoing. Biological outcomes from 204 zygotes obtained in 20 PGS cycles were enrolled. Inclusion criteria were: female age ≤39 years old and at least 6 regularly fertilized oocytes on day-1. Exclusion criteria were: genetic problems, egg donation cycles and severe male factor. Biopsies were performed on 115 blastocysts and analyzed with Next Generation Sequencing. Mean female age was 33.65 ± 3.03 years old.

Participants/materials, setting, methods: All zygotes were split in single (SC) or group (GC) culture (1:1 ratio). Drops of 35 µL and 100 µL of one-step medium at 37°C,6%CO^2,5%$O^2$was used in SG and GC, respectively. Based on the total 2PN, 3-4-5 zygotes were grouped in the same drop in GC (GC-3, GC-4, GC-5 subgroups). All 2PN, both individually (SG) or collectively (GC), were moved in the final drops after the fertilization check without other medium changeover until the blastocyst stage

Main results and the role of chance: A total of 267 mature oocytes were injected with a fertilization rate of 76.4% (N = 204): 100 and 104 zygotes were allocated in SC and GC, respectively. In SC, 95 embryos were obtained with a blastocyst formation rate of 70.5% (N = 67); 57 of them were biopsied from day-5 to day-7 of culture and 22 resulted euploid (38.6%). In GC, 100 embryos were obtained with a blastocyst formation rate of 60.0% (N = 60); 53 of them were biopsied from day-5 to day-7 of culture and 17 resulted euploid (32.1%) (NS). The top quality blastocysts were 32 (47.8%) and 30 (50.0%) in SC and GC, respectively; 50 blastocysts were obtained on day-5 in both SC (74.6%) and GC (83.3%) groups (NS). In the GC subgroups, 48, 36 and 20 zygotes were allocated in GC-3, GC-4 and GC-5, respectively; 46, 36 and 18 embryos were obtained with a blastocyst formation rate of 58.7% (N = 27), 52.8% (N = 19) and 77.8% (N = 14) in GC-3, GC-4 and GC-5, respectively (NS); 21, 18 and 14 of them were biopsied with an euploidy rate of 28.6% (N = 6), 27.8% (N = 5) and 42.9% (N = 6) in GC-3, GC-4 and GC-5, respectively (NS). The quality and the speed of growth were comparable for all blastocysts.

Limitations, reasons for caution: The number of embryos/blastocysts enrolled need to be enlarged. Probably the volume employed in GC is still too high and/or the number of embryos/blastocysts co-cultured is still too low. We are now reducing the volume of group culture to 35 µL and increasing the number of embryos/blastocysts grouped to 4-5-6.

Wider implications of the findings: Actually the choice of culture system is typically a decision based on the laboratory history and routine and/or on the wish to collect data on a single embryo. Data are lacking regarding the influence of the cultures on the biological outcomes, such as the blastocyst formation and the euploidy rates.

Trial registration number: Not applicable.

P-146 Follicular flushing in patients with poor ovarian response: A systematic review and meta-analysis

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Study question: Is the use of double lumen needles and follicular flushing in poor response IVF patients associated with the number of oocytes (cumulus-oocyte-complexes; abbr.: COCs) retrieved?

Summary answer: Flushing with double lumen needles does not increase the mean COC yield in patients with poor ovarian response.

What is known already: Flushing ovarian follicles is widely used, especially in natural-cycle IVF patients or poor responders, as a means to increase the

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number of COCs per aspiration. At least three recent meta-analyses have concluded that flushing does not increase the mean oocyte yield (Wongtra-Ngan et al., 2010; Levy et al., 2012; Roque et al., 2012). Yet, all RCTs but one included in these reviews were performed in normo-responder patients. Recently, further studies have become available. This review collates the evidence on flushing in poor responders.

Study design, size, duration: Multiple literature databases were searched for randomized, fully published studies comparing the outcome of IVF cycles with single lumen needles/no flushing vs. double lumen needles/flushing in poor response IVF patients. Meta-analyses were performed using standard procedures in the software program RevMan v.5.3. All analyses were done per randomized patient, wherever feasible.

Participants/materials, setting, methods: Three RCTs were identified and included in the meta-analysis. Two trials employed a valid randomization method with concealment of allocation, while one study employed pseudo-randomization. All three studies had the primary objective of testing for a difference in oocyte numbers between flushing and no flushing and in all three trials the necessary sample size was determined a-priori. In the control group of all trials, a single lumen needle was used.

Main results and the role of chance: Flushing using double lumen needles does not increase the mean number of COCs per patient undergoing follicular aspiration, but instead there was a trend towards less COCs with flushing (weighted mean difference [WMD]: -0.61 COCs, 95% confidence interval [CI]: -1.38 to 0.15; $p = 0.11$; $I^2 = 48\%$; three RCTs, n = 158). The proportion of randomized patients having at least one COC retrieved was not different between groups (risk difference: -2%, 95% CI: -13 to 8, $I^2 = 38\%$; $p = 0.64$; two RCTs, n = 127). The proportion of randomized patients undergoing embryo transfer was not different between groups (risk difference: -11%, 95% CI: -26 to 5, $p = 0.17$; $I^2 = 32\%$; two RCTs, n = 127). The clinical pregnancy was not different between groups (relative risk: 0.82, 95% CI: 0.52 to 1.30, $p = 0.4$; $I^2 = 87\%$; three RCTs, n = 158). The procedure duration was significantly increased with flushing (WMD +2.4 minutes, 95% CI: 1.8 to 2.9; $p < 0.001$; $I^2 = 0\%$; three RCTs, n = 158).

Limitations, reasons for caution: The combined sample size is large enough and the individual trials sufficiently homogenous in outcome to conclude with confidence that flushing in poor responders does not increase the COC yield. The combined sample size is still insufficient to allow conclusions on pregnancy likelihood.

Wider implications of the findings: Double lumen needles/flushing should not be employed for follicular aspiration in patients with poor ovarian response, since a positive effect could not be shown for any of the outcomes. Double lumen needles and flushing media are costly, while flushing increase effort for the team, and procedure duration for the patient.

Trial registration number:

P-147 A trial to predict blastocyst development of human embryos by Chip-sensing Embryo Respiratory Monitoring System

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Study question: Does the Chip-sensing Embryo Respiration Monitoring system (CERMs) enable prediction of frozen-thawed embryo development to blastocyst stage and safely applicable to clinical IVF?

Summary answer: Oxygen consumption rate correlated to developmental capability of embryos to the blastocyst stage, and CERMs can be safely applied to clinical IVF program.

What is known already: Recently, we developed a non-invasive chip-sensing embryo oxygen consumption measurement device, CERMs, in which circumvent the differences in inter-observer subjective view. CERMs enabled measure the oxygen gradient formed by its respiration as sensitive as the scanning electrochemical microscopy (SECM) technique. Oxygen consumption rates measured by CERMs correlated with in vitro embryo development to blastocyst stage, albeit with the limited numbers of samples.

Study design, size, duration: This is a prospective original research study. The oxygen consumption rate of redundant frozen-thawed human embryos was assessed using CERMs. An impact of its measurement by CERM on *in vivo* development was assessed by the mouse embryo transfer. The endpoints for the study were to assess feasibility of prediction of embryo development to blastocyst stage by CERMs, as well as recovering mouse fetuses.

Participants/materials, setting, methods: The oxygen consumption rates of 30 and 51 human embryos that were frozen-thawed on 2nd and 3rd day after fertilization, were measured by CERMs at 6, 24, 48, 72 and 96 hours after thawing with standard morphological evaluation. Embryos that have reached blastocyst stage and those of arrested were divided into two groups (blastocyst and arrested, respectively) and were analyzed. Mouse blastocyst obtained by IVF was transferred to synchronized surrogate and fetuses were recovered.

Main results and the role of chance: The characteristics of the patients (age distribution, infertility factor and type of assisted conception) were comparable in both groups. Oxygen consumption rates of both D2 and D3 human embryos that have reached the blastocyst stage were significantly higher than those of arrested embryos at 48, 72 and 96 hours after thawing ($p < 0.05$). Thus, irrespective to frozen stage of embryos, *in vitro* development of frozen-thawed human embryos to the blastocyst stage would have higher respiratory activity at 48 h after thawing. However, some portion of 48 h hours after thawing of D3 embryos would have reached to blastocyst stage. Therefore, we focused on D2 frozen-thawed embryos and the probability of reaching blastocyst stage increased significantly with every 1 fmol/s increase of oxygen consumption rate (odds ratio (OR) = 1.978; 95% CI, 1.020–4.577; $p = 0.04$) albeit with the limitation that this theory is applicable at 48 h after thawing. Based on the ROC curve, the cut-off oxygen consumption rate value was 5.0 fmol/s (sensitivity = 0.89; specificity = 0.55; AUC = 0.74). We recovered mouse foetuses that were measured oxygen consumption rates by CERMs. Thus, measurement procedure is not harm for subsequent embryonic development in vivo.

Limitations, reasons for caution: A correlation between oxygen consumption and the *in vivo* viability of embryos remains unknown. Individual respiratory activity of mouse embryos might be too small to evaluate this. Thus, clinical trials would be desirable to address this question. The effect of frozen-thaw manipulations on the respiratory activity is also unknown.

Wider implications of the findings: Selection of quality embryos by CERMs may have an impact on obtaining better clinical outcomes, albeit with clinical trials being required. CERMs may be useful for further optimization of culture condition or enable the omission of long-term *in vitro* embryo culture by early determination of quality embryo.

Trial registration number: not applicable.

P-148 Gonadotropin releasing hormone agonist (GnRH-ag) triggering in in-vitro maturation (IVM) cycles: new insights into physiology

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Study question: What is the outcome of GnRH-ag triggering in IVM cycles?

Summary answer: In IVM cycles, GnRH-ag triggering shows a significantly higher rate of metaphase II eggs on the day of retrieval and a significantly higher fertilization rate.

What is known already: hCG has been the traditional triggering of ovulation mode in both IVF and IVM cycles. Recently, hCG triggering is challenged and is often replaced by GnRH-ag triggering. In IVM even small follicles respond to hCG priming and reach the MII stage at recovery with no need for in vitro maturation. Furthermore, these in vivo matured eggs retrieved in an IVM cycle are developmentally superior compared with those which are matured in vitro. Since there is a lack of information regarding the use of GnRH agonist in these circumstances we report our unit's experience in the IVM practice.

Study design, size, duration: a retrospective cohort analysis of 60 IVM cycles between 2015–2017 comparing cycle outcome by mode of triggering.

Participants/materials, setting, methods: 44 patients going through IVM cycle in a tertiary university affiliated hospital. Indications for treatment were a

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pure ovulatory disorder, a mixed indication including an ovulatory component and oncology patients performing fertility preservation. Our IVM protocol includes gonadotropin (rFSH) exposure for 3 days in cases were endometrial lining is thinner than 6 mm after day 6 and only if the cycle is planned for an embryo transfer.

Main results and the role of chance: A total of 60 IVM cycles were included, 20 of them triggered by GnRH agonist (S.C decapeptyl 0.3 mg) and 40 triggered by recombinant hCG (S.C. 250 micrograms). Means and P values are presented hereby for the GnRH-ag triggering group and HCG group performance, respectively: total number of eggs retrieved 9.7, 10.4 (P value 0.6), MII egg ratio on the day of retrieval 0.25,0.14 (P value 0.02), final MII egg ratio 0.53, 0.59 (P value 0.4) and fertilization rate of injected ova :0.8, 0.6 (P value 0.01). In a sub-analysis comparing only patients who were not exposed to gonadotropins (14 patients in the GnRH-ag group, 9 in the hCG group), both retrieval day MII ratio and fertilization rate showed a significant benefit to the GnRH-ag triggering. Only 5 embryo transfers were performed so far for the GnRH-ag group, reflecting the common cancer indication and not the embryological performance; out of those we report one live-birth, one 1st trimester miscarriage and a pending pregnancy test. Despite the small sample size, the day of retrieval maturation rates and fertilization rates reflect a highly significant advantage for the GnRH-ag triggering.

Limitations, reasons for caution: patient allocation was not random and treatment variations were affected by indication. In spite of the low exposure to rFSH of the GnRH-ag group, they presented a better embryological performance.

Wider implications of the findings: These results reflect a possible advantage for GnRH-ag in IVM cycles, especially those performed for fertility preservation. The fact that immature follicles can perform better after exposure to a GnRH-ag triggering is suggestive of the importance of FSH surge in follicular maturation.

Trial registration number: This is not a clinical trial. REB approval number MMC0031-17.

P-149 Impact of conscious sedation during oocyte retrieval on clinical pregnancy rate in assisted reproductive technology? An equivalence study in France

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Study question: Does using conscious sedation during oocyte retrieval change clinical pregnancy rate per aspiration cycle.

Summary answer: There is no reduction of clinical pregnancy rate per aspiration cycle with conscious sedation during oocyte retrieval.

What is known already: Oocyte retrieval is an important step of assisted reproductive technology (ART). In France, general anesthesia is most commonly used for transvaginal oocyte aspiration. Conscious sedation is the most popular method of analgesia used in ART in many countries. Cochrane systematic review didn't recommend a technique compared to another. Although conscious sedation is associated with better patient satisfaction score. However, few studies have evaluated this technology at centers with french practices.

Study design, size, duration: We performed a retrospective cohort study in our ART center. We compared two periods: period 1 from 01/01/09 to 12/31/11 and period 2 from 01/01/13 to 12/31/16. Primary outcome was clinical pregnancy rate per aspiration cycle. Clinical pregnancy was defined as the presence of a heart beat in US-scan. We performed a non-inferiority study. Equivalence between two rates was established if they differed by less than 25% with reference rate (period 1).

Participants/materials, setting, methods: We included consecutively all patients who underwent transvaginal oocyte aspiration in our ART center. During period 1, general anesthesia protocol associates midazolam 1-2 mg I.V. and alfentanil 1 mg I.V. During period 2, conscious sedation associates premedication (prazepam 10 mg P.O. and ketoprofène 100 mg P.O.) and an association during procedure with midazolam 1 mg I.V., nalbuphine 0,2 mg/kg I.V., ondansetron 4 mg IV and medical nitrous oxide and oxygen mixture. Different steps were carried out according to french or international guidlines.

Main results and the role of chance: We performed 1371 aspirations during period 1 and 1495 aspirations during period 2. After exclusions, we analyzed respectively 1338 and 1452 procedures. There was no difference about cause of infertility. We have gradually changed our practices over the years with an increasing use of antagonistic protocol (6.80% vs 25.12%, $p < 0.001$) but there was no difference in oocytes collected count (9 VS. 10, $p = 0.008$). They were no difference in semen parameters even if we changed technic for semen preparation. Although fertilized oocytes count was greater in period 2 (4.9 vs. 5.1, $p = 0,008$), there was no difference in numbers of oocytes 2PN and embryos obtained. There were more transferred embryos and frozen embryos in period 2 (1.6 vs. 1.7, $p < 0.001$ and 1.1 vs. 1.3, $p = 0,002$). Overall, clinical pregnancy rate per aspiration cycle was 37% in Period 1 and 35% in Period 2.

Limitations, reasons for caution: We chose to study two three-year periods in order to limit selection bias and mandatory variations in ART performance. This leads to variations in practices. However, there is no current scientific evidence of a difference in efficacy for these variant parameters from one period to another.

Wider implications of the findings: Firstly, absence of significant differences between the two groups allows us to validate our protocol. Then we will be able to distribute it to other centers. Finally we will try to improve it.

Trial registration number:

P-150 Different GnRH analogs effects on the level of apoptotic and developmental cumulus cells gene expression in ART cycle

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Study question: Whether different GnRH analogs(agonist or antagonist) prescription can affect cumulus cells gene expression?

Summary answer: Cumulus cells gene expression is significantly different following different type of GnRH analogs administration

What is known already: GnRH (gonadotropin realizing hormone) agonist (GnRH-A) and antagonist (GnRH-ANT) are widely used in ovarian stimulation cycle in order to suppress the endogenous luteinizing hormone (LH) surge. Different characterization and mechanism of action between GnRH analogues in IVF (in vitro maturation) cycle have been well defined.The presence of GnRH receptors on the surface of ovarian epithelial cells and granulosa (GC) and cumulus cells (CC) was detected. Cumulus cells play an important role in follicular development, Based on this background present study proposed to investigate GnRH agonist and antagonist effects on cumulus cell gene expression and IVF outcome.

Study design, size, duration: Sixty 60 infertile patient were enrolled in the study based on our exclusion and inclusion criteria. The patient were divided in two groups randomly, 28 of them allocated to the GnRH agonist group by 0.1 mg/day S.C. triptorelin and 150–225 IU S.C rFSH Gonal-F, administrating based on standardized long protocol. The patient 32 women allocated in GnRH antagonist and treated by (0.25 mg/day) Cetrotide and 150–225 IU S.C rFSH Gonal-F, according to the fixed protocol.

Participants/materials, setting, methods: Defined Clinical and embryological parameters were compared. Percentage of Metaphase II (MII) oocytes,

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fertilization rate, grade A and AB embryo percentage on Day3 post-insemination were assessed as embryological parameter in each groups. Pregnancy rate, and the number of ovarian follicles before OPU were investigated as clinical aspect of our study. In order to molecular assessment BAX, BCL-2, survivn, ALCAM and VCAN were evaluated in Cumulus cells by performed Real time PCR.

Main results and the role of chance: Molecular evaluation: relative gene expression of Bax gene expression was significantly higher in CCs of patient received GnRH agonist (39.1 ± 2) in compare with GnRH antagonist (27.01 ± 4.2) group. ($P < 0.001$). Higher Bcl-2 mRNA expression in CCs of GnRH antagonist group (61.4 ± 2.2) versus GnRH agonist (44.3 ± 4.2) were observed significantly ($P < 0.001$). The ALCAM expression (16.8 ± 0.6 vs 22.2 ± 1.3) was observed significantly different in GnRH agonist and antagonist groups respectively. Significant difference in VCAN (40.5 ± 7.9 vs 41.8 ± 6.7) and survivine (64.8 ± 6 vs 66.9 ± 7.6) between GnRH agonist and antagonist was not determined respectively.

Clinical parameters evaluation: Although the number of dominant ovarian follicle, pregnancy rate are more in GnRH antagonist group but differences was not statistically significant

Embryological evaluation: The mean number of obtained COC percentage of MII oocyte, PN and grad A embryo did not significantly differ between GnRH agonist and antagonist

Limitations, reasons for caution: To accurately the moral issues The protocol of present randomized study was approved by the Ethics Committee of Tehran University of Medical Sciences, informed consents was fulfilled and signed by all of the participants.

Wider implications of the findings: there is an article that indicat GCS apoptosis rate was not correlated to oocyte maturity, quality and pregnancy outcome. But strong correlation of CCS apoptosis and poor oocyte quality was concluded by several studies.

Trial registration number: 29126.

P-151 A single cell analysis of cumulus cells from cumulus-oocyte complexes by flow cytometry

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Study question: Are cumulus cells from cumulus-oocyte complexes (COCs) a heterogeneous population of cells?

Summary answer: Cumulus cell samples prepared from COCs included heterogeneous cell populations. Flow cytometric analyses revealed differential antigen expressions on the surface of cumulus cells.

What is known already: Previous research has tried to predict oocyte quality by analysing the oocyte-surrounding cumulus cells using a non-invasive assay. Transcriptomic analyses of the cumulus cells found candidate genes that could predict oocyte quality. However, these results are still controversial. Previously, single cell analysis of cumulus cells has not been performed. Flow cytometric analysis could provide biological information from cumulus cells and enable the purification of cumulus cells for a precise transcriptomic assay to predict oocyte quality.

Study design, size, duration: From June to December 2016, cumulus cells were collected from intracytoplasmic sperm injection-treated patients who provided informed consent. The age of patients ranged from 30 to 46 years.

Participants/materials, setting, methods: Flow cytometric analysis was performed on cumulus cells from 22 patients. In cases where patients gave more than 2 COCs, the cumulus cells derived from the COCs were pooled. After hyaluronidase treatment of the COC, cumulus cell samples were collected for staining with antibodies and analyzed by flow cytometry (Gallios, Becton-Dickinson, USA).

Main results and the role of chance: The cell suspension collected from the COCs included 63.1% ± 15.95% dying/dead cells stained with propidium iodide. In the propidium iodide-negative living cells, CD45-positive blood cells and CD235a-positive erythrocytes were detected, suggesting that blood cells contaminated the suspension during oocyte retrieval. After screening out blood cells, non-hematopoietic living cells were detected to be two or three times larger than hematopoietic cells. These non-hematopoietic cells expressed CD49a (95.9% ± 5.43%), activated leukocyte cell adhesion molecule (ALCAM, 75.5% ± 17.96% of non-blood living cells), and CD56 (40.8% ± 17.75%) on the cell surface. The non-hematopoietic cells also expressed follicular stimulating hormone receptor (FSHR) which has been reported to be expressed in cumulus cells, although hematopoietic cells had no expression of FSHR. These data indicate that CD49a and ALCAM are good markers of cumulus cells and that cell purification by cell sorting to eliminate dead cells and blood cells would be helpful for performing precise gene expression analyses of cumulus cells, in order to search for oocyte quality-relevant genes.

Limitations, reasons for caution: The current work is a pilot study of a small number of samples and a larger analysis of cumulus cells from individual COCs should be done in the future.

Wider implications of the findings: Flow cytometry would be useful, not only for precise transcriptomic analyses, but also for a quick investigation of cumulus cell status, e.g. percentage of apoptotic cells or status of mitochondrial activation could predict oocyte quality.

Trial registration number: Not applicable.

P-152 Treatment with GM-CSF improves pregnancy rates in poor responders

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Study question: Is the inclusion of GM-CSF in embryo transfer media associated with increased clinical outcomes rates in patients with poor responders IVF attempts?

Summary answer: Better results in terms of clinical pregnancy and ongoing implantation rate were observed after treatment of GM-CSF in embryo transfer media.

What is known already: Cytokines drive the dialogue between the embryo and endometrium and are increasingly expressed throughout embryo development. GM-CSF is known for its importance in the development of blastocysts and in normal fetal and placental development. It is reported that inclusion of GM-CSF in embryo culture media significantly increases ongoing implantation rates in women who have previously experienced miscarriage. However, it is still unclear whether poor responders IVF attempts should be another indication for the use of GM-CSF.

Study design, size, duration: This prospective study was performed between January 2016 and September 2016 at a reproductive center. All patients involved gave written consent, and institutional review board approval was granted. This study includes 55 non-treatment and 60 treatment couples.

Participants/materials, setting, methods: A prospective study was conducted including patients with a poor responders following conventional ICSI in our center. In the treatment cycles, embryos were exposed to a commercially available ready-to-use 2 ng/ml GM-CSF for 10 min immediately before ET. ET was performed either on Day 2 or Day 3. Clinical pregnancy and ongoing implantation rates were compared, p-value of < 0.05 was considered statistically significant.

Main results and the role of chance: The average female age, number of oocytes retrieved, embryo developmental stage and number of embryos transferred did not vary significantly among the groups. Fertilization and cleavage rates did not differ. However, Clinical pregnancy rates (22/60 (38.3%) vs 17/55 (30.9%), and ongoing implantation rates (18.9 ± 5.3 vs 14.2 ± 4.8) were higher in GM-CSF treatment group compared to control, but these differences did not reach statistical significance.

Limitations, reasons for caution: This is an observational study with limited number of patients, offering preliminary evidence for the efficiency of GM-CSF in embryo transfer media in patients with poor responder IVF attempts. This study is still not powered to exclude possible associations between take home baby rate and perinatal outcomes on babies born.

Wider implications of the findings: The findings of this study indicate that GM-CSF treatment improves the rates of pregnancy and ongoing implantation rates in poor responders. However, this treatment does not seem to completely resolve the poor responders. Further investigations are necessary to determine the effects of GM-CSF treatment of the culture conditions.

Trial registration number: NA.

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P-153 Defining a novel method to describe giant oocytes by cytoplasmic diameter -Comparison of the mitotic spindle diameter by fluorescence immunostaining-

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Study question: Is an oocyte cytoplasmic diameter ≥130μm useful as a means of defining Giant Oocytes (GO)?

Summary answer: Using a cytoplasmic diameter ≥130μm is a useful criterion for defining a GO.

What is known already: The diameter of a human oocyte is approximately 110μm, but larger diameter oocytes have been documented in ART treatment cycles and referred to as Giant Oocytes (GO). GO appear to be a product of the cytoplasmic fusion of two primary oocytes, and there are examples of both ova in which some mitotic spindles which are fused or not fused. Accordingly they have a higher risk of aneuploidy than normal oocytes. However, the criterion for identification of GO are not clear, and the classification is often dependent on the experience of the embryologist.

Study design, size, duration: We examined 37,315 oocytes collected from 4,160 ART cycles which underwent controlled ovarian stimulation between January 2014 and March 2015. The cytoplasmic diameter of oocytes that were judged to be larger than normal was measured. 56 MII phase oocytes analyzed had a diameter of ≥130μm and were classified as GO. MII oocytes from conventional IVF cycles in which fertilization did not occur were used as a control group.

Participants/materials, setting, methods: Oocytes defined as GO had further measurements taken (overall diameter- cytoplasmic diameter and including the zona pellucida). Furthermore, these oocytes were fixed and immunofluorescence staining was carried out. They were stained with an α-Tubulin antibody and DAPI. Spindles were classified into three groups GO1 (1 spindle present), GO2 (2 spindles present) and control oocytes. The size of the spindle was measured by the distance between the two spindle poles, and chromosomes align distance equatorial plane.

Main results and the role of chance: GO had a cytoplasmic diameter of 144.3 ± 10.7μm and a total diameter including the zona pellucida of 205.5 ± 22.2μm. After staining of the 56 GO, it was possible to analyze 47. Of these 25 were classified as GO1 and 22 oocytes were GO2. The average equatorial plane diameter of the spindle was 18.0 ± 3.3μm in GO1, 11.7 ± 2.2μm in GO2 and 12.3 ± 1.3μm in control oocytes. The average diameter between the two spindle poles was 19.1 ± 2.2μm in GO1, 14.5 ± 3.2μm in GO2 and 13.2 ± 2.0μm in control oocytes. GO1 were significantly different in these parameters compared to the other groups ($p < 0.05$).

Limitations, reasons for caution: In this study, we compared only the size of the mitotic spindle. Future studies should consider tests, such as Next Generation Sequencing to identify chromosome content.

Wider implications of the findings: Based on the results there were two types of GO, one of which had one large spindle and the other had two normal size spindles. From these findings, it is anticipated that the chromosomal content of GO would be up to 2 times higher than normal ova.

Trial registration number: None.

P-154 Automatic vitrification and warming of ovarian and testicular tissues

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Study question: Previously we reported automated vitrification of oocytes/embryos. Here we address whether automated vitrification and warming can be used for ovarian cortical strips and testicular extracts.

Summary answer: This study is the first report describing a device allowing successful automation of both vitrification and warming procedures for gonadal tissue.

What is known already: Vitrification is the method of choice for preserving oocytes and embryos and is slowly gaining acceptance also for gonadal tissue. However, the procedure is cumbersome, requires highly skilled personal and is not standardized thus producing variable results. Having an automated device allowing the precise exposure of the tissue to cooling and warming solutions is desirable, particularly for preserving fertility for cancer patients. We have developed a fully automated device (Sarah, Fertilesafe Ltd., Israel) for both vitrification and warming for gonadal tissue slices in addition to oocytes and embryos.

Study design, size, duration: Ovarian strips cut into 400 μm slices were prepared from 6 weeks old BL C57 female mice ovaries (n = 4). Testicular tissue extracts were prepared from testis of slaughtered lambs (n = 2), using an 18 g needle. Slices were either grown in culture or underwent vitrification and warming with a new automated robotic device (Sarah). The gonadal tissue was inserted into 0.3 ml straws (CBS, IMV, France) comprising a special capsule with 50 μm pores and loaded in the robotic device.

Participants/materials, setting, methods: Straws loaded in the Sarah device were exposed to increasing concentration of equilibrium and vitrification solutions followed by plunging into LN slush at the last stop. Automated warming started by exposing the straws for 1 minute to reducing concentrations of warming solutions at 37°C until completion into holding medium. Ovarian slices were in-vitro cultured for 14 days and evaluated by L/D fluorescent staining, fixation and H&E histology. Testicular tissue was evaluated by Hoechst vital staining.

Main results and the role of chance: Cooling and warming rates measured using 50 μm T-type thermocouple were over 4500°C/min using LN and over 10,000°C/min when straws were plunged into LN slush (-208°C). After warming, spermatogonial cells recovered from the seminal tubules showed high viability rates (23/25 (92%), 44/50 (88%), 23/30 (83.33%) compared to fresh controls (100% viable). After warming, the vitrified ovarian cortical strips showed over 90% survival of antral follicles (79/89) after 2 weeks of in vitro culture. Slides of ovarian strips after 14 weeks in culture stained with H&E showed the following number of antral follicles per 1 mm^2 – Fresh controls (n = 5): 2.6 ± 1.5; 1.6 ± 0.5; 1.3 ± 0.5; 2.6 ± 0.5; 1.6 ± 0.5. Vitrified/warmed (n = 5): 2 ± 1; 3 ± 1; 3 ± 1; 3.6 ± 0.5; 3 ± 1. These results show that in the vitrified/warmed slides there were slightly more antral follicles compared to the slides of the fresh controls. Since these results were not statistically different that the vitrification/warming process did not damage the ovarian slices.

Limitations, reasons for caution: These preliminary successful results with a newly automated device for both vitrification and warming were obtained on animal gonadal tissue and should be corroborated in human tissues.

Wider implications of the findings: Here we report for the first time a method that can successfully vitrify and warm ovarian and testicular slices by a fully automated, operator-independent process. The standardization and simplification of the vitrification/warming procedures of gonadal tissue can be of immense benefit for cancer patients worldwide.

Trial registration number: N/A.

P-155 Improvement of final oocyte maturation with combination of a gonadotrophin releasing hormone (GnRH) agonist with a human chorionic gonadotrophin (hCG) trigger in in vitro fertilization

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Study question: Do mature oocytes have a better rate of associating a double ovulation triggered in patients with a history of low proportion of mature oocytes?

Summary answer: The double trigger in comparison to the release by hCG only obtains 1,46 times more mature oocytes IC 95% (1,18-1,80) p < 0,001

What is known already: The fertilizable oocytes in in vitro fertilization (IVF) or in intracytoplasmic sperm injection (ICSI) have to be at the stage of metaphase II. In case of repeated immature oocyte retrieved, it was assumed that by prolonging the time between ovulation triggering and oocyte pick up (OPU) and the GnRH agonist trigger, with the consequent simultaneous induction of an FSH surge, the "double trigger" could overcome oocyte meiotic maturation resulting in successful aspiration of mature oocytes, pregnancy, and delivery (*Beck Fluchter* 2012).

Study design, size, duration: It is a retrospective monocentric study between May 2014 and September 2016, which compares the same patient, an attempt with a classic trigger (hCG alone) with a double trigger in the following attempt. In total, 56 patients were included consecutively. That's why the patients in pre-implantation genetic diagnosis (PGD) were included, although the rates of pregnancies are not taken account due to the risk of lacking in healthy embryo transfer.

Participants/materials, setting, methods: Patients had at least one attempt of IVF-ICSI under antagonists or agonist with a rate of immature oocytes ≥ to 40 % and had a double trigger for the following attempt. The ovarian stimulation was realized under antagonist and the double trigger associated hCG (hCG 5000 or 10000 UI or hCG recombinante *Ovitrelle* 250 UI) and agonist of the GnRH (*Decapeptyl* 0,2 mL). The main criteria was the percentage of mature oocytes retrieved.

Main results and the role of chance: The number of mature oocytes obtained by double trigger was 7,4 on average versus 5,1 in the classic group trigger with a relative risk (RR) of 1,46 IC 95 % (1,18-1,80) p < 0,001. There was no difference between both groups concerning the duration of the stimulation, the total dose of gonadotrophin received and the hormonal dosages before the triggering (LH, estradiol progesterone). The number of zygotes obtained in the first day (2PN) was 5,2 in the double trigger group versus 3,4 in the classic release group RR [1,54 (1,18-2,01] p0,002. Also for the number of embryos obtained in the second day: 5,5 versus 3,4 RR [1,66 (1,29-2,13)] p < 0,001 and the number of frozen embryos: 1,6 versus 0,6 RR [4,89 (2,32-10,30)] p < 0.001, there was not a significant improvement of pregnancy rates OR [11,1 (2,45–50,44)] p0.003.

Limitations, reasons for caution: It is a retrospective study which compares the results of the same patient. There is an inherent bias. The rates of the pregnancy rate are biased because of patients' inclusion in PGD. All the frozen embryos were not transferred, which does not report on cumulative pregnancy rates.

Wider implications of the findings: The double trigger allows for obtaining a larger number of mature oocytes and embryos to be transferred. However, our study could not show if there was also an improvement of the cumulative rates of pregnancies. Due to the encouraging results, a randomized prospective study must be established.

Trial registration number: Not applicable.

P-156 The prediction of sperm fertility potential from infertile men in real-time during intracytoplasmic sperm injection

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Study question: Which male features have a predictive value for fertility potential and the occurrence of a pregnancy in infertile men undergoing intracytoplasmic sperm injection (ICSI)?

Summary answer: Follicle stimulating hormone (FSH), age, male infertility factor (e.g. oligozoospermia,asthenozoospermia,teratozoospermia,normozoospermia, and oligoasthenoteratozoospermia), testosterone and luteinizing hormone (LH), head size, and vacuole were relevant predicting parameters.

What is known already: The most prediction models of reproductive medicine are focused on female parameters and treatment success, and there are a few studies about the effect of male factors, and sperm parameters on treatment success from infertile couples with male factor infertility. Currently, there is also no prediction model for sperm fertility potential and the occurrence of pregnancy in real-time during ICSI.

Study design, size, duration: During 2012 to 2014, 209 patients with male factor infertility at the infertility therapy center of Alzahra (Iran, Rasht) were included in this study. To assay of male features, female factor infertility was excluded in this study.

Participants/materials, setting, methods: Selected sperms for intracytoplasmic injection were analyzed using sperm morphology analysis (SMA) algorithm in real-time during ICSI. Therefore, the characteristics of sperm parts (e.g. head and acrosome size, vacuole, neck, cytoplasmic droplet, and tail shape) were determined. According to selected sperm dataset and patient information, the fertility potential of each sperm and pregnancy chance of embryo resulted of that sperm were predicted. Measuring was done by CorrelationAttributeEval and Ranker of WEKA software.

Main results and the role of chance: The effect of male features such as FSH (0.074), age (0.07), male infertility factor (0.04), testosterone (0.04), LH (0.038), and head size (0.034) were weighted more than the other parameters for formation of a zygote, respectively. Also, the occurrence of a pregnancy from embryos resulted of injected sperm was associated with male BMI (0.17), testosterone (0.1), age (0.04), FSH (0.04), the head size (0.02), and vacuole (0.013) of spermatozoa for ICSI.

Limitations, reasons for caution: This study is focused on men's demographic and motile sperm morphologies in real-time during ICSI. The genetic information has not been considered because the selection of sperm during ICSI is performed based on morphology parameters.

Wider implications of the findings: This model enables researchers to evaluate the impact of morphological sperm features and men's demographic on fertility potential in infertile men in real-time during ICSI. Therefore, it can help to embryologist for selection of the best sperm in real-time during ICSI and could promote the outcomes of assisted reproductive techniques.

Trial registration number: Not applicable.

P-157 Distinctive characteristics of extracellular vesicles derived from spent medium from day 3 and day 5 embryos

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Study question: Are human embryo extracellular vesicles (EVs) carriers of information mediating maternal-embryonic communication?

Summary answer: Molecular characterization of human embryo-derived EVs reveals a number of biomolecules (proteins, mRNA) that may suggest an emerging way of embryo-maternal cross-talk.

What is known already: Successful embryo implantation and consequent pregnancy is critically dependent on a two-way communication between the maternal uterus and the blastocyst. However, given the ethical restrictions and the lack of mechanistic studies, the identification of key embryonic signals remains so far elusive. There are plenty of evidence indicating that EVs shuttle biomolecules that can profoundly affect the phenotype and activity of their target cells. EV secretion has been reported for most cell types including embryonic stem cells and *in vitro* produced embryos derived from some mammalian species. However, to date no comprehensive data have been reported regarding human embryo-derived EVs.

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Study design, size, duration: Embryo spent culture medium samples employed in this study were obtained from patients referring to the Centro Scienze Natalità of the San Raffaele Hospital in Milano, Italy, with an indication to ART. Spent culture medium was collected from embryos at the cleavage stage or from expanded blastocysts without signs of degenerating cells.

Participants/materials, setting, methods: Conditioned medium was collected from embryos cultured to day 3 and day 5 after fertilization, pre-cleared of debris and subjected to a series of ultracentrifugations in order to isolate EVs. The characterization of EVs was performed by Nanoparticle Tracking Analysis and transmission electron microscopy. The presence of specific EVs proteins and RNAs was investigated by western blot and RT-PCR. The uptake of fluorescent labeled EVs by primary endometrial cells was monitored by using Vybrant™ DiO.

Main results and the role of chance: Conditioned media from non-manipulated human embryos cultured *in vitro* for 3 or 5 days contain EVs with a diameter ranging from 50 to 200 nm with most of them smaller than 100 nm. Western blotting demonstrated the presence of CD9 and CD63 markers in embryo-derived EVs. The exosome marker protein ALIX was detected in both Day 3- and Day 5-EVs confirming the presence of bona fide exosomes in the EV preparations from embryo secretome. *POU5F1* and *NANOG* mRNAs were detected in both Day 3-EVs and Day 5-EVs, indicating the presence of both transcripts in EVs from embryos at different developmental stages. The embryonic origin of these EVs was confirmed by the presence and their enrichment in the non-classical HLA-G protein at appropriate stages of development. In order to examine whether EVs derived from embryos could be uptaken by primary endometrial cells and thus potentially establish a communication with the maternal side, EVs labeled with a fluorescent dye were co-cultured with primary endometrial cells. Uptake of dye-labeled embryo-derived EVs by primary endometrial cells was also demonstrated with a fluorescence intensity signal greater for cells treated with vesicles derived from blastocysts.

Limitations, reasons for caution: Some data were obtained from the analysis of pooled human embryo culture media instead from medium from a single embryo culture. Lower amount of starting material can impact the quality of the result and therefore we are confident that using pooled medium may, at this stage, be more informative.

Wider implications of the findings: Our finding showing that *in vitro* cultured human embryos can secrete EVs that can be uptaken by the maternal side raises some exciting possibilities regarding their potential therapeutic use as a co-factor for promoting the establishment of a successful pregnancy.

Trial registration number: -.

P-158 A prospective randomised trial comparing embryo development in the MINC incubator versus the EmbryoScope incubator

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Study question: Does culturing embryos in the EmbryoScope versus the MINC benchtop incubator increase the number and quality of blastocysts formed on Day 5?

Summary answer: Culturing embryos in the EmbryoScope rather than the MINC incubator significantly increased the number and quality of blastocysts formed on Day 5.

What is known already: It is unknown whether culturing embryos in the EmbryoScope or the MINC incubator provides a better environment for the developing embryo and hence an improved outcome for patients. There is a popular view that the EmbryoScope is superior with some clinics reporting 60–70% success rates using this device compared to 30–40% using the MINC incubator. These increased success rates are thought to be due to embryos remaining in a constant environment and more information being available for embryo selection.

Study design, size, duration: Between January 2015 to November 2016, 81 couples undergoing IVF or IVF with ICSI were recruited and consented to participate in the study prior to oocyte recovery. Of these 81 couples, 51 continued to meet criteria and their 585 embryos were randomly allocated to the EmbryoScope or the MINC, with 289 cultured in the EmbryoScope and 296 in the MINCs.

Participants/materials, setting, methods: Participants were aged 42 years or under, had an antral follicle count of ≥12 and ≥6 2PN zygotes. Zygotes were cultured individually in 25 µl of G1+ (Vitrolife) followed by 25 µl of G2+ (Vitrolife) in either slides (EmbryoScope) or culture dishes (MINC). Embryos were graded by an embryologist on days 3 and 5. Differences in outcomes were analysed using Chi squared test.

Main results and the role of chance: The proportion of embryos cultured in the EmbryoScope that developed into blastocysts on day 5 was significantly higher than those allocated to the MINC incubator (159/289 (55%) EmbryoScope versus 133/296 (45%) MINC; P = 0.015). The same applied to the total number of blastocysts (192/289 (66%) EmbryoScope versus 170/296 (57%) MINC; P = 0.025). Furthermore, significantly more embryos formed blastocysts suitable for cryopreservation on D5 following culture in the Embryoscope (90/289 (31%)) versus MINC (69/296 (23%), p = 0.033), however, this difference did not remain significant when blastocysts grades were further subdivided. Culturing embryos within EmbryoScope did not significantly increase the blastocyst utilisation rate (117/192 (61%) EmbryoScope versus 100/170 (59%) MINC, P = 0.231), the number of blastocysts frozen (99/192 (51%) EmbryoScope versus 80/170 (47%) MINC; P = 0.392) or transferred (18/192 (9%) EmbryoScope versus 13/170 (8%) MINC, P = 0.558). Furthermore there was no significant difference in pregnancy rate per embryo transfer (71% EmbryoScope versus 73% MINC, P = 1.00) or clinical pregnancy rate (59% EmbryoScope versus 53% MINC, P = 1.00).

Limitations, reasons for caution: Embryo culture in the EmbryoScope was disturbed when slides were removed to change the medium and whilst medium was refreshed in the EmbryoScope, embryos in the MINC were transferred to new dishes. The study was also not powered to determine a difference in clinical outcome parameters.

Wider implications of the findings: Although incubation of embryos in the EmbryoScope increased the number and quality of blastocysts formed, this was not reflected in an impact on pregnancy rates. While this may be due to insufficient statistical power, both incubators provided good outcomes.

Trial registration number: ISRCTN73037149.

P-159 Blastocoel expansion as a morphokinetic marker of genetic quality in embryos from donor eggs

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Study question: How does blastocyst formation time and the rate of subsequent blastocoel expansion correlate with euploidy in donor embryos having genetic screening

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Summary answer: Euploid embryos tend to form blastocysts sooner and expand more rapid than aneuploids, enabling a morphokinetic analysis identifying subgroups enriched for euploidy

What is known already: Egg donation is an important option for infertile couples. Embryo ranking for single embryo transfer remains a central challenge to minimize risks from multiple pregnancy. Without preimplantation genetic screening (PGS), the laboratory must rank embryos based on morphology. Time-lapse imaging offers one new approach, but its value in embryo selection remains controversial. Blastocoel expansion rate has recently been described in donor egg embryos forming sustained pregnancies (Huang et al., 2016), but there is no information correlating expansion rates with karyotype in wider embryo cohorts. Results here support the hypothesis that expansion rate assessments can improve euploid embryo selection.

Study design, size, duration: This was a retrospective observational study utilizing 91 blastocysts from 15 consecutive egg donation cycles performed in 2016.

Participants/materials, setting, methods: The median age of donors was 24.3 yrs. Retrievals averaged 16.3 mature and 14.2 fertilized eggs. After ICSI, embryos were cultured in an Embryoscope (Vitrolife, USA) with laser zona drilling on D3 and biopsy on D5-D6. Blastocoel expansion was measured as the cross-sectional area (CSA, in u^2) of trophectoderm-enclosed space both within and herniated outside of the ZP. It was measured hourly for 10 hours beginning at blastocyst formation (tB).

Main results and the role of chance: Of 91 embryos biopsied, there were 51 (56.0%) euploid, 30 (33.0%) aneuploid, and 10 (11.0%) mosaic calls. Euploid blastocysts exhibited a statistically greater expansion curve regression slope over the first 10 hours than either aneuploids or mosaics (560 vs. 347 vs. 420, respectively; $p < 0.04$). Euploids also formed blastocysts (tB) earlier (102.4 vs. 111.2 vs. 117.9 hrs, respectively; $p < 0.001$). There were no statistical differences between mosaics and aneuploids in tSB or tB ($p > 0.05$). Based on expansion curve results, a scatter analysis was performed correlating Tb with expansion CSA at 8 hours from Tb, where 86.7% of all euploids formed blastocysts < 112 hours from fertilization. Euploidy was increasingly enriched in subgroups showing increasingly expanded blastocoels. This ranged from 63.6% (at cutoff CSA > 14,000u^2) to 79.3% (CSA > 16000u^2) to 85.8% euploid (CSA > 18000u^2). Although few total euploid embryos (13.3%) formed blastocysts at > 112 hours, they showed remarkably productive expansion with CSA's > 16,000 u^2 8 hours into the expansion period; in contrast, aneuploid/mosaics embryos expanded comparatively more slowly and were distributed more broadly across tB times and CSA range (11,000–23,000u^2).

Limitations, reasons for caution: This was retrospective observational study and further limited by the sample size of both patients and blastocysts. In addition, this is the first application of a new metric of blastocyst expansion to biopsied embryos which limits comparison of results and power analysis.

Wider implications of the findings: The value added by morphokinetic analysis for ranking of embryos for transfer remains challenging and controversial. The results reported here in egg donor PGS cases suggest that both the time of blastocyst formation and a new metric describing blastocoel expansion may be useful for objective, non-invasive embryo selection algorithms.

Trial registration number: No Registered Trial Performed.

P-160 Effects of increasing paternal age on clinical outcomes in vitrified egg donation cycles

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Study question: Does increasing paternal age negatively influence embryo development and assisted reproduction technique (ART) clinical outcomes in egg donation cycles with vitrified oocytes?

Summary answer: In vitrified egg donation cycles there is a significant reduction in clinical pregnancy and implantation rates with increasing paternal age.

What is known already: Literature reports that increasing paternal age is correlated with decrease in ejaculate volume, sperm morphology and motility. Furthermore, increasing paternal age is associated with epigenetics aberrations and DNA mutations/damage along with chromosomal aneuploidies in spermatozoa. Advanced paternal age could also influence embryo ploidy and incidence of different types of disorders like autism, schizophrenia and bipolar disorders. All this factors negatively affect ART success rates. On the other hand it would seem that embryo quality is not related with embryo ploidy. In egg donation cycles, the effect of oocytes ploidy on embryo chromosomal assessment is reduced due to lower maternal age.

Study design, size, duration: From October 2014 to December 2016, 213 egg donation cycles with vitrified oocytes (average 6.62 ± 1.33 per cycle) were retrospectively analyzed. Couples were divided in two groups according to paternal age: group A (100 cycles) and group B (113 cycles). No statistical difference was found in donors' age. Inclusion criteria were: a) more than 4 oocytes surviving thawing and b) use of ejaculate sperm. Data were analyzed with Fisher's exact test.

Participants/materials, setting, methods: Paternal age was 30–44 (40.09 ± 3.14) and 45–72 (49.42 ± 4.21) years old in group A and B respectively ($p < 0,05$). Semen parameters were equivalent according to WHO 2010 in both groups; donors' age was 27.31 ± 3.57 and 26.3 ± 3.80 years old in group A and B respectively (NS). Oocytes were delivered from Spain by dry-shipper and injected after 2-hour post-thawing incubation. All oocytes were cultured in 6.0%CO_2 and 5.0%O_2 until cleavage stage and transferred. Supernumerary embryos were frozen.

Main results and the role of chance: Thawing survival rates were 92.5% (N = 612/661) and 93.7% (N = 704/751) in groups A and B respectively (NS). In group A and B fertilization rate was 75.2% (N = 460/612) and 78.4% (N = 522/704), respectively (NS). Top quality embryos on day 2 were 50.6% (N = 402) and 52.1% (N = 448) in group A and B, respectively (NS) and top quality embryos on day 3 were 54.2% (N = 364) and 52.1% (N = 385), respectively (NS). On day 3, 97 and 108 fresh embryo-transfers were performed in group A and B respectively. In group A 1,81 ± 0.44 embryos/cycle and in group B 1.79 ± 0.52 embryos/cycle were transferred. Clinical pregnancy rates were 49.4% (N = 48/97) and 36.1% (N = 39/108) in group A and B, respectively ($p < 0.05$). Implantation rates were 35.5% (N = 64/180) and 22.6% (N = 45/199) in group A and B, respectively ($p < 0.05$). Use of donor oocytes minimize maternal aneuploidy contribution and this data show that advancing paternal age could influence sperm quality and ploidy rate of obtained embryos and therefore their competence according to clinical pregnancy and implantation rates and the whole performance of ART.

Limitations, reasons for caution: Due to cleavage stage embryo-transfer program, blastocysts development wasn't analyzed and the relationship between increasing paternal age and blastocyst formation rate and morphology wasn't investigated. With advancing paternal age also maternal age of the recipient increases therefore a maternal effect could be implicated in the clinical outcomes.

Wider implications of the findings: Egg-donation success rate could decrease with advancing paternal age. Final aim will be defining a universal cut-off for advanced paternal age and investigating correlations between oocyte vitrification, increasing paternal age and their effect on ploidy. Future plans are to analyze semen samples fragmentation and sperm maturity for sperm nuclear assessment.

Trial registration number: Not applicable.

P-161 can transfer of a poor quality embryo along with a top quality embryo influence outcome during fresh and frozen IVF cycles

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Study question: To evaluate the effect of double embryo transfer (DET) with a poor quality embryo (PQE) along with a top quality embryo (TQE) on IVF outcome.

Summary answer: DET with PQE plus TQE does not increase the live birth rate but increases multiple births when compared to single embryo transfer (SET) with TQE.

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What is known already: While SET is the recommended approach to achieve single live births during IVF, DET is considered, especially when there are only PQEs available in addition to one or no TQE on the day of transfer to maximize the treatment success. It is not clear whether transferring a PQE along with a TQE can influence the outcome during fIVF and FET cycles.

Study design, size, duration: A review of prospectively collected data of all the blastocyst transfers as part of fIVF (n = 939) and FET (n = 1009) cycles performed between 2010 and 2016 was undertaken. A total of 1948 cycles were analysed.

Participants/materials, setting, methods: Only one cycle per participant was included. SET with TQE (group1) was set as control and outcome for SET with PQE (group2) and DET with 2 TQEs (group3), PQE plus TQE (group4) and 2 PQEs (group5) were compared. Odds ratios were calculated after controlling for age using logistic regression analysis.

Main results and the role of chance: The live birth rates for group4 (DET with PQE plus TQE) were statistically similar to group1 (SET with TQE) during fIVF (26.5% Vs 33.7%; OR: 0.95(0.53–1.7)) and FET (24.2% vs 32.7%; OR: 0.75(0.48–1.2)), although there was a trend for lower success. Conversely, multiple births were higher in group4 for fIVF (19% vs 4.7%; OR: 2.9(1.3–6.6)) and FET (10.3% vs 2.6%; OR: 2.4(1.2–4.9)). The live birth rates for group2 (12.2% for fIVF and 14.6% for FET) and group5 (21.2% for fIVF and 14% for FET) were lower and for group3 were higher (40.8% for fIVF and 40.3% for FET) when compared to group1. Multiple births were significantly higher with DET regardless of the embryo quality compared to SET with TQE.

Limitations, reasons for caution: The study was limited by the observational design and a randomized controlled trial is warranted to confirm the findings. However, we made effort to limit the bias by including consecutive cycles, only one cycle per participant and by performing the statistical analysis by controlling for confounders like age.

Wider implications of the findings: The data from the study do not support the idea of DET with one PQE along with a TQE, when there is a dilemma where there is only one TQE and one or more PQE remaining available for transfer during fIVF and FET cycles.

Trial registration number: 0.

P-162 Oocyte meiotic spindle structure gradation and its effect to the quality characteristic of the embryos

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Study question: Does the oocyte meiotic spindle structure possibly serve as prognostic marker in blastocyst formation?

Summary answer: Various types of spindle organizations predict the morphological quality of the blastocysts.

What is known already: Well known that oocyte meiotic spindle visualisation is very useful for a safer performance of intracytoplasmic sperm injection (ICSI). Due to polarized light microscopy it was found that correctly shaped oocyte's spindle is related to better outcomes of assisted reproductive technologies (ART) (Matsunaga R., 2015; Korkmaz C., 2011). But there is no clear assessment criterion for evaluating the oocyte's spindle structure with respect to the development of blastocyst.

Study design, size, duration: This study involved 389 oocytes obtained from 40 women who were treated with IVF/ICSI cycles at our medical center from October to December 2016. The mean age (± SD) of the included women was 33,7 ± 7,6. Due to spindle structure, we evaluated the number of zygotes, embryos on cleavage stage and AA category blastocyst.

Participants/materials, setting, methods: Oosight imaging systems™ (USA) was used for obtaining polarized light. The structure was evaluate and classified as the following spindle's grade characteristics: A - compact rhomboid spindle with defined edges, B - modifiedspindle form with blurred edges, C - weak spindle visualization, D - spindle on the 1stpolar body border and cytoplasm (telophase I), E - spindle not visualized. Statistical analysis was carried out using Shapiro-Wilk test for normality, Chi-square test, Criterion φ-angular Fisher transformation.

Main results and the role of chance: In accordance with structure spindle criteria evaluation suggested by us, the number of oocytes distributed in the following way: A graded spindle had 156 oocytes, B graded – 96, C graded – 92, D graded – 12, E graded – 33 oocytes. Thus oocyte distribution into grades was uneven and complies as 0,4:0,25:0,23:0,03:0,09 (p = 0,001). The mean age (± SD) of the included women had no statistically significant difference (SSD) between the groups with different oocyte grades and was 30,70 ± 3,8, 30,05 ± 4,3, 31,27 ± 3,6, 32,0 ± 3,1, 31,9 ± 4,7 years respectively. The number of fertilized oocytes in grades A, B, C were comparable (87,5%, 87,5%, 89,1%) and had SSD relative to the indicators of the oocyte spindle grades D and E (50,0%, 9,7%) (p = 0,004). Same regularity was observed for the three-day developing embryos - A:B:C (82,1%:87,5%:85,9%) versus D:E (33,3%:63,6%), (p = 0,001). Estimation of the blastocysts number by morphology on the fifth day after fertilization showed that the proportion of AA category blastocysts obtained from A graded oocytes consisted 51,0% from the tota AAl blastocysts amount (n = 110) and significantly higher than the blastocysts number of AA category that was forming from B (25,5%) and C (21,7%) graded oocytes (p = 0,01).

Limitations, reasons for caution: It was found that the dividing spindle is sensitive to the changes in environmental conditions, particularly to temperature. The spindle depolymerization begins due to decreasing temperature of below 37°C, which impairs the visualization quality. So the duration of the spindle structure evaluation should be very short.

Wider implications of the findings: The visualization of the oocyte meiotic spindle and prediction of the blastocysts obtained according to spindle structure grade could help to understand the effectiveness of the ART using cytoplasmic donation techniques in the group of patient carriers of the mitochondrial diseases above 40 years of age.

Trial registration number: None.

P-163 The effects after intracytoplasmic morphologically selected sperm injection (IMSI) on euploidy rate of embryo

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Study question: Can intracytoplasmic morphologically selected sperm injection (IMSI) technique improve the euploidy rate of embryo in preimplantation embryos?

Summary answer: We observed that sibling embryos performed IMSI showed significant higher euploidy and good quality rate compared to control embryos.

What is known already: It is well known that the sperm vacuoles were marked as zones without chromatin in the sperm nucleus which may prove chromosomal or DNA defects. Also, such morphological defects of sperm nuclei could correlate with higher aneuploidy rate in sperm.

IMSI allows the selection of motile spermatozoa without DNA fragmentation and head vacuoles at high magnification. Although some studies have been reported the advantages of IMSI, the correlation of IMSI and euploidy rate of embryo remains unclear.

Study design, size, duration: This study performed in 208 sibling oocytes of 20 ICSI cycles from March 2016 to April 2016 in the Fertility center of CHA Gangnam Medical Center. The oocytes were randomly assigned to IMSI and conventional ICSI.

Participants/materials, setting, methods: IMSI was carried out at high magnification (6000X); whereas, conventional ICSI was performed at 400X magnification. Only good quality spermatozoa (normal head shape and one or two small vacuole) were selected for injection. Embryo development was checked on day 3 and embryo biopsy for PGS were performed on day 3 - day 5. And then, biopsied embryos were cultured individually in continuous single culture medium (CSC medium, Irvine scientific, CA).

Main results and the role of chance: No difference was observed in the rates of fertilization (77.08% vs. 81.25%) and biopsied embryo (71.6% vs.

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65.9%). But IMSI showed a higher incidence of the rates of embryo quality (48.7% vs. 40.7%) and euploidy (30.2% vs. 15.0%) as compared with conventional ICSI.

Limitations, reasons for caution: Further studies with larger sample are needed. In addition, this study was restricted to sibling embryos, so further studies with implantation rate and pregnancy rate are needed.

Wider implications of the findings: Our results represent that microinjection of selected spermatozoa with morphologically normal nuclei improves the incidence of normal embryos. This study highlights IMSI-PGS strategy could be one of the favorable options for the patients having a higher risk of aneuploidy such as aged women, severe male factor and repeated implantation failure.

Trial registration number: Not applicable.

P-164 Polyunsaturated fatty acid concentration in the human follicular fluid may affect fertilisation

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Study question: Does the concentration of polyunsaturated fatty acids in the follicular fluid affect fertilisation and the quality of embryos?

Summary answer: The concentration of polyunsaturated fatty acids in the follicular fluid plays an important role in fertilisation.

What is known already: The metabolite composition of the follicular fluid, which represents the intrafollicular environment, may be an important factor affecting fertilisation and subsequent early embryo development. Polyunsaturated fatty acids are essential fatty acids that are not synthesised in the body, but are utilised as an energy source for processes from oocyte nuclear maturation to embryonic development. There is a study stating that linoleic acid and arachidonic acid in the follicular fluid are correlated with the fertility percentage. However, published data to support claims relating to effects on reproductive health are lacking.

Study design, size, duration: A prospective study was conducted on 10 women eligible for in-vitro fertilisation and embryo transfer between August 2015 and December 2015. Ten serum and 52 follicle fluid samples were collected and analysed. This study was approved by the clinical research ethics investigation committee at university of the Ryukyus.

Participants/materials, setting, methods: Two millilitres of follicular fluid was collected from six consecutive follicles of each patient. Each individual follicle was aspirated independently and matched to an oocyte growing in this particular follicular milieu.The fatty acid concentration in each follicle was analysed using gas chromatography, and its association with fertilisation and embryonic quality was studied.

Main results and the role of chance: The concentration of 22 polyunsaturated fatty acids was significantly lower in the follicles, at approximately one-third their concentration in the serum. The concentrations of linoleic acid and arachidonic acid, both of which are n-6 type polyunsaturated fatty acids, in the follicular fluid of fertilised oocytes were 228.7 μg/ml and 73.8 μg/ml, respectively, and were significantly lower than those in the follicular fluid of unfertilised eggs [247.6 μg/ml ($p = 0.03$) and 80.9 μg/ml ($p = 0.03$), respectively]. Similarly, the concentrations of eicosapentaenoic acid, and docosahexaenoic acid which are n-3 type polyunsaturated fatty acids, were significantly lower in the follicular fluid of fertilised oocytes than in the follicular fluid of unfertilised oocytes. The concentration of polyunsaturated fatty acids in the follicular fluid played no significant role in determining the embryo quality.

Limitations, reasons for caution: The sample size was small, and the number of good quality embryos was less. These might be some reasons why the relationship between the embryo quality and polyunsaturated fatty acid concentration in the follicular fluid could not be established.

Wider implications of the findings: The results suggest that specific fatty acids are utilised more during fertilisation and play an important role in fertilisation. The intake of these fatty acids, particularly essential fatty acids, may improve fertilisation.

Trial registration number: 498.

P-165 The autologous mitochondrial transfer into mature oocytes is an attractive option in patients who produce poor quality embryos

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Study question: The aim of this study is to investigate the effect of autologous mitochondrial transfer into mature oocytes on human embryonic development.

Summary answer: The present study suggested that autologous mitochondrial transfer into mature oocytes improved embryonic development from the women with previously poor quality eggs.

What is known already: It has been shown that mitochondrial function in mature oocytes plays an important role for the development after fertilization in mammals. Autologous germ line mitochondrial transfer into mature oocytes is a method to inject mitochondria retrieved from own egg precursor cells into oocytes. However, there are not enough reports on the effects of this treatment regarding embryonic development.

Study design, size, duration: A total of 85 oocytes retrieved from 13 patients (29 cycles) who underwent IVF treatment with mitochondria transfer into mature oocytes were included in the analysis. The study period was between February and November 2016.

Participants/materials, setting, methods: All participants had history of failed IVF. Their previous IVF data showed the problems of poor quality in their oocytes and/or embryos. The developmental competences of the Day3 embryos were compared between the group A (IVF treatment with mitochondria transfer into mature oocytes) and group B (their previous IVF data). Embryos were evaluated as eligible when they reached to at least 5 cells cleavage stage and being grade 1 to 3 on Veeck's classification.

Main results and the role of chance: The average age of the patients was 37.8 ± 5.4 years old, ranging from 31 to 46 years old. Seven patients were in the 30 s, and 6 patients were in the 40 s.

The rate of all eligible embryos was 64.5 ± 20.9% in group A and 42.5 ± 29.9% in group B, and the difference was significant ($p < 0.05$). The rate of eligible embryos in patients in their thirties was 66.5 ± 22.0% in group A and 31.9 ± 24.5% in group B, and the difference was significant ($p < 0.05$). However, the rate of eligible embryos in patients in their forties was 62.2 ± 21.3% in group A and 54.8 ± 33.1% in group B, and the difference was no significant.

Limitations, reasons for caution: This is a descriptive study with limited number of patients. It possibly includes the difference in the patients' background.

Wider implications of the findings: The study indicated that autologous mitochondrial transfer into mature oocytes could be an effective treatment to improve the quality of the embryos derived from the women with poor quality oocytes or embryos in their previous IVF cycles. This treatment would be more effectively indicated for younger age patients.

Trial registration number: None.

P-166 Embryonic survival, development and cryoinjury of repeatedly vitrified mouse preimplantation embryos

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Study question: Do three times vitrified mouse 8-cell embryos have great cryoinjury, low survival, and subsequent poor pregnancy success?

Summary answer: Repeated vitrification of 8-cell mouse embryos even up to three times appears to be efficient to preserve their survival and developmental potential.

What is known already: Survival rate and developmental potential are similar between once frozen and twice frozen mouse embryos.

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Study design, size, duration: Prospective study using six hundred 8-cell stage embryos obtained from 60 female mice. Study duration was several weeks as the interval between vitrification and warming was one week and the interval between warming and re-vitrification was one hour.

Participants/materials, setting, methods: Six hundred 8-cell stage embryos were obtained from 60 female mice and randomly assigned to control and three experimental groups. Embryos were vitrified by indirect methods. The developmental outcomes such as survival rate, blastocyst-forming rate, and the percentage of hatching/hatched blastocyst were assessed. The cell numbers of hatching/hatched blastocyst were counted after nuclear staining. From hatching/hatched blastocysts, the mRNA expressions for Cirbp, Casp3, Sod1, Gpx3, and Cat were quantified by real-time quantitative RT-PCR.

Main results and the role of chance: In once, twice, or three-time vitrified mouse 8-cell stage embryos, survival rates, blastocyst-forming rates, the percentages of hatching/hatched blastocyst, and the cell counts were all similar when compared with non-vitrified control group. The mRNA expression levels of Cirbp, Casp3, Sod1, Gpx3 and Cat were not affected.

Limitations, reasons for caution: Only survival rate and developmental potential were evaluated in vitrified mouse 8-cell embryos. Further studies are necessary to evaluate live birth rates using three times vitrified 8-cell embryos in humans and to assess long-term safety outcomes.

Wider implications of the findings: If corresponding results in humans, clinicians confidently will decide to thaw more embryos than intended for transfer in order to increase the pregnancy rate by the selection of the morphologically highest quality embryo. Extra embryos may be refrozen up to three times without compromising own survival rate and developmental potential.

Trial registration number: This study is not a clinical trial.

P-167 Conventional IVF revisited: Is ICSI really better for non-male factor infertility? Randomized controlled double blind study

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Study question: Is ICSI really better than conventional IVF for non-male factor infertility?

Summary answer: IVF should be the choice of assisted reproductive technique in properly selected non-male factor infertility cases.

What is known already: Although total fertilization failure is the major drawback for the embryologists and the IVF practitioners, based on the existing literature, the overall risk/benefit analysis still favors conventional IVF in non-male factor infertility cases. But according to the ESHRE EIM database pertaining to 1997–2012, the percentage of IVF use has been continuing to decrease steadily which is under massive scuritinization.

Study design, size, duration: In this double blind RCT, 1133 patients started an ART program from March 2015 were evaluated and 138 non-male factor patients with women age ≤42 were included in the study. Exclusion criteria: history of total fertilization failure, number of retrieved cumulus-oophorus-complex (COCs) < 6 and patients who would undergo total cryopreservation for any reason. We present the preliminary data and the study is ongoing at the moment.

Participants/materials, setting, methods: Sibling COCs were randomly allocated to IVF or ICSI. The decision to transfer IVF or ICSI embryo(s) was made depending on the quality of the embryos. In case of transferring two embryos, pure IVF or pure ICSI embryos were chosen. Neither the clinician nor the patients were aware of the group the embryos belong to.

Main results and the role of chance: Demographic variables, ovarian reserve and infertility etiologies were similar for IVF-ET (#64) and ICSI-ET (#74) groups. Duration of stimulation, total gonadotropin dosage, peak estradiol levels were also similar. Mean number of COCs (18,95 vs 19,24 p = 0,856) and number of embryos transferred (1,81 vs 1,81 p = 0,980) were similar, the ratio of good quality embryos/total embryos (56,89% and 55,97%) were similar (p > 0,05). Clinical pregnancy (63% vs 49% p = 0,103) and implantation rates (31% vs 28% p > 0,05) similar. Abortion rates (12,5% vs 8,1% p = 0,394) were also similar.

A total of 1306 COCs were allocated for IVF while 1331 COCs were denuded for ICSI. Fertilization rate was significantly higher in ICSI group (56,20% vs 63,78% p < 0,05). There were ten total fertilization failure, all in IVF group. Although the fertilization rate was higher for ICSI, excluding the cases with total fertilization failure revealed similar fertilization rates.

Limitations, reasons for caution: Higher sample size for both groups would provide more accurate comparison of the two techniques.

Wider implications of the findings: We provide the first data comparing the outcome of IVF and ICSI techniques from Turkey a country with a very high proportion (98%) of ICSI cycles. Cost advantage, feasibility and the similar clinical outcome makes IVF the choice of technique in properly selected non-male factor infertility cases.

Trial registration number: GTB20150304.

P-168 Transfer of a single vitrified-thawed blastocyst does not increase twinning rate

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Study question: Does transfer of a single vitrified-thawed blastocyst cause an increase of the twinning rate compares to a fresh blastocyst transfer?

Summary answer: Twinning rate does not increase after a vitrified-thawed blastocyst transfer compared to a fresh blastocyst transfer

What is known already: Transferring a single blastocyst has been shown to significantly reduce multiple pregnancy rates, but there is a probability of twinning even in this case. There are conflicting reports regarding treatment methods in ART and their involvement in increasing the twinning rate and especially very little data concerning the influence of vitrification of blastocyst on the twinning rate.

Study design, size, duration: This study is a retrospective cohort study assessing twin pregnancies following fresh or vitrified-thawed single blastocyst transfer. Data from 2010 till 2016 was analysed.

Participants/materials, setting, methods: A total of 22668 patients in Altravita IVF clinic who following ICSI and transfer with a fresh or vitrified-thawed single blastocyst. 70 of them demonstrated twin pregnancy at the 7-week ultrasound. Vitrification (Kitazato, Japan or Cryotech, Japan) was used as the method of cryopreservation. For statistical analysis, z-score was used to determine correlation between type of transfer and twinning rate.

Main results and the role of chance: The twinning rate after single blastocyst transfer is 0,3% and does not exceed the twinning rate after natural conception. Multiple pregnancies were registered in 25 of 9507 patients after a single fresh blastocyst transfer (0,26%). After single vitrified-thawed blastocyst multiple pregnancy was registered in 45 cases (0,34%) of 13161 and the difference between two groups of patients was not significant. There was also no elevated percentage of multiple monochorionic pregnancy rate between the two groups of patients (0,2%).

Limitations, reasons for caution: A few multiple pregnancies can be dizygotic because of spontaneous pregnancy during the embryo transfer cycle

Wider implications of the findings: Estimation of risks of twinning after single embryo transfer gives the possibility of planning the optimal number of embryos for a transfer.

Trial registration number: no.

P-169 Tocotrienol-rich fraction supplementation improves oocytes quality in aging mice and upregulates the expression of anti-aging genes in mouse ovary

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Study question: Does tocotrienol-rich fraction (TRF) supplementation improves oocytes quality and delaying the consequences of aging by upregulating the expression of anti-aging genes in mouse ovary?

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Summary answer: Tocotrienol-rich fraction supplementation improves oocytes quality in aging mice and delaying the consequences of aging by up-regulating the expression of anti-aging genes in mouse ovary.

What is known already: Female reproductive aging resulted from oxidative stress will lead to a decline in biological function of ovaries, produce low quality of oocytes which eventually contributes to infertility. Low expression of anti-aging genes is directly related to the poor development of embryos retrieved from aging mice. Tocotrienol-rich fraction, a reputable antioxidant prevents the retardation of embryogenesis and pregnancy failure following various oxidative stress conditions. The proposed mechanisms of action of TRF is by lowering the plasma levels of malondialdehyde (MDA) (an oxidative stress biomarker) as well as cause a decrease in embryo fragmentation due to DNA damage.

Study design, size, duration: Thirty-two female mice (*Mus musculus*) were equally divided into four groups. In Experiment 1: six-week- old young mice were used as a negative control and eight-month-old aging mice were used as a positive control. While in Experiment 2: six-month-old aging mice were used; i.e. control group and group supplemented with TRF at the dose of 150 mg/kg. Supplementation of TRF was given orally for two months.

Participants/materials, setting, methods: At the end of the supplementation period, mice were superovulated and euthanized. The ovaries and oocytes were collected for the gene expression analysis (SIRT1, CDKN2A and E2F1 genes) as well as assessment of oocyte quality.

Main results and the role of chance: The percentage of normal morphology of oocytes were significantly reduced and the fragmented oocytes were significantly increased in the aging group when compared to the young group respectively. No significant difference were noted in the percentage of normal and fragmented oocytes between control and TRF-supplemented group. The percentage of DNA damage in oocytes were significantly higher in the aging when compared to the young group. Interestingly, the percentage of DNA damage in oocytes from TRF-supplemented group was significantly lower as compared to control group. Viable ovarian tissues were used for the gene expression analysis. Aging caused down regulation of anti-aging gene, SIRT1 and upregulation of CDKN2A and E2F1 genes, the two genes that that downregulate the expression of SIRT1 gene. Conversely, TRF supplementation upregulates the expression of SIRT1 gene while the expression levels of CDKN2A and E2F1 were decreased.

Limitations, reasons for caution: Gene expression analysis can only be done using ovarian tissue and not using oocytes as large quantity of oocytes is required for gene expression analysis.

Wider implications of the findings: Tocotrienol-rich fraction supplementation improves the quality of oocytes in aging mice by lowering the percentage of DNA damage. TRF exerts anti-aging effect on the ovary by up-regulates the expression of anti-aging genes in the ovary. The preservation of the production of good quality oocytes by TRF will potentially improve fertility.

Trial registration number: Not applicable.

P-170 In vitro culture in the presence of amorphous calcium carbonate improves mice embryos compaction and hatching rates

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Study question: In the present study, we wanted to find out whether adding amorphous calcium carbonate (ACC) to the culture media affects mice embryos growth rates.

Summary answer: We have found that adding ACC to commercial media culture have improved mice embryos compaction and hatching rates compared to culture media without ACC.

What is known already: Culturing embryos for 1 week has become a standard in assisted reproduction technology (ART). One condition that is well known to affect the in-vitro culture (IVC) of embryos is the pH It is known that low pH may activate a lysosome proteases such as cathepsin family and it was shown that high cathepsin levels is inversely correlated to embryonic quality, most likely due to the cleaving E-Cadherin thus impairing the embryos ability to compact. ACC is more soluble than crystalline calcium carbonate (CCC) and adding it to the media minimize pH fluctuations.

Study design, size, duration: Experimental research was done of 6–8 weeks old mice (CBA x BL C57) superovulated (n = 10) with PMSG and hCG. Male mice proven to be fertile were then put together with the superovulated females. Females were euthanized after 24 hours (approximately 36 hours post-coitus) and embryos at 2 cell stage were retrieved from the oviducts. The embryos were assessed for compaction, blastocyst and hatching rates.

Participants/materials, setting, methods: Embryos from 5 females were divided into 3 groups: (1) 40 embryos were transferred to 20 µL drops with Quinn's Advantage cleavage media (SAGE, Origio, Denmark), overlaid with mineral oil, cultured at 37.0°C under 5% CO_2 and atmospheric oxygen. (2) 122 embryos, same as group 1 with the addition of 1.7 mM of ACC (3) 42 embryos, same as group 1 with the addition of 1.7 mM of crystalline calcium carbonate (CCC). Embryos were cultures until blastocyst/hatching stage.

Main results and the role of chance: Embryos development in cleavage media was compared to embryos development in cleavage media supplemented with 1.7 mM of ACC or with 1.7 mM CCC. The number of embryos at each stage and their portion from the initial number was calculated. The data was statistically analyzed using JMP software. We observed enhanced embryonic growth in the media comprising ACC and CCC at all stages compared to embryos grown with cleavage media only, with an advantage to ACC which was especially enhanced at the hatching rate. CCC showed visible particles in the media whereas ACC is transparent due to its amorphous structure.

Embryonic Stage	Cleavage Media (CM)	CM & 1.7 mM ACC	CM & 1.7 mM CCC
Compaction	30.2% ± 7.9% **B**	81.2% ± 11.9% **A**	85% ± 3.0% **A**
Blastocysts	87.7% ± 6.2% **A**	96.4% ± 3.6% **A**	100% ± 0% **A**
Hatched	57.8% ± 14.25% **AB**	88.7% ± 7.4% **A**	83% ± 1% **AB**

Table 1: IVC of mice embryos grown either in Quinn's Advantage cleavage media (CM) or embryos grown with CM supplemented with amorphous calcium carbonate (ACC) or CM supplemented with crystalline calcium carbonate (CCC). The data is presented as mean ± St. Error mean. Different letters represent statistical differences (P<0.05).

Limitations, reasons for caution: The main limitation of this study is that the cleavage medium used (SAGE Quinn Cleavage) is designed for human IVC and not for mice embryos. A human embryo study should be done as well.

Wider implications of the findings: The results of this study suggest that adding amorphous calcium carbonate (ACC) to commercial media improves embryos in-vitro growth and development with an enhanced advantage at the hatching rate which can lead to higher implantations and pregnancies rates.

Trial registration number: The experiments were approved by the Israeli ethics committee for animal experiments and the authorization number is IL-15-04-119.

P-171 Embryo morphokinetics are affected by culture media type: an interim analysis of a sibling oocyte study

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Study question: Are the morphokinetic profiles of preimplantation embryos altered when cultured in three different, commercially available culture media using a sibling oocyte study design?

Summary answer: Morphokinetic parameters are significantly influenced by culture media type including time to six-cell (t6), seven-cell (t7), morula (tM) and time between nine-cell and morula (t9-tM).

What is known already: With the advent of time-lapse systems, many are seeking to determine more effective mechanisms for embryo selection by using an embryo's morphokinetic profile to predict viability. However, data presented thus far suggests that treatment, environment and patient parameters

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can affect an embryo's early morphokinetic profile indicating that the use of standardised morphokinetic embryo selection algorithms (ESAs) may not be clinically effective.

Study design, size, duration: An ongoing sibling oocyte study commencing August 2016 where oocytes/embryos following ICSI/IVF fertilisation check were randomised to three culture media; G-TL™, SAGE-1-Step™ and Continuous Single Culture®(CSC). Nineteen absolute and interval morphokinetic parameters were assessed for differences in medians using the related-samples Friedman's two-way analysis of variance by ranks. Blastocyst formation rate (BFR), utilisation rate (UR) and incidence of abnormal cleavage events were analysed using Chi-square tests for homogeneity. All results were considered significant at $p < 0.05$.

Participants/materials, setting, methods: Patients (n = 28) undergoing their first treatment cycle of conventional IVF or ICSI where both patients were < 37 years, fresh, autologous gametes were used and six oocytes injected (ICSI) or three embryos created (IVF) contributed 307 oocytes resulting in 259 embryos. Absolute (t2, t3, t4, t5, t6, t7, t8, t9, tM, tSB, tB) and interval parameters (s2, s3, cc2, cc3, cc4, t9-tM, tM-tSB, tSB-tB) were assessed. Time zero was defined as pronuclear-fading.

Main results and the role of chance: Of 259 embryos, 36% were cultured in G-TL, 32% in SAGE-1-Step and 32% in CSC. Of the nineteen morphokinetic parameters assessed four were found to vary significantly between media types (respective means (h), p-value); t6 (24.37, 24.05, 28.35, $p < 0.001$), t7 (31.43, 29.20, 30.09, $p = 0.047$), tM (58.80, 56.77, 53.35, $p = 0.002$) and t9-tM (11.43, 9.51, 6.34, $p = 0.001$). All other morphokinetic parameters were not significantly different between the three culture media ($p > 0.05$). Nine of the 28 treatments were ICSI, contributing 90 oocytes and 85 embryos, with all others contributed from IVF. Eight treatment cycles resulted in no embryo transfer (ET) due to a clinical contraindication. One day 3 and 19 day 5 ETs were performed. The BFR was not different between any culture media at 67.4%, 78.5% and 72.9%, respectively ($p = 0.281$). The UR (embryos transferred and cryopreserved) were significantly different; 38.3%, 65.1% and 58.3%, respectively ($p < 0.001$). A total of 73 embryos underwent an abnormal division event (cell lysis, direct cleavage, chaotic cleavage, absent cleavage and reverse cleavage); 43.8%, 23.3% and 32.9%, respectively ($p = 0.130$).

Limitations, reasons for caution: The data examined is limited in terms of sample size. Although significance has been shown in a number of morphokinetic parameters, significance may be found in other parameters where a larger sample size is examined.

Wider implications of the findings: This investigation allows for the control of confounding factors of patient or treatment origin therefore, it is surmised; any observed effect is a true reflection of the culture media. This implies that the development and validation of ESAs must be specific, robust and prospective before being introduced for clinical use.

Trial registration number: NA.

P-172 Risk factors analysis for Early Embryonic Arrest

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Study question: What are the risk factors for early embryonic arrest (EA) in a population receiving fertility treatment?

Summary answer: Maternal Age, number of retrieved oocytes, basal AMH level, fertilization rate, treatment of infertility and Semen manipulation were associated with increased risk of EA.

What is known already: Normal embryonic development is the key step to establish a successful pregnancy. It is estimated that about 30%–60% of the human embryos arrest at different early stages of development. Although some gene mutation could explain a fraction of EA occurrence. However, there was lack of systematic analysis on the risk factors.

Study design, size, duration: We performed a retrospective cohort study on patients with primary infertility in CITIC-Xiangya Reproductive and Genetic Hospital from 2013 to 2015, involving a total of 20,364 cycles.

Participants/materials, setting, methods: The patients with all Day3 embryos arrest at/before the second cleavage were regard as EA Patient. Logistic regression analysis was performed adjusting for Maternal Age, Maternal Body Mass Index (BMI), Maternal Hormone Level of basal condition/the day of HCG administration, treatment of infertility(IVF-ET/ICSI/Half ICSI/IVM/PGD/PGS), Semen manipulation, number of retrieved oocytes, fertilization rate and cleavage rate.

Main results and the role of chance: 713 of 20,364 (3.5%) patients were positive for EA. Univariate analysis shows all the above risk factors have significance ($p<0.05$) except basal Estradiol/prolactin and Progesterone level and cleavage rate. In a logistic regression analysis, adjusting for confounding factors, demonstrated that IVM (OR 0.31, 95%CI 0.16–0.58), Swim-up for semen manipulation (OR 0.30, 95%CI 0.13–0.63), higher Maternal Age (OR 1.50, 95% CI 1.40–1.61), higher LH level after HCG administration(OR 1.03, 95%CI 1.02–1.03), lower number of retrieved oocytes (OR 0.41, 95%CI 0.37–0.46), lower basal AMH level (OR 0.96, 95%CI 0.94–0.99) and lower fertilization rate (OR 0.96, 95%CI 0.95–0.96) were positively associated with EA.

Limitations, reasons for caution: This dataset for the risk factor analysis just from one center. It should validated using the dataset from the other center.

Wider implications of the findings: This analysis will help to predict the probability of EA occurrence, and may help to develop some clinical intervention treatment, such as reasonable selection of treatment of infertility and Semen manipulation according to the maternal age, AMH level and number of retrieved oocytes.

Trial registration number: Not applicable.

P-173 Mitochondria membrane potential and developmental ability of mouse oocytes were enhanced by high hydrostatic pressure treatment

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Study question: Does high hydrostatic pressure (HHP) treatment affect to the function of organelles and embryonic development of mouse oocytes?

Summary answer: HHP treatment induced increase of mitochondrial membrane potential (MMP) in mouse unfertilized oocytes and enhancement of the developmental ability to blastocyst stage.

What is known already: A novel technique termed high hydrostatic pressure (HHP) treatment has been reported to improve the survival rate after cryopreservation of several mammalian gametes and embryos. However, the mechanism of the improvement by HHP treatment and the effect on the function of organelles such as nuclear, mitochondria, and cytoskeleton have not yet been cleared. In addition, available information about the effect of HHP to unfertilized oocytes is very limited.

Study design, size, duration: Unfertilized oocytes obtained from female C57BL/6J mice were treated with different pressures (10, 20, 30, and 40 MPa for 30 min at room temperature), and non-treated oocytes were used as control (n = 50–60 oocytes/group). The metaphase-II (M-II) spindle morphology, MMP, and developmental ability were compared between each group.

Participants/materials, setting, methods: Morphology of MII spindle was examined immunohistochemical staining with tubulin specific antibody. For evaluation of MMP, oocytes were stained wit JC-1 dye (5 μg/mL, 15 min) and the ratio of red to green fluorescence of JC-1 was calculated and compared between each group. In addition, oocytes were fertilized by ICSI with spermatozoa derived from C57BL/6J male mice and cultured in KSOM medium for 4 days to estimate the developmental ability.

Main results and the role of chance: After HHP treatment, all oocytes survived and any morphological change did not observe in each HHP condition. Immunostaining analysis revealed that HHP treated oocytes had normal barrel shape M-II spindle with aligned chromosomes in the equatorial plane. MMP of oocytes treated with HHP at 30 MPa showed significantly higher than that of control and other conditions. There was no significant difference in fertilization rate between each HHP group and control. The blastocyst rate of oocyte treated with HPP at 30 MPa was significantly higher than other HHP conditions and

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control (10 MPa: 58.3%, 20 MPa: 56.1%, 30Mpa: 67.9%, 40Mpa: 61.1%, control: 56.9%, $p < 0.05$).

Limitations, reasons for caution: This study was conducted using a mouse model with artificially induced the high hydrostatic pressure condition. This finding does not directly represent human infertility.

Wider implications of the findings: This study may provide one of reason for the improvement of survival rate after cryopreservation of gametes and embryos by HHP treatment. Further, HHP treatment could be a new approach for improvement of the developmental ability of oocytes compromised by some defects such as aging and oxidative stress.

Trial registration number: Not applicable.

P-174 Nrf2, a downstream molecule of Sirt1, regulates Cyclin B1 for spindle organization during mouse oocyte meiosis

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Study question: The role of Nrf2 in oocyte meiosis and oocyte aging.

Summary answer: Our data indicate a role for Nrf2 during oocyte meiosis and uncover a striking beneficial effect of increased Nrf2 expression on aged oocytes.

What is known already: Good oocyte quality is a central component of female fertility. Advanced reproductive biotechnologies depend on a sufficient source of oocytes. Nuclear factor-E2 related factor 2 (Nrf2) is an important transcription factor. Lines of studies showed that Nrf2 plays a critical role in the regulation of oxidative stress, aging-associated diseases and inflammation.

Study design, size, duration: We investigated the effects of Nrf2 on oocyte maturation and its possible mechanism using a mouse oocyte model.

Participants/materials, setting, methods: Granular cells of 62 women (age from 22 to 49) were collected for studying the relationship of Nrf2 and woman age. Oocytes from 6–8 weeks and 8–10 months old mouse were used in this study. A series of biological methods was applyed, including oocyte collection and culture, micro injection, RNA interference, western blotting, immunofluorescence and Confocal microscopy, quantitative real-time PCR.

Main results and the role of chance: We show that the expression of Nrf2 is related to female age in ovarian granular cells. Our data demonstrated that Nrf2 depletion disrupted oocyte maturation and spindle/chromosome organization (36.5 ± 6.2% vs. 9.4 ± 5.4% control, $p < 0.05$) by regulating the expression of Cyclin B1. Depleting sirt1 resulted in reduced Nrf2 expression, which indicated that there was a Sirt1-Nrf2-Cyclin B1 signaling pathway in mouse oocytes. Additionally, we find lower Nrf2 protein level in oocytes from aged mice by immunoblotting and that maternal age-associated meiotic defects can be ameliorated through overexpression of Nrf2 (52.3 ± 6.8% old vs.30.2 ± 4.0% old+Nrf2; $P < 0.05$), providing support for the hypothesis that decreased Nrf2 is one of a number of factors contributing to oocyte age-dependent deficits.

Limitations, reasons for caution: Using animal model and in vitro experiments.

Wider implications of the findings: The present results will help us to improve oocyte quality of aged women to provide new theoretical basis and ideas.

Trial registration number: None.

P-175 Assisted oocyte activation is beneficial for patients with total fertilization failure in IVF cycle caused by developmental arrest in sperm condensation/pronuclear formation stage

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Study question: To investigate candidate patient population with total fertilization failure (TFF) in IVF cycle who might be beneficial from assisted oocyte activation (AOA) in the next cycle.

Summary answer: By Total Fertilization Failure Arrest Test (TFFAT), AOA is beneficial for the TFF patients with all oocytes arrested in sperm condensation or pronuclear formation stage.

What is known already: TFF occurs in 2-3% of IVF cycles. The majority of the patients could benefit from ICSI in the next cycle and had normal fertilization rate. Despite the success of ICSI, TFF still occurs in 1–3% of next ICSI cycles with enough oocyte number and these patients may seek for a third treatment attempt. ICSI-TFF mainly caused by deficiency in sperm activation factors or lack of oocyte calcium oscillation. ICSI followed by AOA can restore fertilization in many sperm-related deficiency cases. But, currently there is no accurate analysis available to predict the TFF risk for second ICSI attempt.

Study design, size, duration: A prospective study was conducted in TFF patients (n = 83) underwent IVF cycle at the Reproductive and Genetic Hospital of CITIC Xiangya from August 2015 to March 2016.

Participants/materials, setting, methods: 661 unfertilized oocytes from IVF-TFF patients were donated for TFFAT. The stage of fertilization arrested in was judged by Immunofluorescent staining using anti-H3K27me3 and anti-Protamine antibodies. Patients with sperm penetration barrier were suggested to use ICSI in the next cycle. Others with sperm discondensation or pronuclear formation problem were suggested to perform ICSI-AOA. The main outcome parameter was fertilization rate. The minor outcome parameters were cleavage rate, good-quality embryo rate, TFF rate and low-fertilization rate.

Main results and the role of chance: By TFFAT, 77% of TFF patients were arrested in the stage of sperm penetration. 4.6% of TFF patients were arrested in the stage of sperm condensation and other 12.4% were arrested in the stage of pronuclear formation. Some of TFF patients (n = 27) had already finished the next cycle when the TFFAT was diagnosed. In conventional ICSI group (fertilization arrest in sperm penetration, n = 21) with 158 oocytes, the normal fertilization rate was 95.5%, cleavage rate was 98.5% and good quality embryo rate was 57.6%. In ICSI-AOA group (fertilization arrest in sperm condensation or pronuclear formation, n = 6) with 70 oocytes, the normal fertilization rate was 90.7%, cleavage rate was 95.3% and good quality embryo rate was 68.3%. All patients in two groups didn't meet TFF cycle or low fertilization cycle again.

Limitations, reasons for caution: The current work is a prospective study of a small number of ICSI-AOA patients. Larger trials with an increased number of patients are needed to confirm our findings.

Wider implications of the findings: TFF in previous IVF cycle can be largely avoid in subsequent ICSI cycles by TFFAT. Moreover, our findings uncovered the different causes of TFF patients in IVF cycle.

Trial registration number: None.

P-176 Influence of culture length on blastocysts implantation potential: analysis of 781 euploid blastocysts obtained in 629 PGS cycles

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Study question: Does the time required to reach blastocyst stage influence the implantation potential?

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Summary answer: It seems that the time needed to reach the blastocyst stage is deeply linked to the embryo implantation potential in an inversely proportional trend.

What is known already: Embryo morphology has always been considered an important predictor of successful implantation and pregnancy. However, the Preimplantation Genetic Screening (PGS) technology proved that this parameter seems to have quite low predictive value on implantation potential. Few studies focused on the comparison of pregnancy rates obtained with blastocysts vitrified on day 5 or day 6, demonstrating that the latter have a statistically significant lower value. Until now little is known if the clinical outcomes could be different transferring an euploid embryo obtained after 5, 6 or 7 days of culture.

Study design, size, duration: In this retrospective study, 781 cryopreserved euploid blastocysts obtained in 629 PGS cycles were transferred in single frozen-mbryo transfers performed from April 2011 to October 2016. All blastocysts were biopsied and vitrified on day-5,-6 or -7 of culture (BD5, BD6, BD7 groups); blastocysts were further divided on the basis of their quality in A, B and C grades. Clinical pregnancy (CP), advanced pregnancy (over 3 months) (AP) and abortion rates were compared between the groups.

Participants/materials, setting, methods: Mean female age was 35.7 ± 3.96 years. In order to classify the blastocysts on the basis of their morphology, the expansion grade, the inner cell mass (ICM) and the trophoectoderm (TE) quality were analyzed. Grade-A included expandend blastocysy and good or very good ICM and TE quality; grade-B included not expanded blastocysy and good or very good ICM and TE quality; grade-C included all expansion grades and poor or very poor ICM and TE quality.

Main results and the role of chance: Out of the 746 analyzed blastocysts, 463, 287 and 31 were in BD5, BD6 and BD7 groups, respectively. In BD5, 122 (26.3%) grade-A, 259 (55.9%) grade-B and 82 (17.7%) grade-C blastocysts were obtained. In BD6 78 (27.2%) grade-A, 88 (30.7%) grade-B and 121 (42.2%) grade-C blastocysts were obtained. In BD7, 7 (22.6%) grade-A, 1 (3.2%) grade-B and 23 (74.2%) grade-C blastocysts were obtained. In BD5 grade-A, grade-B and grade-C, 66 (54.1%) 154 (59.5%) and 36 (43.9%) CP, 61 (50%), 139 (53.7%) and 29 (35.4%) AP and 5 (4.1%), 15 (5.8%) and 7 (8.5%) miscarriages were obtained, respectively. In BD6 grade-A, grade-B and grade-C, 37 (47.4%), 49 (55.7%) and 50 (41.3%) CP, 33 (42.3%), 42 (47.7%) and 42 (34.7%) AP and 4 (5.1%), 7 (7.9%) and 8 (6.6%) miscarriages were obtained, respectively. In BD7 grade-A, grade-B and grade-C, 2 (28.6%), 1 (100%) and 6 (28.6%) CP, 2 (28.6%), 1 (100%) and 3 (13.0%) AP and 0 (0%), 0 (0%) and 4 (17.4%) miscarriages were obtained, respectively. Both CP and AP resulted higher in BD5 compared to BD6 ($p = 0.03$ and $p = 0.02$, respectively) and to BD7 ($p = 0.005$ and $p = 0.001$, respectively);CP and AP resulted higher in BD6 compared to BD7 ($p = 0.05$ and $p = 0.02$, respectively).

Limitations, reasons for caution: The main limitation of morphology assessment is that it is an operator-dependent static system. Furthermore, some blastocysts were already formed on the day antecedent the biopsy without reach enough expansion to perform safely the technique, therefore being cultured one day more and consequently counted in the subsequent group.

Wider implications of the findings: Our data show a strong correlation between the day needed to reach blastocyst stage and implantation potential. Trasferring an early (day-5) euploid blastocyst is most clinically advantageous than a blastocyst obtained later (day-6 or day-7) and it should be considered when more blastocysts are available for the transfer.

Trial registration number: not applicable.

P-177 Blastocoele re-expansion speed is correlated with pregnancy outcome in frozen embryo transfer cycles

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Study question: Is the blastocoele re-expansion speed after warming correlated with clinical outcomes in frozen embryo transfer (FET) cycles?

Summary answer: For blastocyst with poor morphological score, blastocoele re-expansion speed is positively correlated with clinical pregnancy rate in FET cycles.

What is known already: Previous studies suggested that the blastocysts with incomplete blastocoele re-expansion had poor clinical outcomes compared with the fully expanded blastocysts in FET cycles, however, the blastocoele re-expansion speed was not involved in most studies.

Study design, size, duration: This is a retrospective study including all FET cycles with single blastocyst transfer from Jan, 2015 to Apr, 2016. Only the fully expanded blastocysts (grade 4 according to Gardner's scoring system) before vitrification are selected. Photographic images of all warmed embryos have been taken at 1 hour after warming. A total of 553 warmed blastocysts are recruited in this study.

Participants/materials, setting, methods: Blastocysts were divided into three groups according to blastocoele re-expansion level (BRL) 1 hour after warming: 75%–100% blastocoelic re-expansion, 50%–75% blastocoelic re-expansion and 0–50% blastocoelic re-expansion. The BRL was calculated as the ratio of the expanded blastocoelic cavity diameter to the whole blastocyst diameter. Classified by blastocyst morphological score, clinical pregnancy rates were compared among the three groups

Main results and the role of chance: The age, BMI and infertility period of patients had no differences in three groups. For blastocysts with good morphology score (≥ 4BB), the clinical pregnancy rates were 57.1% (28 / 49), 56.3% (36 / 64) and 33.3% (3 / 9) for blastocysts with 75%–100%, 50%–75% and 0–50% blastocoelic re-expansion, and no difference was found. For blastocysts with poor morphology score (< 4BB), the clinical pregnancy rates were 46.2% (55 / 119), 37.3% (91 / 244), 23.5% (16 / 68) for blastocysts with 75%–100%, 50%–75% and 0–50% blastocoelic re-expansion, blastocysts with faster-speed blastocoele re-expansion had a higher clinical pregnancy rate ($P < 0.05$).

Limitations, reasons for caution: This study is limited to its retrospective design and small sample size.

Wider implications of the findings: This study suggested that the speed of blastocoele re-expansion had a predictive value for developmental potential of blastocysts with poor morphological score in FET cycles.

Trial registration number: Not applicable.

P-178 Long-term vapor storage in liquid nitrogen for human vitrified embryos

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Study question: Is vapor cryopreserved LN2 storage beneficial for vitrified human embryos that are frozen compared to vitrified human embryos having direct contact with LN2

Summary answer: There are no significant differences compared to human embryos being cryopreserved in LN2 vapor storage and having direct contact with LN2.

What is known already: Embryos having direct contact within the LN2 storage has a chance of cross contamination and biohazard issues during long term storage. Embryos in the LN2 vapor storage will have an indirect contact with the LN2 phase and will have no cross-contamination or biohazard.

Study design, size, duration: There was a set-up for investigated vapor with vitrification protocols for safe and stable frozen processing for in vitro fertilized embryo storage. This study was done over six months with 740 embryos.

Participants/materials, setting, methods: This study is a retrospective study and has been approved by the IRB at the CHA Fertility Center Seoul. The embryo has underwent vitrification for long term storage with vapor or direct contact in LN2. After the thawing of the embryo, we checked on the survival

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ratio. Then we kept maintaining the embryo transfer of the surviving embryo. We transferred only one or two embryos per patient and kept analyzing the implantation and pregnancy ratio.

Main results and the role of chance: A total of 740 fertilized human embryos are then vitrified frozen, then are stored by vapor (n = 151 cycles) or direct contact (n = 160 cycles) for 6 months in LN2. The presented study shows that the human blastocyst stored in LN2 vapor storage is able to retain full well development. Survival was 97.8% (in LN2 vapor storage) and 97.6% (LN2 storage with direct contact), and the vapor storage of human embryos had no significantly different survival rates after long term storage. For one blastocyst transfer, clinical pregnancy was 50.0% in vapor, 54.0% of direct contact (p = 0.72). Therefore, two blastocyst transfers show that clinical pregnancy was 66.3% by vapor and 63.8% by direct contact storage in LN2 (p = 0.76). There also were no significant differences between the LN2 vapor storage and the storage that allowed the embryos to have direct contact with LN2 in regards to the implantation and the clinical pregnancy of embryo transfer. Especially, age dependent pregnancy ratio also had no significantly different patterns between vapor and direct contact storage methods. Vapor storage systems thus represent a useful alternative for safe and effective long-term storage of vitrified human embryos that can avoid cross contamination chances from having direct contact with LN2.

Limitations, reasons for caution: This study is retrospective analysis.

Wider implications of the findings: Embryo development is not usable for being a predictor of the aneuploidy embryo.

Trial registration number: This is not a critical trial.

P-179 Clinical validation of a noninvasive biomarker for embryo selection based on thermochemiluminescence analysis of the embryo spent culture medium

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Study question: To determine if the oxidative stress and oxidisability of each embryo's spent culture media can be considered a good biomarker of implantation potential

Summary answer: The transferred embryos which have a lower oxidative stress (higher oxidation potential) in their culture medium show a higher implantation potential

What is known already: Since the development of Time-lapse technology, we have achieved more accurate information about embryo morphology and additional morphokinetic events. One major achievement is to transfer the embryo with highest implantation probability. However, it can be improved by the use of supplementary tools based on embryo spent culture media analysis. New noninvasive analysis strategies such as the oxidation potential of the culture medium may provide valuable data of the embryo quality without disturbing culture conditions

Study design, size, duration: TCL (thermochemiluminescence) is an oxidative stress (OS) determination technique of biological samples. It is based on heat oxidation induction, which generates the formation of electronically Excited Species (EES). A Retrospective study focused on the search for novel indicators of embryo quality was performed based on that OS analysis concept. The study included a total of 400 embryo spent culture media samples. All the embryo transfers were at blastocysts stage

Participants/materials, setting, methods: Embryos were cultured in independent wells in Embryoscope Incubator (Vitrolife,Denmark). TCL-device was able to analyzed 368 of 400 samples obtained. A minimum of 15 µl/embryo CCM-medium (Vitrolife) were necessary for measurements. Two groups were formed according to embryo destiny: transferred+vitrified (T+V group) and discarded (D group), 159 vs. 83 embryos, respectively. Photons emitted per second (cps) were measured during oxidation. Parameters recorded were: TCL-amplitude after 50 seconds (H1), 100 seconds (H2) and 280 seconds (H3).

Main results and the role of chance: Differences were found when the oxidation parameters results were related with implantation rate. These values were obtained from 76 transferred embryos, retrospectively analyzed. From a total of 49 implanted embryos, the 69% of them showed H1 > 77 cps, H2 > 90 cps values were collected from the 70% of the implanted embryos and H3 > 88 cps was registered in 76% of them. A consistent difference was also found when the average of TCL's settings between T+V and D group were compared. The results were distributed as follows: H1: 95.17 cps vs. 90.51 cps; H2: 94.05 vs. 88.82 cps; H3: 112.64 vs. 88.06 cps, belonging to T+V and D group, respectively. The Embryos growing-up in less oxidized environment (those that present highest oxidation potential) have higher implantation than the rest of cohort's embryos

Limitations, reasons for caution: The retrospective nature of the analysis and the limited sample size, although more data will be collected in future prospective studies

Wider implications of the findings: TCL assay is a fast and reliable method of oxidative stress measurement. It could be used, together with morphokinetic data to select the highest implantation potential embryo of the cohort right before the transfer. Consequently, TCL profile is a good candidate to become a noninvasive biomarker of embryo quality

Trial registration number: N/A.

P-180 Correlation between mitochondrial DNA content, morphokinetic parameters and embryo ploidy status

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Study question: Is mitochondrial DNA (mtDNA) content related with the early embryo development and embryo ploidy status?

Summary answer: Embryos with higher number of mitochondria showed slowed division pattern, however, no relationship was found between mtDNA content and ploidy status of the embryos analyzed.

What is known already: Previous studies indicated that the mtDNA copy number (the value is called as MitoScore) in the embryos could be used to predict their implantation capacity.

According to these studies, an increase in the mtDNA in the embryo is suggestive of an insufficient level of energy and a low implantation potential. Recently, several studies demonstrated that mtDNA quantity might also be associated with embryo ploidy status.

However, to the best of our knowledge, there are no published data on possible effects of the insufficient energy on the early embryo developmental parameters.

Study design, size, duration: Observational study, including 408 embryos of 66 patients, undergoing PGS and mtDNA copy number from April to December 2016.

Embryo development was monitored by a tri-gas time lapse incubator. The analyzed parameters(times in hours)were t_{Pnf} to t_{SB},cc2(t_3-t_2),S2(t_4-t_3) and S3 (t_8-t_5).Mitoscore values were categorized using the median, partitioning the studied population in two groups (group A with the lower mitoscore values and group B with the higher) and compared with ploidy status of the embryos.

Participants/materials, setting, methods: PGS was done with NGS technology on day 3 of embryo development on embryos with ≥5 nucleated blastomers and ‹ 25 % fragmentation. Biopsied blastomers were sent for high resolution NGS and quantification of mitochondrial DNA/nuclearDNA. Morphokinetic parameters and PGS results were annotated and analyzed according to mitoscore values.

Mann-Whitney and Kruskal-Wallis tests were used for comparison of median timings, mitoscores and PGS results.P-value < 0.05 was considered statistically significant.

Main results and the role of chance: The mean age of the female partner was 34.6 years (range 18 – 44 years). Out of the analyzed embryos, 140 (34.3%) were chromosomally normal, 211(51.7%) abnormal and 57(14%) complex abnormal.

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We found that several parameters were significantly correlated with mitoscore values. Timings (median [IQR]) increased with increasing mtDNAcounts: 22.58 h[3.55] in group A compared with 23.58 h[5.23] in group B (p-value = 0.005), 25.14 h[3.78] in group A compared with 26.38 h[5.17] in group B (p-value = 0.003), 35.59 h[5.38] in group A compared with 37.72 h [6.22] in group B (p-value < 0.001), 37.22 h[5.38] in group A compared with 39.34 h[6.42] in group B (p-value < 0.001), 48.25 h[8.86] in group A compared with 50.28 h[11.08] in group B (p-value = 0.002), 51.06 h[8.03] in group A compared with 53.21 h[11.20] in group B (p-value = 0.007), 53.64 h[10.36] in group A compared with 55.58 h[11.90] in group B (p-value = 0.008), 57.38 h[13.56] in group A compared with 60.52 h[17.66] in group B (p-value = 0.004) and 73.03 h[15.61] in group A compared with 75.78 h[17.01] in group B (p-value = 0.009) for t_{Pnf}, t_2, t_3, t_4,t_5, t_6, t_7, t_8 and t_9 respectively.

However, no statistically significant differences were found for the morphokinetics parameters CC2, S3, S2, t_M, t_{SB} and t_{HB}. Additional, no significant differences were observed for mtDNA values according to the groups determined by the variable PGS (p-value = 0.206).

Limitations, reasons for caution: Associations between mitoscore, morphokinetics and ploidy require careful interpretation due to lack of consensus for definitions, variable PGS methodologies, embryo mosaicism, day of biopsy, the sample sizes and time lapse devices used. Larger studies are required to reconfirm the current findings.

Wider implications of the findings: According to these results, euploid and aneuploid embryos have equal mitoscore distributions and therefore altered mitochondrial number and ploidy are independent. However, embryos with higher count of mitochondria take longer time to divide themselves. Out of those results it can be concluded that slow division might cause accumulation of mtDNA.

Trial registration number: Research Ethics Committee Reference No: REFA008.

P-181 Markers of the first lineage separations in human blastocysts

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Study question: Is it possible to identify specific markers of expression of early lineage-specific genes in human blastocyst by immunofluorescence (IF)?

Summary answer: KLF17, SOX17 and GATA2 are respective specific markers of epiblast cells, primitive endoderm and trophectoderm, which can be observed in IF in human blastocysts.

What is known already: Human embryo development begins in transcriptional silence with an oocyte to zygotic transition, followed by a major molecular event named Zygotic Genome activation (ZGA) occurring between the 4- and 8-cell stage for humans. After ZGA, and especially at the morula stage, some differences emerge among embryonic cells, ultimately leading to the formation of 3 cell lineages at the blastocyst stage: pluripotent epiblast cells, primitive endoderm cells and extraembryonic trophectoderm cells, respectively contributing to fetal tissues, yolk sac and placenta.

Study design, size, duration: This study was performed in frozen-thawed human blastocysts donated for research according to French regulation (authorization AG11-0126AMP)

Participants/materials, setting, methods: Frozen-thawed embryos donated for research were fixed in PFA 4% at the morula, early (B2) or expanded (B4) blastocyst stages. The expression of three proteins: GATA2, SOX17 and KLF17 was assayed by immunofluorescence for three embryos of each stage. The following antibodies were used: goat anti Sox17 (1:200, R&D AF1924), rabbit anti Klf17 (1:500, Sigma HPA024629), mouse anti Gata2 (1:50, Sigma WH0002624M1). Images were acquired on an inverted Nikon A1 confocal microscope by confocal microscopy

Main results and the role of chance: KLF17 is strongly expressed in all cells at the morula and B2 stages, and rapidly becomes restricted to the epiblast cells at the B4 blastocyst stage. In contrast, GATA2 is expressed in trophectoderm after B2 stage, and SOX17 is expressed in ICM at the B4 stage. The results were the same for each of the three embryos analyzed for each stage. These results are is in line with single-cell RNA-seq data performed on human embryos (Petropoulos et al., 2016)

Limitations, reasons for caution: This study needs to be continued in a larger number of embryos in order to confirm the specificity and dynamics of expression of each marker. The corresponding gene expression should also be studied with an RNA-Fish approach.

Wider implications of the findings: Understanding cell lineage molecular mechanisms is important to improve embryo quality evaluation and culture in ART. Improving the characterization of human epiblast cell markers is also mandatory to bring new insights into the field of human naïve pluripotent stem cells.

Trial registration number: none.

P-182 Trophectoderm biopsy does not impact embryo viability after vitrification: results from a multicentre prospective study

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Study question: Does trophectoderm biopsy technique increase blastocysts' vulnerability to vitrification/warming injuries?

Summary answer: Trophectoderm biopsy does not impact post-thawing behaviour of vitrified blastocysts. Only blastocyst re-expansion and degeneration grade after warming are predictive of blastocysts' implantation potential.

What is known already: The optimization of preimplantation genetic diagnosis for aneuploidies (PGD-A) cycles requires cryopreservation to obtain a reliable diagnosis, and the employment of an efficient vitrification program to preserve blastocyst developmental competence. Previous studies have shown that trophectoderm biopsy has no impact on embryo viability in fresh cycles, however, there is still a lack of knowledge about the impact of this procedure on blastocyst viability after warming. The developmental potential of warmed blastocysts has been previously evaluated in terms of re-expansion grade that has been reported as a predictive factor of clinical outcomes.

Study design, size, duration: A multicenter prospective cohort study was performed between June 2016 and December 2016, including all the patients who underwent warming cycle with single blastocyst transfer (SET). A total of 383 warmed blastocysts were included in the study (258 from PGD-A cycles and 125 from standard ICSI cycles). 285 SETs had a conclusive clinical outcome when writing this abstract (197 and 88 in PGD-A and standard cycles, respectively).

Participants/materials, setting, methods: The female age was 37.7 ± 3.5 and 34.6 ± 3.9 in PGD-A and standard group, respectively. As primary outcome, the post-warming blastocyst behaviour was evaluated 1.5hr after warming. Blastocysts were graded according to degeneration (absent/partial/full), re-expansion (1 = 80–100%/2 = 20–80%/3 = 0–20%) and morphology grades (excellent/good/average/poor). Secondary outcome was the ongoing implantation (> 14 weeks of gestation). Logistic regression analysis corrected for confounding factors (female age,PGD-A/standard care group,BMI,stimulation protocol,total FSH dosage,main infertility factor,sperm factor,PGD-A indication,incubator,culture media,biopsy/vitrification/warming operator,blastocyst quality) was performed.

Main results and the role of chance: The rate of degeneration after warming was similar in the PGD-A and in the standard groups (n = 2/258, 0.8% versus n = 3/125, 2.4%). Partial (n = 42/258, 16.3% versus n = 24/125, 19.2%) and absent degeneration (n = 214/258, 83.0% versus n = 98/125, 78.4%) rates were also similar.

No difference was found also in the rate of no re-expansion (n = 24/258, 9.3% versus 9/125, 7.2%), partial expansion (n = 78/258, 30.2% versus n = 37/125, 29.6%) and full expansion (n = 156/258, 60.5% versus n = 79/125, 63.2%).

The rates of blastocysts whose morphological quality worsened after warming was similar between PGD-A and standard cycles (n = 65/256, 25.4% versus n = 23/122, 18.9%). Even if either only ICM's quality (n = 37/256, 14.4% versus n = 11/122, 9.0%) or the TE's one was considered (n = 36/256, 14.1% versus n = 16/122, 13.1%).

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The overall clinical outcomes highlighted that not re-expanded blastocysts have a significantly lower ongoing implantation rate than fully expanded ones (n = 3/23, 13.0% versus n = 74/168, 44.0%; p = 0.006). Similarly, also partially-degenerated blastocysts have a significantly lower ongoing implantation rate than fully-viable ones (n = 13/58, 22.4% versus n = 94/227, 41.4%; p = 0.009).

Logistic regression analysis identified the re-expansion grade 1.5hr after warming as the strongest predictor of ongoing implantation. Specifically, not re-expanded blastocysts showed an OR of 0.25 (P = 0.04).

Limitations, reasons for caution: These findings are relative to the trophectoderm biopsy protocol that involves simultaneous zona opening and trophectoderm cells retrieval on fully-expanded blastocysts (no day3 hatching step involved). A higher sample size is advisable to confirm the current results.

Wider implications of the findings: This study provides further evidences that trophectoderm biopsy does not impact embryo developmental and reproductive potential. Furthermore, interesting clues to increase predictive power upon implantation may arise from post-warming blastocysts evaluation which could influence the clinical practice (e.g. warming of an additional blastocyst).

Trial registration number: none.

P-183 Modification of late embryo development after blastomere removal on day 3 for preimplantation genetic diagnosis

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Study question: Is there a difference in late morphokinetic parameters between biopsied embryos on day 3 for PGD and non-biopsied embryos in control group?

Summary answer: Late morphokinetic events at the morula and blastocyst stages occurred significantly earlier in biopsied embryos than in control non-biopsied embryos.

What is known already: Preimplantation genetic diagnosis (PGD) implies embryo biopsy, either on day 3 or at the blastocyst stage. Although trophectoderm biopsy has been shown to yield some advantages over day 3 biopsy, many teams still perform embryo biopsy on day 3, followed by embryo transfer on day 4 or at the blastocyst stage. Although some studies reported delayed compaction and blastulation after Day 3 biopsy, this remains controversial. Time-lapse systems (TLS) bring new insights into embryo development and can be used to revisit the eventual impact of embryo biopsy on subsequent late cellular events.

Study design, size, duration: This case-control monocentric retrospective study was conducted in couples referred for PGD with biopsied embryos (cases) and in ICSI patients with normal caryotype (controls). We analyzed the clinical and biological data of all consecutive patients who had undergone ICSI or PGD cycle with own oocyte and embryo culture performed with the Embryoscope® between May 2013 and April 2016.

Participants/materials, setting, methods: Each embryo was investigated by detailed time-lapse analysis measuring the exact timing of the developmental events up to blastulation in hours after ICSI procedure. All embryos in both groups were cultured in sequential media in the Embryoscope® under low oxygen pressure (6% CO2, 5% O2). Embryo biopsy was performed in decompaction medium, with 1 or 2 blastomeres removed according to developmental stage (< 8 or ≥8 cells).

Main results and the role of chance: Among the 2903 mature oocytes that were microinjected in the PGD group, 1792 were fertilized (61.4%) and 977 were biopsied on day 3 (55%). In the ICSI control group, 3838 oocytes were microinjected, 2241 were fertilized (58.4%) and 1115 had a quality on day 3 compatible with biopsy criteria (50.5%). Both groups were comparable in terms of patients' characteristics and early morphokinetic parameters. Concerning post day 3 development, tM (morula) and tSB (early blastocyst) and tB (full blastocyst) occured significantly earlier in biopsy group than in control group (95.32 ± 0.37 vs 97.17 ± 0.34, 103.1 ± 0.49 vs 106.1 ± 0.39 and 111.2 ± 0.60 vs 113.3 ± 0.43 respectively, p < .05 for all comparisons).

Limitations, reasons for caution: We did not analyze the respective impact of removing 1 or 2 cells on late embryo development. Our control group consisted in a large but not matched group of ICSI cycles. PGS is not authorized in our country.

Wider implications of the findings: Further studies conducted on embryos donated for research could help deciphering the cellular and molecular mechanisms potentially involved in the modification of post-biopsy embryo development. In this respect, the comparison of transcriptomic data obtained in biopsied and non-biopsied embryos from a same cohort could be of interest.

Trial registration number: not applicable.

P-184 Can morphokinetic parameters help in identifying balanced embryos in chromosomal translocation carriers undergoing PGD when PGS is forbidden by regulation?

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Study question: Are there any morphokinetic differences between balanced and unbalanced embryos in chromosomal translocations carriers referred for PGD which could be included in a prediction model?

Summary answer: We found some morphokinetic differences between balanced and unbalanced embryos, but previously published algorithms for aneuploidy screening did not perform well in our PGD population.

What is known already: Preimplantation genetic diagnosis (PGD) is a procedure developed for couples at high risk of transmitting a specific genetic abnormality or of pregnancy loss because of a specific chromosomal translocation, whereas Preimplantation genetic screening (PGS) consists in the selection of euploid embryos for transfer, this last technic being forbidden in some countries. Recently, several ART laboratories implemented time-lapse in order to improve embryo culture conditions and embryo quality evaluation. As PGS is an expensive and relatively invasive procedure, some studies evaluated the predictive interest of time-lapse for embryo ploidy, with promising but still partial results needing confirmation.

Study design, size, duration: This case-control monocentric retrospective study was conducted in couples referred for PGD because of chromosomal rearrangement, either reciprocal or robertsonian. Clinical and biological data of all consecutive patients who had undergone ICSI-PGD cycle with own oocyte and embryo culture performed with the Embryoscope® between May 2013 and April 2016 were analyzed.

Participants/materials, setting, methods: All mature oocytes were injected and cultured in the Embryoscope® (6% CO2, 5%O2) until day 3 where all embryos with at least 6 blastomeres and little fragmentation underwent biopsy of 1 or 2 cells for further genetic analysis of both chromosomes implicated in the translocation by FISH. Each embryo was investigated by detailed time-lapse analysis measuring the exact timing of the developmental events in hours after ICSI procedure.

Main results and the role of chance: Mean female age was 32.0 ± 3.6 years, mean female BMI was 24.3 ± 4.2 kg/m^2. The mean number of oocytes aspirated was 13.7 ± 5.6. A total of 1176 oocytes were microinjected and cultured in the Embryoscope®, among which 749 were fertilized (63.7%) and 427 were biopsied (57%). Among them, 177 displayed a balanced chromosomal status (41.5%).

Some cleavage timings were significantly different between balanced and unbalanced. t3, t5 and t9+ occurred significantly earlier in unbalanced than in balanced embryos. Concerning cell cycle and synchrony, cc2 and interval t5 – t2 were significantly longer in balanced than in unbalanced embryos. However, there was considerable overlap between groups, preventing from building a statistically relevant prediction model.

In a second phase, we tested the performance of a recently published morphokinetic predictive model for embryo aneuploidy (Basile et al., 2014) in our PGD population of couples with chromosomal rearrangement. The respective proportion of balanced embryos was not significantly different in the 4 groups (p > .05 for all comparisons).

Limitations, reasons for caution: The principal limitation of our study is that we only compared balanced and unbalanced embryos for one specific

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chromosomal rearrangement, and not for all chromosomes, as aneuploidy screening (PGS) is not allowed in our country.

Wider implications of the findings: Although promising studies reported a predictive interest of morphokinetic parameters for embryo aneuploidy screening, this strategy needs to be further validated and does not seem to be relevant for the identification of balanced embryos in chromosomal translocation carriers when PGS is forbidden.

Trial registration number: NA.

P-185 Clinical validation of a new automated analysis for embryo selection based on time-lapse technology

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Study question: Can an automated embryo assessment, based on similarity algorithm, classify embryos in regards to their implantation potential as compared to manual annotations or standard morphology?

Summary answer: Utilising two dimensional morphokinetic analysis, blastocysts with a UAD value > 1.03 (a defined arbitrary unit of distance) have significantly lower implantation rate than blastocysts with UAD < 1.03

What is known already: Selecting the best embryo for transfer is needed to achieve the first goal for IVF treatments, implantation. Time-lapse technology has helped to elucidate key events of embryo development but nevertheless, not all apparently good quality blastocysts manage to implant. The development of novel tools that will assist in even better selection of embryos with most implantation potential enable IVF clinics to get closer to that goal

Study design, size, duration: The study was done in two stages. First, a retrospective analysis of embryos from 799 couples belonging to an oocyte donation program was conducted. In the second phase, embryo analysis was performed before embryo transfer in fresh cycles for 108 patients. In all cases, the embryos were assessed by an automated embryo analysis software

Participants/materials, setting, methods: In the first stage, cleavage timings and cell cycle lengths of 3,781 embryos were registered automatically. Morphokinetic parameters were positioned in 2D-plots and the average distances from the data cloud of KID (known-implantation-data) embryos were calculated, followed by the generation of quality ranking system based on implantation outcomes. The same procedure was followed in the second stage, where 147 embryos were transferred according to their ranking position based on their expected implantation potential

Main results and the role of chance: In the first stage, a retrospective quality ranking of embryo implantation potential was generated based on UAD values obtained from 1,021 analyzed KID embryos. The implantation rates obtained in five chosen grades were: A 34%, B 25%, C 24% and D 19% (**A:** UAD < 0.5, **B:** UAD = 0.5 – 1.0, **C:** UAD = 1.0– 3.0 and **D:** UAD ≥ 3.0). These results were comparable with Basile et al. 2015 algorithm and ASEBIR morphologic classification in day 3 of embryo development. When embryos in the second stage were analyzed, a more accurate hierarchy data was generated: **A:** UAD ≤ 0.50, **B:** UAD = 0.50–0.66 **C:** UAD = 0.66–1.03 **D:** UAD > 1.03. KID embryos were distributed in these 4 groups as follows: 50% in Group A, 48% in Group B, 41% in Group C and only 25% in Group D. Most implanted embryos were found in Groups A, B and C (UAD < 1.03), whereas the implantation rate in Group D (UAD > 1.03) was significantly lower, 46% vs. 25% respectively

Limitations, reasons for caution: The retrospective nature of the analysis, although validated throughout an independent dataset, may limit its universal application

Wider implications of the findings: The automated assessment system provided by time-lapse culture and associated software utilizing an automatic two dimensions grading avoids inter-observer biases and achieves more accurate selection of embryos with the highest implantation potential. This in turn can lead to improved clinical outcomes

Trial registration number: N/A.

P-186 Does Kisspeptin/ Kisspeptin Reseptor system has an local direct effect on oocyte maturation and fertilization?

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Study question: Does Kiss1/Kiss1Receptor mRNA expression in oocytes or surrounding cumulus cells (CCs) and follicular fluid or serum concentrations have an direct effect on oocyte maturation or qualitative functions for further steps?

Summary answer: Positive correlation between mean follicular diameters of mature-fertilized oocytes and follicular kisspeptin concentrations, suggests a direct action on oocyte maturation and fertilization.

What is known already: Kisspeptin signalling system is the main regulator of the reproductive axis and is considered to be the most potent modulator molecule for gonadotrophin releasing hormone (GnRH). Although well known Kiss1 reproductive functions at the level of central nervous system, its direct role on ovarian control has not yet been fully elucidated yet. İmmediate gonadal effect for the presence of kisspeptin locally remains unknown.

Study design, size, duration: This was a prospective cohort study of a total of 26 oocytes and 52 cumulus cells, and 52 samples of folliculer fluid retrieved from infertile patients underwent intracytoplasmic sperm injection (ICSI) cylcles.

Participants/materials, setting, methods: Samples provided from infertile women who underwent ovarian stimulation, divided into three groups according to the stage of oocyte maturity and fertilization. Group 1 included 16 immature oocytes with their micro-enviroments, Group 2 included 10 mature-unfertilised oocytes with their micro-enviroments and Group 3 consisted from 26 micro-enviroments of mature fertilized oocytes as a control group. Kiss1/Kiss1R mRNA in oocytes, CCs, serum and follicular fluid levels were measured. The quantitative results were compared within each group.

Main results and the role of chance: The mean follicular diameter was measured as 18 mm ± 1.84 in Group 1, 20.6 mm ± 1.96 in Group 2 and 19.65 mm ± 1.46 in Group 3. A statistically significant difference was determined between Group 1 and 2 ($p < 0.001$) and between Group 1 and 3 ($p < 0.001$). The follicular and serum concentration of Kiss1/Kiss1R showed positive correlation between each other ($p = 0,007$; $p < 0,001$ respectivly). Serum and follicular fluid Kiss1 concentrations were found to be correlated in line with each other, with lowest level in group 3 and the highest in group 1, and there was no significant difference between the groups ($p = 0,301$; $p = 0,862$). Although, follicular concentrations of Kiss1 and Kiss1R and between mean follicle diameter showed significant positive correlation only in Group 3 (mature and fertilized oocytes) (respectively $p = 0,003$; $p = 0,026$). CCs and oocyte Kiss 1 concentrations were also parallel to each other, but they both failed to induce any change on oocyte maturation ($p = 0,568$; $p = 0,181$ respectivly) or fertilization ($p = 0,181$).

Limitations, reasons for caution: The cumulus cells and oocytes were obtained from patient who underwent ovarian stimulation, which in comparison with natural cycles, may have affected gene and protein expression. Also due to the ethical restriction and patient rights, fertilized oocytes regarded as potential embryos and were not analyzed.

Wider implications of the findings: Our data demonstrate that, in addition to crucial effects at the central nervous, kisspeptin/KISS1R systems are co-expressed and are functionally active in follicular development.

Trial registration number: We do not have a clinical trial registration number.

P-187 In vitro exposure to environmental relevant dose of bisphenol A does not influence the rabbit blastocyst transcriptome

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Study question: Does in vitro exposure of preimplantation rabbit embryos to environmental doses of bisphenol A (BPA) affect its gene expression?

Summary answer: Exposure to a concentration of BPA relevant to the human situation does not affect rabbit embryo gene expression.

What is known already: In mice, in vitro studies have shown that a single addition of BPA at the nanomolar range at the start of 2 cell-embryos culture can speed up embryo development while it slows it down at high doses (100 µM) without affecting blastocyst cell numbers. Because mice preimplantation embryo development presents important differences with the human one, our goal was to evaluate the transcriptomic effects of an in vitro exposure to a concentration of BPA relevant to the human situation (2 ng/ml = 8.76 nM) on another mammalian species, the rabbit, assumed as a more relevant model for reproductive and developmental toxicology.

Study design, size, duration: In vivo fertilized one cell embryos recovered from different stimulated females were pooled, randomly distributed by groups of 25 to 35 in 50 µl drops of culture medium G1+/G2+ (Vitrolife, Goteborg, Sweden) containing 2 ng/ml Bisphenol A or not (vehicle only) and cultured until 97 hours post coitum. Then, 4 pools of 9 expanded blastocysts in the control and BPA exposed conditions were constituted and frozen.

Participants/materials, setting, methods: Total RNA was extracted from each pool of exposed and control blastocysts, amplified and labelled using the One Color Microarray-based Gene Expression Analysis low input Quick AMP labeling kit (Agilent®). After purification, cRNAs were hybridized on a customized Agilent Rabbit array (24 K). Transcriptomic analysis was explored using Hierarchical Clustering, Principal Component Analysis and Independent Component Analysis. Standard statistical analyses (Limma R-package) was then performed to identify genes whose expression has been modified between exposures.

Main results and the role of chance: Embryo development and blastocyst rates were unaffected by BPA. Exploratory analysis using Hierarchical clustering or principal component analysis failed to evidence any difference in global gene expression explained by the culture conditions of the blastocysts. Furthermore, applying the False Discovery Rate (FDR) multiple analysis correction method, no gene was evidenced as differentially expressed between exposed and control embryos at the $p < 0.05$ level using the LimmaR-package. If FDR was not applied and the raw p-value was set at < 0.01, only 9 genes with a two-fold ratio (> 2 or < 0.5) of expression between BPA exposed and control embryos were retrieved. RT-qPCR analysis failed to confirm any difference in gene expression for those genes between culture conditions. Therefore, in our hands, BPA at concentration relevant to the human situation had no effect on gene expression of the rabbit blastocyst.

Limitations, reasons for caution: BPA addition was performed at the start of the embryo culture. We cannot rule out a degradation of the toxicant along the 4 days culture period. Furthermore, epigenetic evaluation was not performed in this study so that epigenetic alterations cannot be eliminated.

Wider implications of the findings: This study highlights the necessity to study other mammalian species than mouse when assessing the effect of any potential embryotoxic molecule. The previous results on mice came from BPA exposure beginning at the 2 cell embryo stage i.e at the time of the major phase of embryo genome activation.

Trial registration number: not applicable.

P-188 Possible influence of the different shipment types on the viability of vitrified oocytes

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Study question: During the oocytes shipment, is there any influence related to the different shipment types and oocytes viability when they are thawed?

Summary answer: There is a significant difference between shipment types; we can state the possible influence of the different shipment types on the viability of vitrified oocytes.

What is known already: Oocyte donation has become a fundamental part of ART. The demand for oocyte donation has increased lately, as it has become a treatment option. The introduction of oocyte cryopreservation into donation programmes overcomes many challenges. It simplifies the logistics of ART cycles as there is no need for menstrual cycle synchronization between donor and recipient and it has also led to the development of ova banks. In many European countries, gametes donation 'has recently been approved by law", so the incipient need for egg donors makes many of them turn into cryobanks, often located outside their borders.

Study design, size, duration: 5336 vitrified oocytes have been shipped during the research, from November 2014 until September 2016, in two types of shipments: Standard air delivery by plane via a conventional cargo company; and Lab to Lab, in which a member of Ovobank staff is personally in charge of the door-to-door delivery. The survival data from every centre have been analysed and they have been classified by their shipment type in an Excel table for subsequent analysis.

Participants/materials, setting, methods: Oocytes at methaphase II from donors aged 18–31 were used. They were collected via follicular puncture 36 hours after trigger, then vitrified following Kitazato protocol (Cryotop) and stored in tanks with liquid nitrogen afterwards at Ovobank laboratory (FIV Marbella, Spain). Dry Shipper Dewars containers were used for their delivery. The shipment types were the above mentioned: Standard air delivery and Lab to Lab.

Main results and the role of chance: In the research we have only analyzed the survival rate, since in the other rates there are many factors that may affect the result and not only the shipment.

Out of the 5336 shipped oocytes from November 2014 to September 2016, 5262 thawed have been reported: 2278 thawed oocytes were sent via conventional cargo companies (DHL, TNT or Feddex) and 2984 thawed oocytes were sent via Lab to Lab (by Ovobank staff).

Overall, when the survival rate is analysed according to the type of shipment, it is significantly higher in Lab to Lab deliveries than in standard air delivery (74,9% vs 70,5%).

These data show that oocytes may be damaged if they are shipped via standard carrier. This is probably due to the container handling, the possible exposure to X-rays, the use of commercial airlines, etc.

The safest shipment type according to this data is Lab to Lab, as a member of Ovobank staff is continuously looking after the container during the delivery, from Ovobank laboratory to the recipient clinic laboratory.

Limitations, reasons for caution: The sent oocytes batches come from different donors and stimulations, in addition they have been thawed by different centres and professionals who, even thought they have followed the established protocol, may have limited the obtained results.

Wider implications of the findings: Due to the constant flux of oocytes shipments by Ovobank, we are continuously receiving results from their thawing, so we will have to continue researching, analyzing and going in depth on the influence of the different shipment types on the viability of vitrified oocytes.

Trial registration number: Not applicable.

P-189 GnRH agonist triggering affects early-stage embryo morphokinetic parameters

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Study question: Our objective was to compare embryo morphokinetic of embryos derived from GnRH antagonist cycles triggered with either GnRH agonist hCG, using the time lapse monitoring system (TMS).

Summary answer: hCG triggering was associated with a higher proportion of optimal day-1 embryo morphokinetic parameters compared with GnRH agonist triggering even after adjustment for confounding variables.

What is known already: GnRh agonist triggering elicits an endogenous surge of LH and FSH that remain elevated for 24–36 hours, opposed to the hCG-

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mediated LH activity that persists for several days. Oocyte and embryo kinetics are affected by treatment protocol and ovulation triggering medications.

Study design, size, duration: A retrospective cohort study evaluating all TMS [EmbryoScope, Unisense Fertilitech, Aarhus, Denmark] data of fresh antagonist cycles in women ≤42, between April 2013 and December 2016. Timing of PB-extrusion, PN-fading, cleavage timings (t2-t8), second cell cycle (CC2 = T3-T2) and second synchrony (S2 = T4-T3) durations were compared. Optimal CC2 was defined as > 5 hours and optimal S2 was defined as < 1 hour. KID score was used for assessing embryo quality.

Participants/materials, setting, methods: Embryo morphokinetic parameters and cycle outcome were compared between embryos derived from GnRH antagonist with final oocyte maturation triggered by GnRH agonist (Decapeptyl 0.2 mg) or hCG (Ovitrelle 250 mcg). Multivariate logistic analysis was performed for confounding factors including maternal age, BMI, type of gonadotropin used and estradiol levels.

Main results and the role of chance: We analyzed 8,422 embryos deriving from 727 cycles; 2,406 embryos in the GnRH agonist triggering and 6,016 embryos in the hCG triggering group. The percentage of embryos with optimal s2 duration, symmetric blastomeres and no multinucleation was higher with hCG triggering group. These early kinetic parameters occurring during the first 24 hours from fertilization were the only ones found to be affected by type of medication triggering. Later stage embryo kinetic parameters of cleavage t2-t8 were similar. Differences remained significant in a multivariate model after adjusting for confounding variables that might have an effect on embryo morphokinetics. The proportion of high (4/5) KID score embryos and pregnancy rates per transfer were similar in cycles with final oocyte maturation triggered by GnRH agonist or hCG.

Limitations, reasons for caution: It is yet to be determined if these differences in embryo kinetic parameters are of any clinical significance considering embryo grading (KID score) and pregnancy rates were similar. Furthermore, despite statistical adjustment for confounding variables, the physician's choice of triggering was affected by patient characteristics and response to treatment.

Wider implications of the findings: The TMS allows embryo quality assessment by documenting timing of events and length of intervals in embryo development, providing a more objective scoring of the embryos, for embryo selection. Type of oocyte maturation triggering might influence embryo morphokinetics parameters and should be considered in embryo selection models.

Trial registration number: none.

P-190 Artificial oocyte activation improves morphokinetic parameters and number of quality blastocysts: a sibling oocyte study

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Study question: How the artificial oocyte activation (AOA) with calcium ionophore affects the embryo development and aneuploidy rate?

Summary answer: The AOA embryos developed faster, but morphokinetics were similar to control after arrested embryos exclusion. Euploidy rate was similar, but with AOA blastocysts yield increased.

What is known already: The most of failed ICSI fertilization cases can be explained by the lack of oocyte activation by spermatozoa. The AOA by short exposure of freshly fertilized oocytes to medium containing calcium ionophore can overcome recurrent low or failed fertilization and give successful pregnancy. However the impact of AOA on embryo development is mostly unknown. This study compares morphokinetic, quantitative and aCGH data for embryos obtained after AOA to same patient's embryos fertilized by standard ICSI.

Study design, size, duration: This retrospective study includes total of 62 patients with AOA cycles performed between April 2015 and December 2016. For 39 of them the time-lapse culture data was available for both AOA and standard ICSI embryos. Additionally, for 37 patients the embryo ploidy was established by aCGH after trophectoderm biopsy in both AOA and non-AOA cycles. Data from development of same patient's "sibling" embryos from conventional ICSI were used as a control.

Participants/materials, setting, methods: The indications for AOA were the previous ICSI cycles with poor fertilization, cleavage stage arrest or severe teratozoospermia. Ovarian stimulation, ICSI and embryo culture followed standard protocols. The AOA was done after ICSI by placing oocytes for 15 minutes in the medium containing 10μmol/l calcimycin (A23187). Conventional ICSI was performed in control group. Time-lapse imaging culture was carried out in EmbryoScope™ or MIRI-TL™ until day 5 and trophectoderm biopsy for aCGH was done if required.

Main results and the role of chance: The fertilization rate was significantly higher in AOA group compared to control (71.2% vs 63.1%). After 43 embryo transfers 21 clinical pregnancies were achieved in AOA group, with average of 1.3 embryos per transfer.

According to time-lapse data the AOA embryos were significantly faster than the sibling controls in timing of pronuclear fading, first and second round of mitotic divisions. Interestingly, after the embryos not reaching blastocysts were excluded the both groups showed equal morphokinetic behaviour.

The euploidy rate in the AOA group was 36.1% compared to 32.5% in control group, with no statistically significant difference. However, the yield of blastocysts with good morphology that hatched enough for trophectoderm biopsy was higher after AOA (3.6 blastocysts per cycle compared to 2.7 in control group).

Limitations, reasons for caution: While AOA can help in case of reduced or failed ICSI fertilization, at this moment this procedure is considered as an experimental technique. This study is a retrospective analysis and thus can be a subject to bias.

Wider implications of the findings: The AOA can help patients with previous low or failed fertilisations and infertile couples with severe male factor. The early development dynamics and ploidy of embryos that were obtained with AOA seems to be unaltered by procedure. These data are an additional step towards wider application of this technique.

Trial registration number: Not a RCT trial.

P-191 Relative humidity of outside air influences ongoing pregnancy rates if outside air is not conditioned for humidity before passing through the carbon/potassium permanganate filters

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Study question: What could be the reason for inexplicable fluctuating IVF results in a high tech clean-air ART laboratory with experienced lab technicians and standard operating procedures?

Summary answer: The relative humidity of unconditioned outside air influences efficiency of activated carbon/potassium permanganate filters which can result in significantly impaired ongoing pregnancy rates.

What is known already: It is generally known that air quality and the presence of VOC's can have detrimental effects on embryonic growth and ongoing pregnancy rates. Therefore cleanroom technology, including a high efficiency particulate air filter (HEPA 14) and two types of activated carbon/potassium permanganate (KMnO4) filters can improve implantation rates significantly. The efficiency of these activated carbon filters, however, is highly dependent on the relative humidity and work suboptimal above a humidity of 70%. Therefore, during periods with high relative humidity of outside air, VOC's can enter the IVF laboratory.

Study design, size, duration: In this retrospective cohort study, the influence of the mean daily relative humidity of the outside air near the clinic was correlated with the pregnancy outcome for all 1770 IVF/ICSI cycles with ET performed between January 2014 and September 2016 in a tertiary referral center.

Participants/materials, setting, methods: All 1770 IVF/ICSI transfers were divided into four groups dependent of the mean relative outside humidity on the day of oocyte pick-up. Humidity in Group I (N = 148) < 60%, Group II (N = 235) 60–70%, Group III (N = 992) 70–85% and Group IV (N = 395) > 85%, respectively.

Main results and the role of chance: Comparable figures for cause of infertility, cycle number, mean age, number of oocytes found, fertilization rate and number of embryos transferred were found in the different groups analyzed. IVF results were significantly impaired and inversely correlated to the degree of humidity in the outside air: +BHCG and clinical ongoing pregnancy rate per

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embryo transfer were 39.7% and 31.0% for Group I, 36.9 and 27.8% for Group II, 31.3% and 22.5% for Group III and 29.4 and 17.4% for Group IV, respectively ($P < 0.001$). In Belgium, the mean relative humidity is above 70% during 80% of the time. The outside air quality near our clinic is highly polluted by the industry with VOC's such as benzene, toluene and heavy metals. If the outside air goes unconditioned for humidity through the active carbon filters, these filters work suboptimal to eliminate VOC's.

Limitations, reasons for caution: After multivariate analysis no confounding factors could be found which could have influenced these data.

Wider implications of the findings: Despite the presence of cleanroom air containing HEPA14- and active carbon/potassium-permanganate filters, Coda® tower and Coda® inline filter cartridges, air quality can influence ongoing pregnancy rates if outside air is not conditioned for humidity before filtering. ART centers with inexplicable fluctuating IVF results should control their filter system.

Trial registration number: Not applicable.

P-192 Comparison of differences in IVF outcomes between single frozen-thawed D5 and D6 blastocysts transfer cycles

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Study question: Are there differences in the IVF outcomes of frozen-thawed blastocysts on different developmental stages?

Summary answer: D5 blastocysts transfer resulted in higher pregnancy rate and lower ectopic pregnancy rate than D6 blastocyst, while live brith rate and neonatal outcomes were similar.

What is known already: The IVF outcomes of D5 and D6 blastocyst transfers in different studies using frozen-thawed cycles were contradictory. Some studies reported higher clinical pregnancy rate and higher live birth rate in D5 blastocyst transfer cycles, while others observed no differences between the two groups. Furthermore, none of these studies have addressed IVF outcomes only from single blastocyst transfer cycles.

Study design, size, duration: In this retrospective study, the data recorded during June 2010 to July 2016 were extracted from the database of the Reproductive Center of Sixth Affiliated Hospital, Sun Yat-Sen University in China and in total, 3179 single frozen-thawed blastocyst transfer cycles were analyzed.

Participants/materials, setting, methods: In total, 2337 D5 and 842 D6 single frozen-thawed blastocyst transfer cycles were retrospectively analyzed. The demographic parameters and IVF outcomes including HCG (+) rate, pregnancy rate, first trimester abortion rate, birth rate, birth weight and gender proportion of the D5 and D6 blastocyst transfer cycles were compared.

Main results and the role of chance: The HCG positive rate, mean HCG level, biochemical pregnancy rate and clinical pregnancy in the D5 group were significantly higher than those in the D6 group ($P < 0.001$, < 0.001, $= 0.016$, and < 0.001, respectively). The ectopic pregnancy rate in the D5 group was lower than that in the D6 group($P = 0.03$). The first trimester abortion rate (per HCG positive cycle), live birth rate (per embryo transfer), birth weight and gender proportion did not significantly differ between these two groups.

Limitations, reasons for caution: Although 3179 cycles were included in our study, it is still limited by the retrospective nature and lack of randomization.

Wider implications of the findings: This study is the first to analyze IVF outcomes of single blastocyst cryopreserved during different developmental stages. In the clinic, D6 blastocysts in frozen-thawed cycles had similar birth rate and neonatal outcomes to those of D5 blastocysts.

Trial registration number: None.

P-193 When to decide for single (SBT) or double blastocyst transfer (DBT): the embryologist's view

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Study question: What are the birth rates (BR) and the baby-take-home rates (BTHR) per blastocyst transferred in SBT or DBT in regard to blastocyst quality?

Summary answer: To increase the chance of transferred blastocysts to implant and give a healthy baby, SBT should be performed with exception of low quality blastocysts.

What is known already: Implantation rates are known to be related to blastocyst quality evaluated by morphological criteria. To increase the chance for a pregnancy, DBT is commonly performed, especially after a previous unsuccessful cycle, drastically augmenting the risk for twin pregnancies. Multiple pregnancies are associated with a higher risk for preeclampsia, placental problems, perinatal morbidity, preterm birth and low birth weight. In order to maximize the chance of a blastocyst to implant and result in a healthy baby and to minimize the risk for twin pregnancies, we analyzed clinical outcome of SBT and DBT in relation to blastocyst grading.

Study design, size, duration: A retrospective cohort study was conducted including 992 SBT and 1.768 DBT between January 2010 and December 2014 in our center. SET or DET were performed on basis of patient history, availability of blastocysts and recommendation of the physician, but also substantially on the patients wish. Data were extracted using DynaMed® software, data analysis was performed using SPSS-Statistics Patients with lost-of follow-up for clinical outcome were excluded.

Participants/materials, setting, methods: Blastocyst quality was assessed prior BT according to the Gardner criteria. Top quality (top) included blastocysts with AA, BA or AB grading for inner cell mass and/or trophectoderm, good quality (good) blastocysts BB, BC or CB grading, and low quality (low) included either CC grading or early blastocysts with no expansion. DBT were grouped according to the transferred qualities (e.g. top + good quality). BT was only performed after good endometrial build-up.

Main results and the role of chance: BR significantly increases with DBT when 2 top blastocysts were transferred as compared to 1 top blastocyst (48.8% vs. 38.7%; p-value < 0.05). However, in all other quality groups, DBT did not significantly augment BR compared to SBT. Further, we found in all DBT transfer groups vs. SBT significantly reduced BTHR per transferred blastocysts. BTHR/blastocyst decreased from top blastocyst SBT (39.1%) to 31.0% in top+ top DBT, 27.3% in top+ good, and 22.4% in top+ low quality DBT (p-value < 0.001). With good quality blastocysts BTHR/blastocyst was 33.2% in SBT; 22.7% in good+ good DBT, and 18.2% in good+ low quality. BT (p-value < 0.001). Solely for low quality blastocysts similar BTHR/blastocyst in SBT and DBT were observed (SBT 10.6% vs. 9.7% DBT of 2 low quality blastocysts). Maternal age was considered in statistical analysis. When analysing twin rates, we found twinning rates of 1% in SBT with top, 1.6% with good and 0% in SBT with low quality blastocysts. For DBT, the twinning rates were 27.3% (top+ top); 25.8% (top+ good), 20.5% (top+ low) and 23.5% (for both, good+ good and good+ low). However, DBT of 2 low quality blastocysts showed significantly reduced twinning rates (7.7%) compared to the other groups.

Limitations, reasons for caution: The increase the robustness of our results they should be verified in a larger data set.

Wider implications of the findings: In DET with at least one top or good blastocyst a meager increase in BR but a significant reduction in BTHR/blastocyst was found indicating to reconsider the policy of DBT. Chances to conceive increase with SBT and subsequent cryo-cycles. DBT should be recommended for low quality blastocysts only.

Trial registration number: not applicable.

P-194 Time-lapse Systems for ART – a systematic review

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Study question: Do time-lapse systems (TLS) improve live birth rates (LBR) for couples undergoing assisted reproductive technology (ART)?

Summary answer: There is insufficient evidence of a difference in LBR for couples using TLS versus conventional incubation of embryos.

What is known already: TLS take digital images of embryos at frequent time intervals within an incubator. This allows embryologists to assess the quality of the embryos without removing them from the incubator and potentially select a 'better' embryo. TLS has been widely introduced into clinical practice accompanied by claims of improved clinical outcomes. There is often an additional charge to patients for using the technology. The Cochrane review published in 2015 included 3 randomised controlled trials (RCTs) of 994 couples, and concluded insufficient evidence of differences in LBR, miscarriage, stillbirth or clinical pregnancy between TLS and conventional incubation.

Study design, size, duration: This systematic review has been undertaken using Cochrane methodology. A comprehensive search of published and unpublished RCTs was undertaken. Two authors scanned the titles retrieved by the search and extracted data (SA and PB). Authors were contacted directly to obtain further unpublished data and clarify study methodology. Risk of bias was assessed using the Cochrane tool and GRADE was used to evaluate the overall quality of the body of evidence for the review outcomes.

Participants/materials, setting, methods: This systematic review includes 5 RCTs of 1757 couples. In order to assess the potential advantage of TLS, the studies were grouped into three designs:

Study design 1: TLS with conventional morphological assessment of still images versus conventional incubation and assessment

Study design 2: TLS utilizing a cell-tracking algorithm versus TLS with conventional morphological assessment of still images

Study design 3:TLS utilizing cell-tracking algorithm versus conventional incubation and assessment.

Main results and the role of chance: Considering all trial designs together, there is insufficient evidence to determine a difference in livebirth OR 1.24 (95% CI 1.0–1.54), miscarriage OR 0.9 (95% CI 0.64–1.26,), stillbirth and clinical pregnancy OR 1.17 (95% CI 0.96–1.42,) between TLS and conventional incubation. The evidence was graded very low evidence for all outcomes because of the high risk of selection, performance and attrition bias in 2 studies (Kovacs 2013 and Rubio 2014). There is also significant heterogeneity and indirectness owing to the inclusion of donor oocytes in Rubio 2014.

Considering the results by subgroup, study design 1 and 2 reveal insufficient evidence to determine a difference for all outcomes (moderate quality for trial design 1 and low quality evidence for trial design 2). However, trial design 3 (TLSs utilising cell-tracking algorithms versus conventional incubation and assessment) has an improvement in livebirth (OR 1.48 95%CI 1.15–1.90 very low quality evidence) and clinical pregnancy (OR 1.30 95%CI 1.02–1.67) with TLS. There was insufficient evidence of a difference for miscarriage within this subgroup (OR 0.70 95%CI 0.47–1.05). Both the studies in this analysis were at high risk of bias for selection, performance and attrition bias and have been graded very low quality evidence.

Limitations, reasons for caution: The studies included in analysis for study design 3 are at high risk of bias and represent very low quality evidence. The largest, (Rubio 2014), included women receiving donor oocytes, and livebirth data are only available in abstract form. Another concern was some women were allowed to choose the TLS.

Wider implications of the findings: The high risk of bias in the studies included in subgroup analysis of trial design 3, requires caution. Further robust, well designed, independent and appropriately powered trials should be added to this analysis before purporting improved outcomes with TLS and recommending it to couples as part of an IVF cycle.

Trial registration number: Prospero: CRD42015019962.

P-195 Can follicular fluid anti-Müllerian hormone level be a determinant for pregnancy in women under 35 years of age?

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Study question: Can follicular fluid anti-Müllerian hormone level be a determinant for pregnancy in women under 35 years of age?

Summary answer: The FF AMH levels were found to have a predictive effect on pregnancy in women under 35 years of age.

What is known already: Anti-Müllerian hormone, which is also known as Müllerian-inhibiting substance, is a main regulator in the follicle development (Richardson SJ *et.* al.,1987; Faddy MJ *et.* al., 1992). It is probably produced by preantral and smaller antral follicles independent of follicle-stimulating hormone (FSH) (Hansen KR et al.,2008; Klein NA *et* al., 1996). It has been shown to inhibit the follicle growth and selection, as well as growth from the primordial follicle to the primary follicle (Klein NA *et* al., 1996). Recently, many study groups have adopted *AMH* as a new marker for ovarian reserve and the indicator of the response to gonadotropins.

Study design, size, duration: The samples were collected from each of 61 patients with the age range of 21 to 42 years separately. The oocytes were isolated from *FF*, which did not contain irrigation solution, of the follicles reaching 15 mm in diameter with the help of sterile injectors. The remaining *FF* was centrifuged at 1800 rpm (300 g) for 15 min. Two milliliters from the supernatant parts were stored in liquid nitrogen tanks to be studied later on.

Participants/materials, setting, methods: The study was carried out at Fertijin *In Vitro* Fertilization Center between November 2014 and June 2015. and the *FF AMH* levels of a total of 61 patients (mean age: 33.72 ± 4.82 years; range: 21 to 42 years) were analyzed. The *AMH* levels of the collected samples were studied using an *AMH* kit with Roche Cobas e601 autoanalyzer (*Roche* Diagnostics, *USA*) using electrochemiluminescence immunoassay (*ECLIA*).

Main results and the role of chance: Four groups were formed according to the AMH levels of the FF: very low level group with 27.9% (FF AMH ≤1.0 ng/mL, n = 17), low level group with 41% (FF AMH = 1.0—2.1 ng/mL, n = 25), moderate level group with 15% (FF AMH = 2.1 < X < 3.6 ng/ mL, n = 11), and high level group with 13.1% (FF AMH > 3.6 ng/mL, n = 8). There was no statistically significant difference in the rates of fertilization between the AMH groups ($p > 0.05$). The AMH levels were found to be correlated with oocyte count ($p < 0.01$), female age ($p < 0.05$), embryo quality ($p < 0.01$), and clinical pregnancy rates ($p < 0.035$). The mean age of the group with moderate AMH level was found to be higher, compared to the low level group (34.5 years) ($p < 0.05$). The FF AMH levels in patients under 35 years of age were higher in women who were pregnant than non-pregnant women ($p < 0.01$).

In addition, AMH levels of pregnancies in women under 35 years of age were statistically higher than those of pregnancies ($p = 0.019$, $p < 0.05$). However, there was no significant difference between AMH levels of women who were over 35 years of age according to pregnancy status ($p > 0.05$).

Limitations, reasons for caution: The *FF* samples taken from the follicles larger than 15 mm were included in the study. The *metaphase II* (MII) oocytes obtained from these follicles were fertilized by intracytoplasmic sperm injection. Following the developing embryos, one or two embryo transfers were performed by choosing the best-quality embryo morphologically.

Wider implications of the findings: Several studies have shown that the morphological studies are insufficient to determine the oocyte quality (Lasiene K et al.,2009; Klani S et al 2011). The failure of oocyte morphological assessments to predict *in vitro* fertilization (IVF) outcomes has led the researchers to examine biochemical criteria of follicular fluid (*FF*).

Trial registration number: not applicable.

P-196 When multinucleated embryos create high quality blastocysts they have a high implantation rate

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Study question: Should we transfer high quality blastocysts (HQB) that are created from multinucleated embryos (MnE)?

Summary answer: The implantation and live birth rates obtained following transfer of HQB created from MnE indicate that their transfer is recommended if there aren't non-MnE available.

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What is known already: The presence of multinucleated blastomeres in the first divisions of an embryo is associated with poor implantation rates, low possibilities of creating a blastocyst, an increased rate of chromosomal abnormalities (Van Royen 2003; Munné 1995; Parriego 2013) and miscarriage (Scott 2007; Fauque 2013).

It would appear that many MnE have the capacity to self-correct and form euploid blastocysts which then give rise to healthy live births (Balakier, 2016).

The multinucleation rate is significantly higher in the embryos of women ≥40 years of age than in women ≤35 of age (Balakier, 2016).

Study design, size, duration: Between January 2014 and March 2016, 1607 MnE of > 2 cells were cultivated to day+5. 42.9% of these embryos reached the blastocyst stage and only 8.1% created HQB. In the same period, 70.9% of the non-MnE reached the blastocyst stage and 39.1% created HQB. The pregnancy rate, implantation, miscarriage and live birth rates were analysed following transfer of 78 multinucleated HQB with known implantation data.

Participants/materials, setting, methods: All the embryos came from patients'oocytes aged ≤35 years. Culture was in EmbryoscopeTM incubator with single culture medium (Life Global) to day+5. The number of nuclei per blastomere on day 2 and 3 was assessed, with multinucleation defined as more than 2 nuclei in at least 1 blastomere from the 3 cell stage onwards.

The Gardner (1999) score was used to ascertain good quality: expansion grade ≥3, internal cell mass ≥B and trophoectoderm ≥B.

Main results and the role of chance: 71 embryo transfers took place with a total of 78 MnE transferred, and an average of 1,1 embryo per transfer. The multinucleated HQB had a pregnancy rate of 47.8%, an implantation rate of 44.9%, and a miscarriage rate of 20.6%. The live birth rate was 33.8%.

The results refer to transfers of MnE with known implantation data.

Limitations, reasons for caution: If non-MnE were available, the MnE were not transferred. For this reason the number of cases analysed was low.

Wider implications of the findings: Blastocyst culture selects potentially viable embryos, allowing MnE to self-correct and then create healthy live births. Our results support this. We suggest that HQB created from the MnE of women ≤35 years of age should not be destroyed; if not transferred, they should be vitrified.

Trial registration number: Not applicable.

P-197 Retrospective comparative study between KIDScore Day 3 and KIDScore Day 5

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Study question: Is KIDScore Day 5 better than KIDScore Day 3 to predict the implantation outcome of an embryo?

Summary answer: KIDScore Day 5 has shown significant capacity to predict the embryo implantation outcome compared with KIDScore Day 3, whose capacity of prediction is not significant

What is known already: KIDScore Day 3 is an algorithm given by the embryo kinetics until day 3 widely used to predict the implantation potential of an embryo during a controlled ovarian stimulation cycle for IVF/ICSI treatment.

The new algorithm KIDScore Day 5 uses the same kinetics parameters until day 3 and adds new ones until day 5. Also includes a morphology quality assessment of the embryo on day 5 performed by an embryologist. This additional information could improve the capacity of the algorithm to predict the implantation outcome of the embryo.

Study design, size, duration: This comparative retrospective study was conducted at a university fertility centre over a period of 28 months from June 2014 to October 2016. The study involved 168 embryos with known implantation outcome transferred on day 5 or day 6 to women under IVF/ICSI treatment.

Participants/materials, setting, methods: 168 embryos were cultured in an 'Embryoscope", a time-lapse incubator. Embryo quality was assessed using the manufacturer's algorithm based on morphokinetic parameters to generate the KIDScore. For each embryo a KIDScore Day 3 was obtained, followed by a KIDScore Day 5, with subsequent transfer on day 5 or 6.

Statistical analysis was performed using SPSS. Multivariate and univariate logistic regressions were performed to determine the predictive capacity. The study was completed with an ROC curve.

Main results and the role of chance: Firstly a multivariate logistic regression was done to compare the predictive capacity of each KIDScore. Possible interactions between both variables were excluded, ensuring their independence. For the KIDScore D5, a statistically significant difference was shown in our logistic regression model (p-valor = 0,003) showing a capacity 35% higher than KIDScore D3 to evaluate the implantation potential of the embryos.

Subsequently, the univariate analysis for KIDScore D3 noted a lack of signification (p-valor = 0,412) for the evaluation of our embryos, whereas a statistically significant difference was shown with KIDScore D5 (p valor = 0,002) with an odds ratio of 1,355.

These results were also confirmed by a pertinent comparative ROC curve between KIDScore D3 and KIDScore D5 for the implantation outcomes. In an ROC analysis AUC (Area Under Curve) has been statistically significant for KIDScore D5 (AUC = 0,669; p-value < 0,001) but not-significant for KIDScore D3 (AUC = 0,570; p-value = 0,140).

KIDScore D5 showed better statistical results compared with KIDScore D3 for the implantation outcome. Therefore, we can conclude that it can be used as a better predictive indicator for viability of the embryo in the IVF/ICSI treatments.

Limitations, reasons for caution: The retrospective nature of the study introduces the inherent limitation of selection bias.

Further studies are needed to assess current outcome indicators.

Wider implications of the findings: This could help to select the best embryo to transfer, with a high implantation potential.

Trial registration number: Not applicable.

P-198 Meta-analysis on the effect of blastocyst collapse for human embryo vitrification

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Study question: It is currently unclear if a blastocyst collapse adds to the results of cryopreservation. Does a meta-analysis of existing literature support inclusion of this procedure?

Summary answer: A meta-analysis on predominantly retrospective studies shows improved rates on post-warming survival and clinical pregnancy when performing blastocyst collapse before vitrification.

What is known already: Overall, results from human blastocyst vitrification are good. During vitrification many embryos collapse spontaneously but not all. Induction of blastocyst collapse for vitrification is debated but used frequently.

When blastocysts do not collapse spontaneously during vitrification procedures, water in the blastocoel may not be replaced sufficiently by cryoprotectants. This possibly results in increased risks for ice formation, increasing the risk of cell damage and suboptimal survival.

Study design, size, duration: A literature search was performed using Medline to identify articles published before January 1, 2017 involving a comparison with or without induced blastocyst collapse on human blastocysts before vitrification. Articles meeting these criteria were assessed and studies including data on morphological survival and clinical pregnancy were considered eligible for inclusion.

Participants/materials, setting, methods: Studies including a comparison of vitrification with or without induced blastocyst collapse including results on morphological survival and clinical pregnancy were identified. The outcomes from vitrification with or without collapse were summarized and compared in a meta-analysis with results presented as relative risk (RR) applying the random effect model. Categorical variables were presented as percentages and compared using a 2×2 Chi-square test. The analyses were performed using MedCalc® 17.0.4 with p-values < 0.05 considered statistically significant.

Main results and the role of chance: Six articles were identified comparing the effect of vitrification with or without blastocyst collapse including results on survival and pregnancy rates. Only 1 of the 6 publications was a prospective

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randomised controlled trial (RCT). The meta-analysis involved 2761 and 1654 blastocysts with or without collapse respectively for the comparison of morphological survival and pregnancy was assessed on 1711 and 761 cases respectively. Survival rates were 97.4 % and 87.1 % ($p < 0.0001$) with or without collapse respectively (RR 1.143; CI 1.016–1.285; $p = 0.027$) and pregnancy rates were 50.0 % and 30.5 % ($p < 0.0001$) (RR 1.549; CI 1.273–1.884; $p = 0.001$) for the same groups. The prospective RCT observed a difference in survival favouring blastocyst collapse ($p = 0.007$) but did not show the anticipated increase in pregnancy rates, as obtained from their power calculation ($P > 0.05$).

Limitations, reasons for caution: It seems unlikely that post-warming survival from induced blastocyst collapse itself is affected by a retrospective analysis but other parameters, not controlled for in such an analysis can have an impact. Results on pregnancy outcome have to be taken with caution and should be controlled further in prospective RCTs.

Wider implications of the findings: Not all clinics obtain satisfactory success rates from blastocyst vitrification. Blastocyst collapse before vitrification seems to improve post-warming survival rates and can be considered when optimizing laboratory procedures. Increased post-warming survival may positively affect overall efficiency of an IVF treatment cycle, but this requires further confirmation from large prospective studies.

Trial registration number: not applicable.

P-199 Clinical outcomes of oocytes matured from Metaphase I to Metaphase II on the day of the egg retrieval in ICSI cycles

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Study question: To investigate the clinical outcomes of oocytes matured from Metaphase I (M I) to Metaphase II (M II) on the day of the egg retrieval in ICSI cycles.

Summary answer: Clinical pregnancy rates were not different between oocytes matured from MI to MII on the day of the egg retrieval and M II oocytes.

What is known already: In vitro maturation of MI oocytes for a short period of time may increase the number of available embryos, however, overnight in vitro culture of MI oocytes did not improve results.

Study design, size, duration: This was a retrospective study at a reproductive center. To evaluate the clinical outcomes between MI→MII and M II.

5,976 cycles of 3641 couples
from January, 2010 to December, 2015

Participants/materials, setting, methods: ICSI was performed to MI→MII oocytes and M II oocytes.Day3 good embryo rates, Day5 good blastocyst rates, clinical pregnancy rates, live birth rates and miscarriage rates were compared between two groups.Day3 good embryos included 7–10 cells with grade 1 or 2 by Veeck's criteria. Day5 good blastocyst included better than 3BB by Gardner's criteria.

Main results and the role of chance: The fertility rates were greater in MII group (82.3%: 11958/14531) than MI → MII group (52.0%:183/352) ($p < 0.05$). Day3 good embryo rates were greater in MII group (63.0%: 7457/11845) than MI → MII group (47.0%: 86/183) ($p < 0.05$). Day5 good blastocyst rates were greater in MII group (43.0%: 4816/11197) than MI → MII group (22.5%: 38/169) ($p < 0.05$). Clinical pregnancy rates were 39.0% (2244/5755) in MII group, and 30.3% (10/33) in MI → MII group (n.s). Live birth rates were 23.2% (1338/5755) in MII group, and 27.3% (9/33) in MI → MII group (n.s). Miscarriage rates were 28.2% (633/2244) in MII group, and 10.0% (1/10) in MI → MII group (n.s).

Limitations, reasons for caution: Confirmation of our preliminary data on a large population should be required.

Wider implications of the findings: Although fertility rates, Day3 good embryo rates and Day5 good blastocyst rates were significantly low in MI → MII group, clinical pregnancy rates and miscarriage rates were not different between two groups. Therefore, it was worth transferring blastocysts from MI → MII oocytes, if they developed to good ranked blastocysts.

Trial registration number: Not applicable.

P-200 Subsequent (day 3 and day 5) vitrification/warming does not adversely affect the clinical outcome: A retrospective cohort analysis of 556 cases

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Study question: This study is performed to evaluate the clinical **outcome** of re-vitrification of surplus blastocysts developed from frozen cleaved embrios and transfer at the blastocyst stage

Summary answer: Refreezing surplus blastocysts developed from vitrified/warmed cleavage-stage embryos does not adversely affect the clinical outcome comparing with once-frozen surplus blastocysts transfers in good responder patients.

What is known already: Many IVF clinics lead to use cryopreservation increasingly due to high survival and pregnancy rates after transfer of vitrified embryos. Embryos can be cryopreserved successfully at cleavage or blastocyst stage. There is a tendency to transfer embryos at the blastocyst-stage independently of cryopreservation stage of embryos especially in good responder patients. In many cases surplus embryos are available for re-freezing after transfer. Several studies have already shown good results with frozen embryo transfer cycles in comparison with fresh embryo transfers. However there is little data on re-cryopreservation of human blastocyst developed from vitrified/warmed cleavage-stage embryos.

Study design, size, duration: This retrospective cohort study includes 556 antagonist cycles performed between January 2013 and December 2016 in Bahceci Fulya IVF Centre. Good responder female patients with ≤38 years of age and male partners with normo-oligospermia were enrolled into the study. Exclusion criteria included poor response to controlled ovarian hyper stimulation, repeated implantation failure (≥3 IVF failure), pooling embryos ((≥2 oocyte pick-up cycles without embryo transfer), embryo biopsy for PGD and using testis sperm for ICSI.

Participants/materials, setting, methods: Cycles included in the study were grouped into three: Group I consisted of cycles in which transferred embryos were re-vitrified surplus blastocysts developed from frozen cleaved embryos; Group II included cycles in which surplus embryos were cryopreserved & warmed on day 5/ transferred on day 5; and Group III consisted of cycles in which embryos were transferred on day 5 as fresh, without any prior cryopreservation. Every patient involved in the study only once.

Main results and the role of chance: The differences in clinical outcome among three study groups were compared. Patient characteristics such as age, BMI and sperm parameters were comparable among groups ($p > 0.05$). There were no statistical significant differences among biochemical (BPR), clinical (CPR) and ongoing pregnancy rates in all groups ($p > 0.05$). BPR were similar in Group I (68.2%), Group II (66.5%) and Group III (59.5%) respectively ($p = 0.176$). Similar CPR were observed between Group I (62.9%), Group II (60.0%) and Group III (53.2%) respectively ($p = 0.151$). OPR were not significantly different in Group I (51.7%), Group II (48.0%) and Group III (41.0) respectively ($p = 0.116$). There were no statistical significant differences among biochemical abortion rates in all groups (16.5 %, 18.0% and 20.5% respectively; $P = 0,849$). Clinical abortion rates also were not significantly different in Group I (7.8%), Group II (9.8%) and Group III (10.7%).

Limitations, reasons for caution: The main limitation of our study was its retrospective nature, since numerous confounding factors that can affect the ART outcome may be overlooked. Also, cumulative pregnancy rates of cycles included in the study have not been taken into account in this analysis.

Wider implications of the findings: Our study indicates that subsequent (day 3 and day 5) vitrification/warming does not adversely affect the clinical outcome. Repeated cryopreservation can be done successfully by vitrification. However, well-designed additional studies with large sample sizes will be needed to draw a firm conclusion on the efficiency and impact of re-cryopreservation.

Trial registration number: None.

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P-201 The higher incidence of cleavage failure in oocytes with smooth endoplasmic reticulum clusters (sERC) than in oocytes without sERC in sERC positive cycles

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Study question: Are there any abnormal patterns of embryo development in oocytes with smooth endoplasmic reticulum clusters (sERC)?

Summary answer: Yes. A higher incidence of cleavage failure was seen in oocytes with sERC than in their cohort oocytes without sERC.

What is known already: In human embryos, several abnormal cleavage patterns, such as direct cleavage and reverse cleavage, can be observed during embryo development by means of time-lapse systems. sERC is one of the dysmorphic phenotypes which appears in human oocytes. Significantly reduced pregnancy rates and a comparatively higher number of abnormities in live-birth babies derived from sERC+ oocytes and / or sERC+ cycles have been reported. However, the opposite results have also been reported: showing that healthy babies, derived from sERC+ oocytes, can be born, without any reduced pregnancy rates. Thus, the clinical and scientific impact of sERC+ oocytes remains controversial.

Study design, size, duration: This was a retrospective observational cohort study, involving a total of 361 oocytes, obtained from 68 sERC+ cycles, which had at least one sERC+ oocyte among the retrieved oocytes, observed between January 2011 and December 2016. Unfertilized oocytes and embryos which were not captured by time-lapse observation up to day 4 were excluded from this study.

Participants/materials, setting, methods: Time lapse images were captured automatically every 15 mins, at 7 focal planes using an EmbryoScope®Time-lapse system (Vitrolife, Tokyo, Japan). Any changes in the configuration of the embryos observed by time lapse system were retrospectively analyzed. Statistical analyses were carried out to explore the nature of problems in embryos derived from sERC+ oocytes.

Main results and the role of chance: By careful observation, we have found that reverse cleavage has two different patterns. In one pattern, a cell cleaves into a number of cells greater than three cells. Then the cleaved cells reverse to a normal count of 2 cells or a reduced number of cells from the greatest number of cleaved cells. In the other pattern, a cell cleaves into 2 cells or more, but shortly after, the cleavage reverses to a single cell. The first pattern is considered to be reverse cleavage with completion of mitosis. On the other hand, the latter pattern is considered to be cleavage failure. This includes cells which have had an initiation of cleavage, but failed to cleave completely. In this study, we found that the incidence of mitotic cleavage failure in oocytes with sERC was 31.5% (29/92), which was significantly higher than that of 8.6% (24/279) in oocytes without sERC ($p < 0.0001$). Furthermore, the incidence of meiotic cleavage failure during the second polar body extrusion in oocytes with sERC was 9.8% (9/92), which was significantly higher than that of 2.5% (7/279) in oocytes without sERC ($p = 0.0073$).

Limitations, reasons for caution: Detecting cleavage failure is only possible up to and including the third mitosis due to the difficulty of detection during the fourth mitosis. The study of the incidence of cleavage failure among embryos in sERC negative cycles is under investigation following this study.

Wider implications of the findings: In the case of cleavage failure, a cell can theoretically be tetraploid and may cause abnormal chromosomal configurations. Even if cells with cleavage failure become trophectoderm cells, the embryo can be susceptible to further cleavage failure. For this reason, it is necessary to follow up babies derived from sERC+ oocytes.

Trial registration number: Not applicable.

P-202 Instability of osmotic pressure in microdrops cultured in non-humidified incubators

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Study question: Is the osmotic pressure of culture media stable when using a non-humidified incubator?

Summary answer: The osmotic pressure of microdrops covered with mineral oil was stable in humidified incubators, however, it increased significantly in non-humidified incubators on a daily basis.

What is known already: Humidified incubators are widely used for culturing human embryos. However, recently, non-humidified benchtop incubators have also been used due to their smaller size and lower risk of fungal contamination. Mineral oil is typically used to cover microdrops of culture medium in dishes so as to prevent changes in the osmotic pressure, pH, and temperature of the medium. Although the stability of pH and temperature in microdrops in non-humidified incubators has been verified, it is unclear whether osmotic pressure remains stable. In this study, we measured the osmotic pressure of different-sized microdrops in humidified and non-humidified incubators.

Study design, size, duration: Basic clinical study.

Participants/materials, setting, methods: We compared three incubators: (i) humidified benchtop, (ii) non-humidified benchtop, and (iii) humidified water jacket. Microdrops (50-µl, 100-µl, 200-µl) of a single step medium (A) (standard value, 265 ± 10 mOsm/kg) or a sequential medium (B) (standard value, 290 ± 10 mOsm/kg) were prepared in 35-mm culture dishes and then covered with mineral oil (Naka Medical Co. Japan). After one and two days' incubation, osmotic pressure was measured using an osmometer (Fiske 210®).

Main results and the role of chance: In the humidified benchtop and water jacket incubators, there were slight but non-significant increases in osmotic pressure of up to 4 mOsm/kg over 1 day in both media, regardless of microdrop volume. In contrast, there were significant increases in osmotic pressure in the non-humidified benchtop incubator. In 200-µl drops of medium A, the osmotic pressure increased from 267 to 274 ± 0.4 mOsm/kg after 2 days, while in medium B, it increased from 289 to 299 ± 0.5 mOsm/kg after 2 days. The increase in osmotic pressure of the 50-µl drops was more than in the 100-µl and 200-µl drops. In medium A, 50-µl drops increased from 267 to 283 ± 0.9 mOsm/kg after 2 days, while in medium B, the 50-µl drops increased from 289 to 310 ± 0.8 mOsm/kg after 2 days. In the 50-µl microdrops, the increases in osmotic pressure in the non-humidified benchtop incubator were significantly greater than in the humidified incubators ($P < 0.01$).

Limitations, reasons for caution: In this study, biological data was not collected, e.g. survival rate or growth rate of the embryos under each condition. However, it is generally obvious that large increases in osmotic pressure would be harmful to embryos.

Wider implications of the findings: Since different incubators and culture media may be used in different clinics, careful selection of the appropriate conditions for human embryos is very important for successful treatment at each clinic. Furthermore, it is necessary to describe the details of the culture environment in all publications to compare the data adequately.

Trial registration number: Not applicable.

P-203 Low lactate culture medium improves the development of human preimplantation embryos in vitro

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Study question: Does low lactate culture condition benefit human embryo development in vitro?

Summary answer: Continuous culture using low lactate medium improves in vitro human embryo development over higher lactate concentrations.

What is known already: Mammalian cleavage stage preimplantation embryos characteristically metabolize primarily pyruvate. Glucose consumption is low during the cleavage stage, and increases throughout compaction until becoming the blastocyst's primary metabolite. Lactate and pyruvate concentrations play critical roles in regulating pyruvate and glucose metabolism at different stages during embryo development. The lower concentration of lactate has been suggested to be beneficial for early stage of embryo development. Several groups including our own have demonstrated low lactate culture improved in vitro embryo development in mouse or bovine studies.

Study design, size, duration: A prospective split-case trial of media with low lactate or six times higher lactate was conducted using sibling-oocytes from total of 107 patients ages 23 to 40 at three different clinics. Embryos were

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cultured continuously in microdops overlaid with light mineral oil to blastocysts without media refreshment. Blastocyst development rates and transfer rates were determined after five days of culture. Pregnancy and ongoing pregnancy were monitored after transfer.

Participants/materials, setting, methods: Superovulation, oocyte collection and fertilization by conventional in vitro fertilization or intracytoplasmic sperm injection, embryos culture, freeze, and transfer to recipients were performed per the protocols of each clinic approved under IRB. 2PN zygotes from each patient were randomly allocated into media with low lactate or high concentrations of lactate and cultured under oil. Clinics reported blastocyst rates and transfer rates. Pregnancy and ongoing pregnancy rates were determined by blood test and ultrasound, respectively.

Main results and the role of chance: The blastocyst rate was normalized to the control for each patient at all three clinics collectively, where the high lactate control medium was set to 100%. The low lactate medium normalized average blastocyst rate was 120% over the high lactate control medium. Of all transfers made, 60% were from embryos cultured in the low lactate medium compared with 40% from the high lactate control medium. Of all the pregnancies, 60% were from embryos cultured in the low lactate medium compared with 40% from the high lactate control medium. Of all the ongoing pregnancies, 55% were from embryos cultured in the low lactate medium compared with 45% from the high lactate control medium.

Limitations, reasons for caution: These results suggest that culture media with lower lactate can benefit in vitro human embryo development. Additional studies are required to determine if the low lactate benefit is statistically significant.

Wider implications of the findings: The observed improvement from low lactate medium helps in the exploration and demonstration of how embryo culture media can be optimized to provide an ideal metabolic environment that is more suitable for human embryo development in vitro.

Trial registration number: IRB ID # 5454.

P-204 Clinical application of day 4 embryo biopsy for preimplantation genetic screening and diagnosis (PGS and PGD). A retrospective analysis of our current practice

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Study question: To evaluate the efficacy and outcome of day 4 embryo biopsy.

Summary answer: Embryo developmental potential, pregnancy result and overall cycle outcome suggest that day 4 biopsy can be an alternative stage at which to perform embryo biopsy.

What is known already: Embryo biopsy routinely takes place on day 3 (cleavage stage) or day 5-6 (blastocyst stage); with the latter preferred due to the ability of biopsying more cells, providing increased genetic material for analysis. A limitation of blastocyst biopsy is that several embryos capable of generating a pregnancy may not be biopsied if they fail to reach the blastocyst stage. In two recent studies, embryos biopsied on day 4 have shown no significant difference in embryo development and pregnancy rates compared to embryos that were not biopsied.

Study design, size, duration: A retrospective analysis was undertaken of patients having day 4 embryo biopsy for PGS and/or PGD at Westmead Fertility Centre between February 2014 and December 2017. The cause of PGS included implantation failure, history of recurrent miscarriages and advanced maternal age, whilst PGD was employed for specific gene defects. Average patient age was 37.3 years. A total of 104 PGS and/or PGD cycles were undertaken and 1214 day 4 embryos were biopsied.

Participants/materials, setting, methods: Participants were consented patients undergoing IVF/ICSI cycles combined with PGS and/or PGD. Following fertilisation, fresh or frozen-thawed embryos, cryo-preserved at the 2PN stage for banking purposes, were cultured to day 4. On day 4, all embryos suitable for biopsy were decompacted using Ca/Mg free medium and 2–6 cells were removed for analysis. The genetic analysis was performed on the same day and euploid/non affected embryo(s) were transferred on day 5.

Main results and the role of chance: Of the 1214 biopsied embryos, a total of 864 (71.1%) were at the compaction stage on day 4 with 653 (53.8%) reaching the blastocyst stage by day 5. Following the biopsy, 137 (11.3%) embryos had no amplification of their genetic material, with amplification failure correlated to poor embryo quality. A total of 297 out of the 1214 biopsied embryos (24.5%) had no abnormalities detected following PGS and/or PGD. From those, 102 (34.3%) were transferred on day 5, 138 (46.5%) were vitrified on day 5 and 57 (19.2%) were discarded due to poor quality. Out of the 240 embryos that were utilised, 117 (48.8%) were not expanded blastocysts at day 5 and would not have been biopsied if we were employing a blastocyst biopsy approach. From the 104 patients, 94 had at least one embryo with no abnormalities detected and an average of 1.1 embryos was transferred each time either on day 5 or in subsequent frozen cycles. A total of 61 pregnancies were recorded from 116 embryo transfers (52.6%) with 55 (47.4%) resulting in foetal heart at the 7 week scan. So far 19 live births have been reported with no malformations recorded and within the normal birthweight range.

Limitations, reasons for caution: The results represent the experience gained from current practice at Westmead Fertility Centre and not of a prospective controlled study. No embryos were cultured to day 6, therefore the developmental potential of certain embryos classified as not expanded blastocysts at day 5 of embryo development is unknown.

Wider implications of the findings: Day 4 embryo biopsy provides screening results in a timely fashion, allowing for a day 5 transfer within the same treatment cycle. The combination of this option with embryo developmental potential and pregnancy outcomes, provides an alternative approach to current embryo biopsy practices.

Trial registration number: N/A.

P-205 Morphokinetics in the early cleavage stage predicts formation and quality of the blastocyst stage

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Study question: The aim of our study was to compare whether the morphokinetic data obtained until day 2 could predict the formation and quality of the blastocyst.

Summary answer: The morphokinetic parameters of day 2 embryos are sufficient to predict both blastocyst formation and quality.

What is known already: It has been reported that significant differences in cleavage patterns and morphokinetic parameters between embryos that reached the blastocyst stage and those which arrested their development before the blastocyst stage or which lead to a poor quality blastocyst. Embryos cleaving earlier have a significantly higher chance of achieving optimal blastocyst stage (Meseguer et al, 2011). The blastocyst formation was found to correlate with cc2 (time between division to 2 cells and division to 3 cells), s2 (time between division to 3 cells and subsequent division to 4 cells) and with the duration of the first cytokinesis (Wong, 2010).

Study design, size, duration: This retrospective study performed on 524 patients between August 2013 and September 2016. Embryos were cultured in time-lapse incubator (EmbryoScope™) and blastocysts were scored according to Gardner's classification.

Participants/materials, setting, methods: This study was performed in 586 cycles (n = 4808) that had undergone ICSI treatment in an unselected population. All embryos were cultured after ICSI insemination and assessed in EmbryoScope™ until day 6 and annotated for the pattern time of cleavage. Statistical analysis was performed with SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). Student's t-test was used for statistical analyses. Average hour ± SD from ICSI insemination are reported for all stages.

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Main results and the role of chance: We analyzed 8 parameters and abnormal cleavage events correlated with subsequent blastocyst formation and quality. The mean timings of the tPB2, tPNa, tPNf, t2, t3, and t4 for the embryos those developed into blastocysts were statistically significantly faster than the arrested embryos (respectively; $P < 0.001$). Moreover, the cell cycle of cc2 and synchrony of the cell cycle of s2 were also significantly different (respectively; $P < 0.001$). We compared abnormal cleavage events in embryonic development. There was no difference in the rate of multinucleation at the 2-cell stage (46.1% vs. 49.0%, $P = 0.092$). However, the uneven blastomere size at the 2-cell stage (15.2% vs. 34.2%, $P < 0.001$) and the direct cleavage from 1 to 3-cell (16.5% vs. 40.0%, $P < 0.001$) showed statistically significantly lower rates. We also examined whether the kinetic parameters of early embryos affect the quality of blastocyst. This study showed that cc2, s2 and t2 have the greatest effect on the blastocysts quality (respectively; $P < 0.001$). The quality of the blastocysts was also significantly affected by abnormal cleavage events.

Limitations, reasons for caution: The study was monocentric and retrospective.

Wider implications of the findings: This study is the first observation of a total analysis about the formation and quality of blastocysts using kinetic parameters and abnormal cleavage events collected until day 2. In particular, abnormal cleavage events have a considerable effect on the formation and quality of blastocysts.

Trial registration number: We don't need to trial registration number.

P-206 Are there any morphokinetic difference between euploid embryos those resulted with live birth and miscarriage?

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Study question: What are the morphokinetic factors affecting the miscarriage after euploid embryo transfer?

Summary answer: The T2, blastulation and hatching time of the embryos those resulted with live birth and miscarriage were different.

What is known already: Time-lapse monitoring of embryo development may represent a superior way to culture the embryos and it seems to be a new tool for selecting the embryos within the highest implantation potential. The morphokinetic behavior of the euploid and aneuploid embryos seems to be different.

Study design, size, duration: This retrospective analysis compiled 263 controlled ovarian stimulation(COH) cycles of 247 patients with a blastocyst biopsy in which comprehensive genomic hybridization(CGH) was performed and embryos were monitored with time-lapse technology, from January 2014 to November 2015. Clinical indications for CCS based trophectoderm biopsy were advanced maternal age and reccurent implantation failure. Only euploid embryos resulted with clinical pregnancy were enrolled in the study.

Participants/materials, setting, methods: All patients underwent COH with the GnRH antagonist and recombinant FSH or hMG. Endometrial preparation was done by artificial hormone replacement treatment for ET. Single euploid embryos were transferred in frozen ET cycles.

Main results and the role of chance: The 135 embryos resulted with clinical pregnancy was analysed. Of these 99 embryos transferred were resulted with live birth and 36 embryos were resulted with miscarriage. There were no statistical differences between live birth group and miscarriage group regarding age (31,1 ± 3,6 and 30,4 ± 2,3 years, respectively), female BMI (22,6 ± 3,9 kg/m^2 and 22,9 ± 4 kg/m^2 respectively), antral follicle count (12,6 ± 6,3 and 13,3 ± 5,9 respectively), previous IVF attempt (2,8 ± 1,7 and 3,2 ± 3,1 respectively), day of stimulation (9.5 ± 0.73 and 9.7 ± 0.65 days, respectively), peak E2 (1919 ± 1172 and 2132 ± 1140 pg/ml, respectively), and peak P4 (0.88 ± 0.53 and 0.92 ± 0.49 ng/ml, respectively). The two groups also were similar in the number of oocyte retrieved, and the number of injected MII oocytes. Only T2 time was statistically different between two groups (25,4 ± 2,6 and 23,4–33,2 hours respectively) No difference was found in the synchrony parameters. Blastulation and hatching time of the embryos were different between two groups (92,8 ± 8,1; 101,4 ± 8,6 and 112,8 ± 5,4; 106,8 ± 7,6 respectively p: < 0,001 and p: 0,003).

Limitations, reasons for caution: The study is limited by its retrospective nature. A higher sample size or a prospective design could be used in future studies to corroborate the current findings.

Wider implications of the findings: Our results suggest that morphokinetic parameters have difference in euploid embryos within live birth and miscarriage.

Trial registration number: None.

P-207 Poor quality embryos may interfere with implantation when transferred together with good quality embryos

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Study question: Do poor quality embryos impede implantation when transferred together with good quality embryos?

Summary answer: It seems to be that embryo of poor quality can disrupt implantation of the embryos of good quality in human.

What is known already: Interactions between the competent embryo and maternal endometrium are essential for successful implantation of embyos. In human co-culture model, it was reported that human endometrial stromal cells are biosensors of embryo quality upon differentiation into decidual cells. However, it was not known whether poor quality embryos transferred with good quality embryos interfere with implantation of good quality embryos.

Study design, size, duration: This study was performed from January 2014 to December in our fertility center. In this study, we analyzed the clinical outcomes of 649 cycles transferring 1 or 2 vitrified-warmed blastocyst. The quality of blastocyst was classified into good or poor depending on the cell number of inner cell mass and trophectoderm.

Participants/materials, setting, methods: Cryopreservation was performed using vitrification protocols at blastocyst stage on day 5 of 6 after oocyte retrieval. The day before transfer, warmed blastocysts were cultured in blastocyst medium. One or two blastocysts were transferred using ultrasound-guide. Clinical pregnancy and implantation outcomes were compared between those groups.

Main results and the role of chance: The clinical pregnancy rate of double good blastocysts group was significantly higher than that of mixed quality blastocysts group (64.4% vs. 51.7%, $p = 0.004$); however, there was no significant difference between mixed quality group and single good quality group (51.7% vs. 56.6%, $p = 0.37$). Implantation rate of mixed quality group was significantly lower than double good quality group (36.7% vs. 46.4%, $p = 0.002$) and single good quality group (36.7% vs. 58.1%, $p < 0.001$). This result shows that the poor embryo may interfere with the implantation of the good embryo.

Limitations, reasons for caution: It was reported that endometrium cells in patients experienced recurrent loss fail to distinguish poor embryos. Therefore, it is necessary to confirm the clinical result according to the indication.

Wider implications of the findings: Our study showed that poor quality embryos could interfere with implantation of good quality embryos. Therefore, when only one good quality embryo and the other poor quality embryos were present, transfer of only one good quality embryo seems to be effective in prevention of multiple pregnancy and clinical outcome.

Trial registration number: NA.

P-208 The impact of blastocyst development stage at the time of embryo transfer (ET) on serum human chorionic gonadotropin (HCG) level

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Study question: Does the development stage of the blastocyst on ET day affect the serum human chorionic gonadotropin (HCG) level 9 days later?

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Summary answer: Faster growing embryos produced higher serum HCG level.

What is known already: HCG is secreted by the trophectoderm (TE) of the embryo. Following ET, serum HCG level in women who conceive is affected by a number of factors. Several studies suggested that the HCG levels in frozen ET cycles were higher than that in fresh ET cycles. The HCG levels after the transfer of day-3 or day-5 embryo were similar. It is not known if the transfer of faster growing embryos produces higher serum HCG level than that of slower growing embryos.

Study design, size, duration: The retrospective study involved 283 cycles after single blastocyst transfer in a teaching hospital IVF clinic from January 2015 to July 2016.

Participants/materials, setting, methods: Women who conceived after IVF following the transfer of a single blastocyst were included. The blastocyst was divided into four different stages of development: cavitating, full blastocyst, expanded blastocyst and hatching blastocyst. As part of routine clinical practice, a blood sample was obtained 9 days after blastocyst transfer for HCG measurement to verify if pregnancy has occurred.

Main results and the role of chance: Overall, the median serum hCG level 9 days after a single blastocyst transfer in women who conceived a singleton ongoing intrauterine pregnancy was 177.2 IU/L. The corresponding median HCG levels in the 4 subgroups were: I, cavitating: 98.0 IU/L; II, full blastocyst: 159.0 IU/L; III, expanded blastocyst: 182.5 IU/L; and IV, hatching blastocyst: 201.1 IU/L. There was a significant trend towards higher hCG levels as the stage of blastocyst development on the day of ET become more advanced. The HCG level in subgroup I was significantly ($p < 0.05$) lower than that of group IV, but not significantly different from groups II or III. To conclude, faster growing embryos appeared to produce higher hCG levels 9 days after transfer.

Limitations, reasons for caution: This is a retrospective study; the findings should be considered preliminary pending confirmation from a properly powered prospective study.

Wider implications of the findings: Our data showed that, among embryos that successfully implanted, faster growing ones appeared to be associated with higher hCG levels 9 days after the transfer.

Trial registration number: not required, as the data were collected as part of routine clinical practice.

P-209 Faster and more accurate improved rescue ICSI by using polarization microscope (PLM) and differential contrast microscope (DCM)

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Study question: Is faster and more accurate improved rescue ICSI by observing changing size and position of chromosome and spindle using polarization microscope (PLM) and differential contrast microscope (DCM) possible?

Summary answer: The early and accurate identification of fertilization with DCM and PLM is beneficial for rescue unfertilized oocytes following conventional IVF.

What is known already: Unfertilization after conventional IVF is confirmed by the existence of Metaphase-II (M-II) chromosome and first polar body. Spindle examination in unfertilized eggs using the polarization microscope is useful to assist rescue ICSI. However it has limitations since this apparatus observes only the existence of spindle not the stage of nuclear maturation (M-II, Anaphase-II or Telophase-II). Nomarski differential interference contrast microscopy clarifies each stage of second meiotic cell division.

Study design, size, duration: We performed this retrospective study to investigate the usefulness of chromosomal examination with a differential interference contrast microscope to rescue unfertilized eggs after insemination without using the polarization microscope in 425 M-II oocytes after insemination from 38 couples from January 2015 to December 2016.

Participants/materials, setting, methods: Unfertilized oocytes were identified by the normal width of chromosomes at M-II stage without extrusion of second polar body or white colored spindle with full length adjacent to the cytoplasmic membrane. Fertilized oocytes showed half width of chromosome in a line and shortening spindle 2-3 hours after the start of the fertilization (Anaphase-II).

Main results and the role of chance:

1. The 425 freshly ovulated M-II oocytes were examined. The proportion of unfertilized oocytes that remained at M-II stage was 20.4% (87/425), fertilized oocytes after completion of second meiosis was 79.5% (338/425).

2. The fertilization, cleavage, blastocyst stage rates, pregnancy and miscarriage rates in fertilized oocytes (A) and unfertilized oocytes followed by new rescue ICSI (B) are listed in Table. No 1PN or 3PN oocytes could be observed both in group A and B.

Table Clinical outcome following new rescue ICSI.

	Fertilization rates (%)	Cleavage rates (%)	Blastocyst rates (%)	Pregnancy rates (%)	Miscarriage rates (%)
A(n = 328)	78.5	64.5	45.3	33.4	20.5
B(n = 87)	52.4	62.3	33.2	25.5	25.6

Limitations, reasons for caution: Not all of M-II oocytes could be identified as having completed second meiosis after insemination. Some chromosomes were scattered or degenerated. If spermatozoon penetrated the not fully activated oocyte it showed premature chromosome condensation. These chromosomes are more easily identified with a PLM.

Wider implications of the findings: The identification of nuclear maturation with DCM and PLM beneficial not only for rescue ICSI but also for other procedures that need to identify the nuclear maturation division. Particularly, this procedure could become indispensable in cytoplasmic exchange for the treatment of mitochondrial disease.

Trial registration number: None.

P-210 Successful and safe cryopreservation of low sperm number

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Study question: Can small numbers of spermatozoa be successfully cryopreserved for later use without risk of cross-contamination?

Summary answer: Using the commercial device SpermVD and CBS High-Security tubes, small numbers of spermatozoa can be successfully frozen, without the risk of cross contamination.

What is known already: In cases with low sperm numbers, e.g. extreme oligozoospermia or testicular sperm of non-obstructive azoospermic patients, the standard slow freezing procedure for human sperm remains suboptimal. A methodology that allows the successful freezing of low numbers of individually isolated sperm will provide benefits for the management of male infertility. To date, few attempts have been reported using empty zonae or adapted devices with various recovery and motility rates. Nevertheless, none of these excludes the risk of cross-contamination when stored in liquid nitrogen (LN2).

Study design, size, duration: A new commercial device (SpermVD, MFC Global ltd.) was used as sperm carrier, and stored in two different types of sterile vials: Cryotubes with a screwcap (Thermo-Scientific; closed group) and heat-sealed CBS High-Security tubes (Cryo Bio System; sealed group). The recovery and motility rates were investigated. In cases of immotile spermatozoa, the Hypo-Osmotic-Swelling (HOS) test was performed to indicate sperm viability. The aspiration/deposition of sperm was performed with a Narishige micromanipulator.

Participants/materials, setting, methods: Motile spermatozoa were individually plated in 0.5 µl cryopreservation solution (50:50/Sperm-freezing solution:G-MOPS, Vitrolife) on the 3 wells of the SpermVD device. After 15 min at room temperature (RT), each device was placed inside a vial, closed or sealed and submerged in LN2. For thawing, the vials were removed from LN2, and after 5 min at RT, the spermatozoa were plated in G-MOPS medium. Recovery

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and viability rates were compared (χ^2-test) between the closed and sealed groups.

Main results and the role of chance: A total of 809 spermatozoa were plated on SpermVD devices (n = 20), of which 394 spermatozoa were frozen in closed (n = 10) and 415 spermatozoa were frozen in sealed (n = 10) vials. After thawing, a significantly higher sperm number was recovered from the closed devices (365/394, 92.6% vs 353/415, 85.1%; p = 0.0007). However, a significantly higher motility rate was found in the sealed group as compared to closed group (43/353, 12.2% vs 8/365, 2.2%; p < 0.0002). After performing the HOS test on the immotile spermatozoa upon thawing, the total number of viable sperm (motile + HOS-positive sperm) was still higher in the sealed group (90/353, 25.5% vs 47/365, 12.9%; p < 0.0002). In conclusion, although the recovery rate was higher in the closed vials, the sealed vials performed better in preserving the sperm viability during the freezing/thawing procedure. Taking into account that the sealed vials do not allow cross-contamination in LN2, the combination of the SpermVD as sperm carrier with the CBS High-Security tubes as freezing vials, provides a safe solution for cryopreserving individual spermatozoa in cases with few sperm available.

Limitations, reasons for caution: This study represents the first step in validating the combination between SpermVD and CBS High-Security tubes as a suitable tool for routine cryopreservation of low numbers of spermatozoa. Before implementation in routine clinical practice, the embryological and clinical outcome of the embryos obtained after using this methodology should be evaluated.

Wider implications of the findings: Effective methods to safely cryopreserve single spermatozoa would allow the patients with very low sperm numbers to benefit from multiple ICSI treatments avoiding repetitive surgeries and cancellation of cycles due to the lack of sperm.

Trial registration number: not applicable.

P-211 Clinical outcomes using human euploid embryos after quantification of mitochondrial DNA content as a viability predictor tool

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Study question: Does transfer of euploid embryos with lower mitochondrial DNA (mtDNA) copy number improve the clinical outcomes in elective single embryo transfers (eSETs)?

Summary answer: Transferring of euploid embryos with lower mitochondrial DNA (mtDNA) copy number does not show a statistical improvement in clinical outcomes in eSETs

What is known already: The mtDNA copy number can be quantified in human euploid embryos during pre-implantation genetic screening (PGS) using real-time PCR to predict the viability of the embryos. Based on the results, a transfer priority list can be prepared, where the priority of euploid embryo(s) getting transferred is inversely proportional to the mtDNA copy number present.

Study design, size, duration: This was a retrospective, cohort, pilot study including 45 patients who underwent PGS between the months of March and December 2016 at NOVA IVI Fertility, Ahmedabad, India. 4 patients with Day-3 biopsy, 3 with pending embryo transfers (ETs) and 10 with double ETs were excluded from the study. 28 patients who underwent Day-5/6 trophectoderm biopsy for PGS followed by eSETs in subsequent frozen-thawed cycle were included in the study.

Participants/materials, setting, methods: Post – biopsy, blastocysts (*n* = 143) from patients (*n* = 28) with indications such as advanced maternal age, recurrent implantation failure and recurrent pregnancy losses were vitrified individually on carriers. Euploid embryos with least mtDNA content were then thawed for eSETs and transferred in frozen-thawed cycles. For the study, 4 groups (upto 20, upto 30, upto 40 and > 40) were made based on the mitoscore (MS) values calculated by Igenomics

Main results and the role of chance: The total number of blastocysts biopsied for 45 patients were 202, out of which 121 were found to be euploid (59.9%). To evaluate the efficacy of MS the transferred embryos were divided into four groups viz upto 20, upto 30, upto 40 and > 40. The pregnancy rates (PR) for these groups were 50.0%, 43.8%, 100.0% and 0 % respectively; while the implantation rates (IR) were 16.6%; 37.5%, 33.3% and 0 % respectively.

The present study shows no significant difference in pregnancy rates (PR) on transferring embryos with proper morphology and least MS values as compared to the group with an MS of upto 40 (PR: 50.0 versus 100.0%, *P* > 0.5; MS upto 20 versus upto 40 group). The mean age (± SD) of female patients undergoing the treatment was 34.35 ± 3.05 years.

Limitations, reasons for caution: Given the retrospective nature of the study, limited information was available. A prospective, multi-centre study with a large sample size can be designed to look into the predictive value of MS. Results from Day-3 blastomere biopsy should also be analyzed.

Wider implications of the findings: Along with traditional methods of embryo selection, novel methods that are non-invasive and cost-effective but have higher predictive value for embryo selection should be developed. This would help in escalating the live birth rates in ART, especially in eSETs and would thus help in decreasing the risk of multiple pregnancies

Trial registration number: not applicable.

P-212 The dynamics of in vitro maturation (IVM) and their optimal ICSI timing of germinal vesicle oocytes retrieved from IVM cycles

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Study question: What is the most favorable ICSI timing after the first polar body (1st PB) extrusion in relation to the fertilization and embryo development?

Summary answer: The optimal ICSI timing of the IVM oocytes is 3–6 hours after the polar body extrusion in terms of fertilization and embryo developmental competence.

What is known already: IVM has been less successful than standard IVF in terms of embryo development and clinical outcomes. It has been known that it's because of asynchronous nuclear and cytoplasmic maturation. Although a study has reported the dynamics of IVM of GV oocytes obtained from IVF cycles using time-lapse system (TLS), to date there is no report of dynamics of maturation of the GV oocytes retrieved from regular IVM cycles. In addition, what has not been reported is the optimal ICSI timing after the 1st PB extrusion using the TLS equipment.

Study design, size, duration: The maturation timing process from GV-stage oocytes (n = 162) was recorded following culture in the TLS equipment. Intra Cytoplasmic Sperm Injection (ICSI) was performed at different times after the 1st PB extrusion (n = 100). Fertilization and embryo utilization rates were analyzed to see the optimal ICSI timing after the 1st PB extrusion.

Participants/materials, setting, methods: The oocytes were obtained from PCO(S) patients undergoing FSH/hCG-primed IVM cycles. After removing most of the cumulus cells, GV-stage oocytes were cultured for maximum 30 hours in the TLS. The elapsed time between the 1st PB extrusion and ICSI was recorded. The data was divided into 3 groups depending on ICSI timing after the 1st PB extrusion (< 3 h, 3–6 h and > 6 h). Fertilization and embryo utilization rates were analyzed with ANOVA test.

Main results and the role of chance: Overall maturation rate in vitro was 61.7 % (100/162). The nuclear maturation rate was 0 %, 3.0 %, 40.0 %, 60.5 % and 61.7 % after culturing for 6 h, 12 h, 18 h, 24 h and 30 h respectively. The average time from GV- to Germinal vesicle breakdown (GVBD)-stage was 3.3 hours (± 2.3, range: 0.5–9.3 hours) and GVBD- to MII-stage was 12. 5 hours (± 1.5, range: 7.7–15.6 hours) after beginning culture in IVM media. The fertilization rate of the group who had ICSI 3–6 hours after the 1st PB extrusion was higher (83.1%) than those of other groups (< 3 h: 40%, > 6 h: 64 %). The lower number of good quality morula/blastocyst embryos (0%) developed from the group where ICSI was performed < 3 h after the 1st PB extrusion compared to other groups. There was no difference of embryo utilization rate between 3–6 h and > 6 h groups (47.1% vs. 56.3%).

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Limitations, reasons for caution: Most of CC at GV-stages were removed to see the first PB extrusion in TLS system, which may act differently than oocytes with intact CC. As this was pilot study, the number of oocytes in the study is small.

Wider implications of the findings: This is the first observation describing extensively the IVM timing process of GV-stage oocytes derived from IVM cycles using TLS and suggesting the optimal ICSI timing to improve fertilization and further embryo development. This study may provide essential information for improving laboratory process for human IVM program.

Trial registration number: not applicable.

P-213 Outcomes of cleavage-stage versus blastocyst transfers in patients with low number of fertilized oocytes in two subsequent cycles

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Study question: What is the more successful transfer strategy to adopt in patients with a low number of fertilized oocytes: cleavage-stage or blastocyst transfer?

Summary answer: Ongoing pregnancy rates (OPR) are nearly doubled in patients with blastocyst transfers, either in the first or second ICSI cycle.

What is known already: Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology has been covered by a *Cochrane* review (Glujovsky *et al.*, 2012). These authors provided evidence for a slight benefit in live birth rates of blastocyst transfers, but looking at cumulative pregnancy rates, day 2/3 transfers were more efficient. However, the use of blastocyst culture as a standard treatment option for all patients and especially for poor prognosis patients is still under discussion. As being characterized by a low ovarian reserve, poor prognosis patients may be at risk of being harmed by prolonged embryo culture and having their transfer cancelled.

Study design, size, duration: This retrospective study comprised a period between August 2011 and December 2016. Patients with 1-2 fertilized oocytes and no embryo to vitrify were either transferred on day 2/3 (n = 1418) or on day 5/6 (n = 186) in their first cycle. For their second attempt, patients who received previously a cleavage stage embryo transfer were either transferred on day 2/3 (n = 188) or on day 5/6 (n = 151). Embryos were cultured in low oxygen tension using a single-step culture medium.

Participants/materials, setting, methods: The mean age of patients receiving a cleavage-stage embryo transfer was 36.92 ± 5.46 for the first and 38.28 ± 4.72 for the second cycle. The mean age of patients having a blastocyst stage embryo transfer was 35.52 ± 5.19 for the first and 35.8 ± 4.9 for the second attempt. After a failed cleavage-stage embryo transfer, in the second cycle, the mean number of fertilized oocytes was 1.87 ± 0.98 for cleavage-stage and 4.05 ± 2.64 for blastocyst transfers.

Main results and the role of chance: The OPR was nearly doubled in blastocyst transfers when compared to cleavage-stage transfers, either for the first (33.15% vs. 18.05%; $p < 0.0001$) or for the second cycle (32.45% vs. 18.61%; $p = 0.0034$). The clinical miscarriage rate were higher in cleavage-stage transfers (23.12% and 25.5%, for the first and second cycle, respectively), when compared to blastocyst transfers (18.91% and 16.95%, for the first and second cycle, respectively), although not reaching statistical significance. The cancellation rate between day 3 and 5 was of 39.8% for the first attempt (n = 123) and of 8.48% for the second attempt (n = 14), indicating that prolonging embryo culture two more days allows the selection of poor prognosis patients who will ultimately fail to achieve the blastocyst stage. Moreover, the number of fertilized oocytes doubled in a subset of patients having a blastocyst-stage transfer in their second cycle, when compared to those receiving a second cleavage-stage transfer, showing that a failure after a cleavage-stage embryo transfer does not reduce chances of achieving the blastocyst stage in subsequent cycles for a younger group of poor prognosis patients.

Limitations, reasons for caution: Inclusion criteria favored a group of poor prognosis patients with reduced outcomes. Results were obtained in an IVF program aiming prolonged culture and blastocyst transfer to select viable embryos.

Wider implications of the findings: Blastocyst stage transfers are more successful than cleavage-stage transfers in our cohort of poor prognosis patients. However, transfer cancellation rates are undeniably high when aiming for blastocyst culture, which may be regarded as nonviable pregnancies. The effect of prolonged culture needs to be addressed in prospective randomized trials.

Trial registration number: Not applicable.

P-214 Clinical validation of extend algorithm from embryo viability assessment system; a blastocyst analysis

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Study question: Is there a correlation between the automatic classification provided on Day3 by the Eeva-Xtend algorithm (Early embryo viability assessment) with blastocyst rates and quality?Is this model useful for embryo selection?

Summary answer: There is a direct correlation according to the automatic test for blastocyst formation and quality.This test is potentially useful to distinguish embryos with similar morphology.

What is known already: Wong et al(2010) developed an algorithm to classify embryos according to their probability of reaching blastocyst stage based on the combination of: P2(t3-t2) and P3 (t4-t3). Using these two parameters, a first algorithm was developed classifying embryos according to their probability of reaching blastocyst stage as High or Low, with an improved version that included a Medium category. Different groups have demonstrated the utility of this classification as an improvement for embryo selection and reduction in variability among embryologists(VerMilyea et al 2014,Conaghan et al 2013,B. Aparicio-Ruiz et al 2016).Recently an improved version of the algorithm (Xtend) has been up-graded on Eeva-system

Study design, size, duration: Retrospective cohort study, from June 2016 to January 2017.

Participants/materials, setting, methods: University-affiliated infertility clinic. An improved version of this algorithm was developed taking into account new parameters to select embryos. The values of P2 and P3 are complemented with egg age, number of cells on day3 and a texture image analysis that is correlated with fragmentation.The study includes 96 consecutive ICSI cycles from the egg donation program in which embryos were incubated in a conventional incubator with especially designed scopes that used an automatic cell-tracking software.

Main results and the role of chance: A total of 96 patients generated 776 embryos. Out of those, 68.4% developed to the blastocyst stage. When categorizing according to EEVA: 19.7% embryos were labelled as 1, 17.9% were labelled as 2, 16.7% were labelled as 3, 23.7 % were labelled as 4 and 22.0% were labelled as 5. The blastocyst formation rate according to the five EEVA categories was: 1 = 89.1%; 2 = 82.4%; 3 = 80.3%; 4 = 61.3%: 5 = 40.9 % $p < 0.0001$. Development to "good morphology" blastocyst (defined by A /B inner cell mass and trophectoderm morphology) from total embryos was also analysed with decreasing percentages as we moved on between categories. More specifically: 1 = 55.9%; 2 = 52%; 3 = 40%; 4 = 25.6%; 5 = 13.2% $p < 0.001$.

Limitations, reasons for caution: The retrospective nature of this study may be a reason for caution; nevertheless, we are presenting the first results of this new algorithm. The classification system itself has some errors due to difficulties in cell tracking, generating "none result", however we only included cases where a classification was provided.

Wider implications of the findings: Our study demonstrates that embryo selection by an automated time-lapse system supported by the use of a new algorithm is related with higher probability not only of reaching blastocyst stage but of developing into good quality blastocysts. Our aim is to keep increasing the sample size to consolidate our hypothesis.

Trial registration number: 1406-VLC-045-BA

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P-215 Patient over 40 can be treated more effectively with conventional IVF compared to ICSI in many of the cases

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Study question: Is increased female age really an indication of intracytoplasmic sperm injection (ICSI) or can be conventional in vitro fertilization (IVF) applied effectively?

Summary answer: Increased female age does not seem to be an indication of ICSI.

What is known already: Although ICSI was originally developed to treat severe male factor infertility, nowadays, it is commonly used in many other cases such as poor responders and advanced patient age. According to the literature, fertilization rate following ICSI is lower compared to conventional IVF. ICSI may have more potential risks too. There seems to be an increase in de novo sex and autosomal chromosome aberrations after ICSI. Increased risk of imprinting disorders in ICSI children has also been reported. However, in Europe ICSI is used in about 70% of the cases, far more than what the frequency of the male factor explains.

Study design, size, duration: Data of 559 IVF cycles performed at our division between 2011 and 2016 were retrospectively analyzed. Cycles were only included in the study if patient age exceeded 40, and ejaculated semen was used for the fertilization. Oocytes were fertilized by ICSI in 465 cases and by conventional IVF in 94 cases.

Participants/materials, setting, methods: Cycles were classified into two groups according to the fertilization method (IVF and ICSI Groups). In order to be able to compare fertilization rates, it was calculated as the number of retrieved oocytes divided by the number of normally fertilized ones (2PN). Clinical pregnancy rate and embryology data were also assessed. The formers were analyzed using Pearson's Chi-squared test, while cell numbers, fragmentation rates and morphology scores were compared using Mann–Whitney U test.

Main results and the role of chance: Patient age was lower (41.4 ± 1.6 vs. 42.4 ± 1.9; $p < 0.001$), total oocyte count was higher (9.7 ± 3.7 vs. 5.0 ± 3.8; $p < 0.01$) in the IVF group. Fertilization rate was significantly higher following conventional IVF compared to ICSI (63.7% vs. 56.0%; $p < 0.01$). Quality of the developing embryos did not show any difference on Day 2 and 3. No differences were found between neither the numbers of blastomeres (Day 2: 3.7 ± 1.4 vs. 3.8 ± 1.4; $p = 0.410$; Day 3: 6.5 ± 2.2 vs. 6.8 ± 2.3; $p = 0.128$) nor fragmentation rates (Day 2: 15.1 ± 10.7% vs. 15.4 ± 10.5%; $p = 0.539$; Day 3: 16.1 ± 11.8% vs. 15.6 ± 10.7%; $p = 0.648$). Morphology scores were also similar (Day 2: 2.1 ± 0.9 vs. 2.1 ± 0.8; $p = 0.954$; Day 3: 2.1 ± 0.8 vs. 2.2 ± 0.7; $p = 0.084$). Multinucleated embryos occurred in the same proportion (18.9% vs. 16.2%; $p = 0.144$). Clinical pregnancy rate however, was significantly higher following conventional IVF (39.1% vs. 17.6%; $p < 0.01$).

Limitations, reasons for caution: Differences in oocyte count and age affect the outcomes, however the latter should not explain the higher fertilization rate following IVF. These could be eliminated by the matching of the cycles, but that would have cause an even more significant bias: the difference in the quality of the sperm samples.

Wider implications of the findings: ICSI should not be chosen evidently as the method of fertilization in case of patients over 40. However, we have to stress the strong need of prospective randomized trials to confirm this finding and to clarify the evident indications of ICSI once and for all.

Trial registration number: -.

P-216 No benefit of transferring more than one embryo even without using preimplantation genetic screening

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Study question: Does double embryo transfer (DET) result in increased clinical pregnancy rate compared to elective single embryo transfer (eSET) when embryo selection is solely based on morphology?

Summary answer: It does not seem to have any benefit of transferring more than one embryo even when preimplantation genetic screening (PGS) is not available.

What is known already: There is a growing body of evidence that elective single embryo transfer (eSET) does not reduces the chance of pregnancy when embryo selection is supported with preimplantation genetic screening. Alhtough, eSET is widely promoted by scientific societies and health care professionals because of the associated adverse events of twin pregnancies, the main concern expressed by patients is still the potential decrease of chance due to the reduced number of embryos transferred. Here we analyze data from four consecutive years evaluate the efficiency of eSET in fresh and frozen cycles without using PGS for embryo selection.

Study design, size, duration: Data from all stimulated eSET (having at least two embryo available for ET) and double embryo transfer (two embryos were transferred) cycles were retrospectively analyzed between 2013 and 2016. Cycles with donor oocytes, day 3 ET and PGS were excluded. Also, frozen embryo transfer (FET) cycles where no embryo was transferred in the fresh cycle were analyzed using the same inclusion criteria.

Participants/materials, setting, methods: From all ETs, 86,57% (290/335) of fresh, autologous, non-PGS cycles resulted in a day 5 ET, and 97,93% (284/290) was either a single or double embryo transfer. Eighty-seven ETs were excluded due to only one embryo available for ET. A total number of 29 FET with eSET and 30 with DET were also analyzed. Clinical pregnancy, implantation and twin pregnancy rates were compared using Fisher exact test. A $p < 0,05$ was considered significant.

Main results and the role of chance: We did not find any significant difference in female age (35,53 ± 5,093 in eSET vs. 35,43 ± 4,472 in DET) between the two groups. Clinical pregnancy (CPR) and implantation rates (IR) were similar (44,44% (52/107) CPR and 44,44% (52/107) IR in eSET group vs. 52,52% (73/139) CPR and 35,25% (98/278) IR in DET group, respectively; $p > 0,05$). Also, CPR and IR did not differ when patients were further stratified based on their female age. Patients with advanced maternal age (≥38) showed no difference in CPR and IR in eSET and DET groups (34,21% (13/38) CPR and 34,21% (13/38) IR in eSET group vs. 41,67% (20/48) CPR and 29,16% (28/96) IR, respectively; $p > 0,05$). A similar phenomena was observed analyzing data from the younger age (≤37) group (49,37% (39/79) CPR and 49,37% (39/79) IR in eSET group vs. 58,24% (53/91) CPR and 38,46% (70/182) IR, respectively; $p > 0,05$). A significantly increased twin pregnancy rates were observed in the DET group compared to the eSET group (none (0/52) in eSET group vs. 34.24% (25/73) in DET group; $p < 0,0001$).

Limitations, reasons for caution: Although, data from four consecutive years were analyzed the number of cycles is still below the desired number of procedures.

Wider implications of the findings: These results show that in our setting adding another embryo to the embryo transfer does not seem to markedly increase clinical pregnancy rate but dramaically increses twin pregnancy rate. This is an important piece of information advising patients before ET even when advanced embryo selection techniques are not available.

Trial registration number: -.

P-217 ART media act as modifiers that substantially influence embryo survival

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Study question: Do monozygotic twin embryos become functionally different if cultured in different ART media?

Summary answer: Genetically identical mouse embryos from the same fertilization event accrue different cell numbers in the three founder lineages, and progress differently after implantation.

What is known already: While there is growing consensus that *in vitro* culture influences the biological properties of the embryo, a direct and specific effect of ART culture media is subject of debate. A major confounder is

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represented by inter-embryo heterogeneity, whereby potential effects of media are superimposed with the different qualities of embryos arising from independent fertilization events of distinct oocytes. A critical parameter that contributes to define an embryo's potential is the formation of the epiblast among the founder cell lineages.

Study design, size, duration: Mechanical bisection of two-cell mouse embryos resulting in monozygotic twins, cultured in parallel in different media and then transferred to the uterus as blastocyst pairs. Transfer to uterus puts the quality of these embryos to the ultimate test of totipotency. Embryos are handled and analyzed respecting the pair associations.

Participants/materials, setting, methods: Two-cell embryos were derived from hybrid inbred oocytes (B6C3F1; stimulated cycle) and outbred sperm (CD1). Seventy-two pairs of sister blastomeres were cultured individually zona-free in pair combinations of three media (GM501, SAGE 1-step, KSOM(aa)), yielding 131/144 blastocysts. The blastocyst pairs were assessed immunocytochemically for the number of cells present in the founder lineages (epiblast, NANOG-positive; primitive endoderm, SOX17-positive; trophectoderm, CDX2-positive). Additional 164 pairs of monozygotic blastocysts were transferred to the uterus (1 pair/recipient).

Main results and the role of chance: Multivariate analysis of variance revealed that the effects of media ($p = 0.0043$) exceed those of inter-embryo heterogeneity associated with the independent fertilization events ($p = 0.0540$). The number of 4 epiblast cells which is considered as the minimum threshold for further development is achieved more often in twin blastocysts cultured in ART media (GM501 = 5.33 ± 0.46 cells; SAGE 1-step = 3.92 ± 1.08 cells) compared to the mouse medium KSOM(aa) (3.58 ± 1.08 cells). Preliminary embryo transfer data show that more twins are delivered to term when ART media are used. When both twins developed in the same ART medium, 86 pairs were transferred to uterus resulting in 28 live-borns of which 6 were monozygotic pairs. When both twins developed in KSOM(aa) medium, 48 pairs were transferred to uterus resulting in 10 live-borns of which 1 was a pair. When twins developed one in ART and the other in KSOM(aa) medium, 30 pairs were transferred to uterus resulting in 9 live-borns of which 2 were a pair. Experimental control is inherent in this setting, given the same zygotic origin and genetic identity of the twin and cotwin, and the same uterus for both twins. Therefore, the functional responses observed in this study can only be driven by the respective culture media.

Limitations, reasons for caution: This study was conducted in both an experimental twinning model and an animal model. Naturally, monozygotic twins are, other than in humans, exceptional in mice. Our mouse results suggest that medium-induced embryo diversification could happen also in human ART, however, the final effect may or may not be the same.

Wider implications of the findings: These results, if reproduced in independent studies in other species including human, would prove beyond reasonable doubt that embryo culture media are modifiers, since they cause monozygotic twins to diversify. As such, ART media have a substantial influence on survival of embryos, and diminish their natural selection.

Trial registration number: Not applicable.

P-218 Nucleolar morphology, aneuploidy and maternal age: are they correlated?

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Study question: Could nucleolar morphology be correlated with embryo ploidy?

Summary answer: Poor nucleolar morphologic scores were associated with higher aneuploidy rates in < 40, but not in ≥40, year old patients.

What is known already: The single euploid embryo transfer has been shown to increase the implantation rate and decrease the risk of multiple pregnancies. Nevertheless, many patients do not have access to Preimplantation Genetic Screening (PGS) technologies. In this sense, a better understanding of the correlations between morphology and ploidy can be useful to select the embryo more likely to be euploid. Despite the great interest that blastocyst morphology has received, a very few number of studies have related the nucleolar morphology to chromosomal content of embryos.

Study design, size, duration: This is a retrospective observational study performed from January 2013 to December 2016 in order to assess the relationship between maternal age, standard morphology and ploidy. The morfology parameters of 338 embryos (zygote, cleavage stage and blastocyst) from 129 PGS cycles (array-CGH or NGS) were analyzed. Besides, frozen embryo transfers (FET) of 77 euploid blastocysts were analyzed, where it was possible to track the implantation outcome.

Participants/materials, setting, methods: Advanced maternal age (> 35; 48.1%) and repeated implantation failure (19.2%) were the main PGS indications. Prior to trophectoderm biopsy (day-5 or day-6), zygotes were classified according to the Z-score system and cleavage stage embryos (day-3) based on number of blastomeres and fragmentation (top, average and poor quality). Furthermore, blastocysts were scored according to Gardner and Schoolcraft grading system and categorized in BL1 (AA, AB or BA), BL2 (BB or CB) and BL3 (BC or CC).

Main results and the role of chance: The mean maternal age was 37.1 ± 4.1 years old. The overall euploid rate was 56.8%. Poor nucleolar and blastocyst morphology, but not cleavage stage embryo morphology, were associated with higher aneuploidy rates. Z1 = 34.6%, Z2 = 43.2% and Z3/Z4 = 71.8%; ($p < 0,001$); Top = 44.1%, Average = 41.1% and Poor = 25% ($p = 0,6871$); BL1 = 35.2%, BL2 = 42.8% and BL3 = 67.4% ($p < 0,005$). A strong correlation was observed between advanced maternal age and higher aneuploidy rates, especially in women aged 40 years or older (71,8%; $p < 0,0001$). Therefore, patients were divided into two groups (< 40 years old and ≥40 years old) to assess the influence of maternal age on the correlation between morphology and ploidy. While nucleolar morphology was predictive of aneuploidy rate in the < 40 years old group (Z1 = 26.9%, Z2 = 36.4% and Z3/Z4 = 69.2%; $p < 0,001$), this association was not present in the ≥40 years old group (Z1 = 71.4%, Z2 = 67.5%, Z3 = 76.9%; $p = 0,8042$). The same pattern was observed for blastocyst morphology as reported before. Interestingly, a trend towards increased aneuploidy rates, correlated with poor nucleolar scores, was observed even when comparing same quality blastocysts. BL1/Z1 = 31.2%, BL1/Z2 = 35.9%, BL1/Z3,Z4 = 60% ($p = 0,2015$); BL2/Z1 = 33.3%, BL2/Z2 = 42.1%, BL2/Z3,Z4 = 72.7% ($p < 0,01$); BL3/Z1 = 60%, BL3/Z2 = 66.6%, BL3/Z3,Z4 = 83.3% ($p = 0,3469$). Finally, nucleolar and blastocyst morphology did not affect the implantation rate in FET of euploid blastocysts.

Limitations, reasons for caution: The study is limited by its retrospective nature and by the subjective assessment of morphology. A higher sample size or a prospective randomized design could be used in future studies to corroborate the current findings.

Wider implications of the findings: The present data suggest that a combined assessment of nucleolar and blastocyst morphology should be used when selecting embryos for transfer in < 40 year old patients who do not have access to PGS. Moreover, nucleolar and blastocyst morphology should be applied to choose the embryos undergoing biopsy and chromosomal analysis.

Trial registration number: NA.

P-219 Supernumerary vitrified blastocysts can be effectively diagnosed after warming by fast qPCR-based aneuploidy testing and transferred without the need of a second vitrification round

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Study question: Is qPCR-based chromosomal analysis on vitrified blastocysts with subsequent euploid single embryo transfer (SET) an efficient approach for patients needing preimplantation genetic diagnosis for aneuploidies (PGD-A)?

Summary answer: In patients with previous fresh IVF-failure(s), supernumerary vitrified blastocysts can be effectively diagnosed after warming and transferred without the need of a second vitrification round.

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What is known already: PGD-A is a technique whose application in IVF treatments is constantly growing nowadays, while vitrification is a consolidated, safe and efficient strategy for oocytes/embryos cryopreservation. Since supernumerary embryo cryopreservation is a common strategy in IVF, many patients have a cohort of untested cryopreserved embryos. Thus, for patients with a history of fresh IVF failures and/or miscarriages, an attractive approach may involve the warming and chromosomal testing of those embryos with subsequent euploid SET

Study design, size, duration: Longitudinal cohort study involving 51 patients (female age at oocyte retrieval:35.9 ± 3.5,23.6–42.0). Candidate patients had untested vitrified embryos from a previous IVF cycle and a history of IVF failures (1.4 ± 1.5,0–6) and/or miscarriages (0.5 ± 0.7,0–3). All PGD-A cycles on warmed blastocysts with subsequent euploid SET between April2013-October2016 were included. The primary outcome measure was ongoing pregnancy rate per euploid SET (> 12 weeks of gestational age). Secondary outcomes included biochemical pregnancy loss and miscarriage rates

Participants/materials, setting, methods: Trophectoderm biopsy was performed after warming and blastocyst full re-expansion. Chromosomal analysis was conducted by qPCR in 4 hours and subsequent euploid SET was performed with no need for a further vitrification-warming cycle. Clinical outcomes were compared to vitrified-warmed euploid SETs from 447 patients (female age:36.5 ± 2.8,25.6–44.0) undergoing PGD-A with an indication of history of IVF failures (1.9 ± 1.8,0–11) and/or miscarriages (0.7 ± 1.0,0–5) between April 2013-October 2016.

Main results and the role of chance: 115 blastocysts survived warming (survival rate after warming: 98.3%; n = 115/117,95%CI = 93.9%-99.8%) and underwent trophectoderm biopsy after full re-expansion. No blastocyst degenerated after biopsy. 66 blastocysts were diagnosed euploid by qPCR and 40 subsequent euploid SETs were performed. 26 euploid blastocyst were re-vitrified. Ongoing pregnancy rate for the study group was similar to the historical control group, namely 42.5% (n = 17/40; 95%CI = 27.0%-59.1%) and 40.9% (n = 244/597; 95%CI = 37.7%-45.8%), respectively (NS). No differences between the two groups were also found for the biochemical pregnancy loss rates: 5.0% (n = 1/20; 95%CI = 0.1%-24.8%) and 7.7% (n = 23/300; 95% CI = 4.9%-11.3%) and miscarriage rates: 10.5% (n = 2/19;95%CI = 1.3%-33.3%) and 11.9% (n = 33/277; 95%CI = 8.3%-16.3%) for the study group and the historical control, respectively (NS).

Limitations, reasons for caution: Data are needed to assess implantation potential for those blastocysts undergoing a second round of vitrification. Vitrified oocytes or embryos cryopreserved at earlier preimplantation developmental stages were not included.

Wider implications of the findings: Trophectoderm biopsy after warming and fast qPCR aneuploidy testing with subsequent euploid SET is an efficient strategy to rescue untested cryopreserved embryos when indicated.

Trial registration number: none.

P-220 BMP4 plays a role in apoptosis during early human embryonic development

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Study question: Does BMP4 preferentially induce differentiation of the cleavage stage blastomeres towards trophectoderm (TE) or inner cell mass (ICM) in the blastocyst?

Summary answer: BMP4 has no effect on first lineage differentiation, but triggers apoptosis in the early embryo. Apoptotic embryos express higher levels of *BMP4* than control blastocysts.

What is known already: Bone morphogenetic proteins (BMPs) are multifunctional growth factors that belong to the TGFβ family. Several BMP pathway components are expressed at early stages of development but their role during human preimplantation development remains unknown.

Study design, size, duration: Cryopreserved cleavage-stage day 3 embryos were warmed and cultured for 1, 2 or 3 days with human recombinant BMP4 protein (100 ng/ml) (n = 46). Control embryos (n = 42) were cultured in parallel without BMP4 supplementation.

Participants/materials, setting, methods: The experiments were approved by the Local Ethical Committee and the Federal Committee for research on human embryos. The embryos were donated for research after the legal storage period of 5 years by patients who signed an informed consent. Post-warming, the further developmental rate of the embryos was assessed in both groups. Gene expression was analyzed after 24 and 48 h of culture using qRT-PCR and immunocytochemistry. TUNEL-assay was performed after 72 h to evaluate apoptosis.

Main results and the role of chance: Supplementation of BMP4 to the culture medium significantly decreased the blastocyst rate ($P < 0.01$) (11.76% vs 50%). A significant increase in the amount of TUNEL positive cells was observed after 72 h of BMP4 supplementation ($P < 0.01$) (100% vs. 3.77%). No differences in the expression of differentiation genes were found between control and BMP4-treated embryos after 72 h. Immunocytochemistry for the ICM marker NANOG and the TE marker CDX2 was performed to confirm that BMP4 does not influence the first lineage differentiation, i.e. the segregation of the ICM and TE. Remarkably, a significant increase in *MSX2* expression ($p < 0.05$) after 24 h of culture and a significant decrease of *SIRT1* expression ($p < 0.05$) after 48 h of culture were found. MSX2 has been shown to be a transcriptional regulator in the programmed cell death pathway and SIRT1 is a deacetylase controlling P53 activity inducing the transcription of proapoptotic genes. Finally, apoptotic embryos (n = 5) expressed significantly ($p < 0.05$) higher levels of *BMP4* than control blastocysts (n = 5). These results suggest that BMP4 plays a role in apoptosis during early human preimplantation development.

Limitations, reasons for caution: This study is descriptive as functional studies are difficult to perform in human embryos. Currently, we do not have the possibility to knock down or over-express genes in human preimplantation embryos.

Wider implications of the findings: Comprehensive understanding of differentiation and apoptosis processes will help us to understand causes of unsuccessful human preimplantation development *in vitro* and potentially lead to predictive markers for embryos that show DNA damage, early signs of apoptosis and/or developmental arrest.

Trial registration number: /.

P-221 Influence of culture length on blastocysts implantation potential: analysis of 781 euploid blastocysts obtained in 629 PGS cycles

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Study question: Does the time required to reach blastocyst stage influence the implantation potential?

Summary answer: It seems that the time needed to reach the blastocyst stage is deeply linked to the embryo implantationpotential in an inversely proportional trend

What is known already: Embryo morphology has always been considered an important predictor of successful implantation andpregnancy. However, the Preimplantation Genetic Screening (PGS) technology proved that this parameterseems to have quite low predictive value on implantation potential. Few studies focused on the comparisonof pregnancy rates obtained with blastocysts vitrified on day 5 or day 6, demonstrating that the latter havea statistically significant lower value. Until now little is known if the clinical outcomes could be differenttransferring an euploid embryo obtained after 5, 6 or 7 days of culture.

Study design, size, duration: In this retrospective study, 781 cryopreserved euploid blastocysts obtained in 629 PGS cycles weretransferred in single frozen-mbryo transfers performed from April 2011 to October 2016. All blastocysts-were biopsied and vitrified on day-5,- 6 or -7 of culture (BD5, BD6, BD7 groups); blastocysts were furtherdivided on the basis of their quality in A, B and C grades. Clinical pregnancy (CP), advanced pregnancy (over3 months) (AP) and abortion rates were compared between the groups.

Participants/materials, setting, methods: Mean female age was 35.7 ± 3.96 years. In order to classify the blastocysts on the basis of their morphology,the

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expansion grade, the inner cell mass (ICM) and the trophoectoderm (TE) quality were analyzed. Grade-A included expandend blastocysy and good or very good ICM and TE quality; grade-B included notexpanded blastocysy and good or very good ICM and TE quality; grade-C included all expansion grades andpoor or very poor ICM and TE quality.

Main results and the role of chance: Out of the 746 analyzed blastocysts, 463, 287 and 31 were in BD5, BD6 and BD7 groups, respectively. InBD5, 122 (26.3%) grade-A, 259 (55.9%) grade-B and 82 (17.7%) grade-C blastocysts were obtained. In BD678 (27.2%) grade-A, 88 (30.7%) grade-B and 121 (42.2%) grade-C blastocysts were obtained. In BD7, 7(22.6%) grade-A, 1 (3.2%) grade-B and 23 (74.2%) grade-C blastocysts were obtained. In BD5 grade-A,grade-B and grade-C, 66 (54.1%) 154 (59.5%) and 36 (43.9%) CP, 61 (50%), 139 (53.7%) and 29 (35.4%) APand 5 (4.1%), 15 (5.8%) and 7 (8.5%) miscarriages were obtained, respectively. In BD6 grade-A, grade-B andgrade-C, 37 (47.4%), 49 (55.7%) and 50 (41.3%) CP, 33 (42.3%), 42 (47.7%) and 42 (34.7%) AP and 4 (5.1%),7 (7.9%) and 8 (6.6%) miscarriages were obtained, respectively. In BD7 grade-A, grade-B and grade-C, 2(28.6%), 1 (100%) and 6 (28.6%) CP, 2 (28.6%), 1 (100%) and 3 (13.0%) AP and 0 (0%), 0 (0%) and 4 (17.4%)miscarriages were obtained, respectively. Both CP and AP resulted higher in BD5 compared to BD6 ($p = 0.03$and $p = 0.02$, respectively) and to BD7 ($p = 0.005$ and $p = 0.001$, respectively);CP and AP resulted higher in BD6compared to BD7 ($p = 0.05$ and $p = 0.02$, respectively).

Limitations, reasons for caution: The main limitation of morphology assessment is that it is an operator-dependent static system.Furthermore, some blastocysts were already formed on the day antecedent the biopsy without reachenough expansion to perform safely the technique, therefore being cultured one day more andconsequently counted in the subsequent group.

Wider implications of the findings: Our data show a strong correlation between the day needed to reach blastocyst stage and implantationpotential. Trasferring an early (day-5) euploid blastocyst is most clinically advantageous than a blastocystobtained later (day-6 or day-7) and it should be considered when more blastocysts are available for thetransfer.

Trial registration number: Not applicable.

P-222 Adding a low-quality embryo to a high-quality embryo in embryo transfer does not improve pregnancy rates and increases multiple gestation rates

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Study question: Does the addition of a low-quality embryo in fresh day 3 embryo transfer affect ongoing pregnancy and multiple pregnancy rates in women undergoing IVF/ICSI?

Summary answer: The addition of a low-quality embryo in fresh day 3 embryo transfer does not increase ongoing pregnancy rates but increases multiple gestation rates.

What is known already: In recent years, double embryo transfer (DET) and single embryo transfer (SET) have become the most common embryo transfer strategies in IVF/ICSI. When analyzed per first transfer only, pregnancy rates after DET increase considerably compared to SET, but also multiple gestation rates increase. These conclusions are based on randomized controlled trials (RCTs) in which patients with two or more high-quality embryos were included. It is unknown whether the addition of a low-quality embryo affects ongoing pregnancy and multiple gestation rates. Given the relatively large group of patients with one or no high-quality embryo, this is an important question to address.

Study design, size, duration: Multicenter retrospective cohort study of 5050 patients receiving 7252 fresh embryo transfers on day 3 after fertilization in IVF/ICSI cycles from 2012 to 2015 in two academic hospitals in Amsterdam, The Netherlands.

Participants/materials, setting, methods: Cases were women that received fresh day 3 SET or DET with any combination of high-quality embryos (7–9 blastomeres, < 20% fragmentation) or low-quality embryos (all other embryos). Outcomes were ongoing pregnancy rate (OPR), miscarriage rate and multiple gestation rate. We used a generalized estimating equations model adjusting for age, number of oocytes and center of treatment.

Main results and the role of chance: In our database, DET with two high-quality embryos resulted in a higher OPR (OR 1.42, 95% CI 1.17–1.73), while DET with one high- and one low-quality embryo resulted in a lower OPR (OR 0.67, 95% CI 0.49–0.93) compared to SET with one high-quality embryo. However, SET in patients that had only one high-quality embryo available resulted in a lower OPR compared to SET in patients that developed multiple high-quality embryos (OR 0.51, 95% CI 0.38–0.69). After adjusting for this confounder we found that the difference between DET with one high- and one low-quality embryo and SET with one high-quality embryo was no longer statistically significant (OR 0.72, 95% CI 0.49–1.07). If only low-quality embryos were available, DET did also not increase the OPR as compared to SET with one low-quality embryo (OR 0.86, 95% CI 0.57–1.31). Multiple gestation rates were higher in all DET groups compared to both SET groups (all comparisons $p < 0.0001$). Meanwhile, the miscarriage rate was lower when one low-quality embryo (OR 0.52, 95% CI 0.41–0.67) or two low-quality embryos (OR 0.59, 95% CI 0.39–0.90) were transferred compared to transfer of one high-quality embryo.

Limitations, reasons for caution: Limitations to this study include the retrospective design and potential unascertained confounding factors.

Wider implications of the findings: Our study suggests that, in contrast to RCTs with high-quality embryos, in patients with one or no high-quality embryo, the addition of a low-quality embryo in embryo transfer does not improve pregnancy rates but increases multiple gestation rates. Future prospective studies should include embryo quality when comparing SET and DET.

Trial registration number: not applicable.

P-223 Hyaluronan enriched embryo transfer media improves Reproductive outcomes in frozen thawed cycles for couples with repeated implantation failure

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Study question: Objective: To examine the effect of hyaluronan enriched embryo transfer media on reproductive outcomes in couples with failed implantations

Summary answer: Hyaluronan enriched transfer media seems to favor better ongoing pregnancy rates with reduced first trimester miscarriages in couples with history of failed implantations.

What is known already: Successful implantation requires a competent blastocyst interacting with receptive endometrial lining, followed by invasion of trophoblast. Hyaluronan, a glycosaminoglycan increases in the female reproductive tract at the time of implantation and helps developing 'sticky' matrix on endometrial wall, there it is postulated that media enriched with Hyaluronan may improve the implantation. We assessed the role of Hyaluronan in failed implanation group.

Study design, size, duration: Design: Retrospective study

Patients: Couples with recurrent implantation failures after two attempts of blastocyst transfers done in 2015 & 2016 were included in this study (n = 44). They were offered hyaluronan enriched embryo transfer media for their subsequent embryo transfer. These group of women were compared against a control group (n = 100) who had history of failed implantations and went ahead for subsequent transfer without hyaluronan enriched media.

Participants/materials, setting, methods: Interventions: Both groups had frozen embryos transfers at blastocyst stage. All patients had double embryo transfer. Embryos were transferred with or without hyaluronan enriched media. Reproductive outcomes in both the groups were compared

Main results and the role of chance: Results: Study group (n = 44) had a Clinical pregnancy rate was 65.9% [29/44], miscarriage rate of 4.54% (2/44)

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and an ongoing pregnancy rate of 61.36% (27/44). In control group (n = 100) clinical pregnancy rate was 62% [62/100], 19% miscarriage rate (19/100) and an ongoing pregnancy rate of 43% (43/100). Statistical significance was noted with miscarriage rates and ongoing pregnancy rates (p values - 0.02 and 0.04 respectively).

Limitations, reasons for caution: Smaller size of study group.

This study did not look at live birth rates as the results are still pending. It would be interesting to see if use of hyaluronan can improve take home baby rates.

Wider implications of the findings: Considering the reduced miscarriage rates in study group, it would be interesting to explore the role of hyaluronan enriched transfer media in RPL women.

Trial registration number: Not Applicable.

P-224 Morphological grade impact on clinical outcomes in single or double blastocyst transfers

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Study question: Are there advantages in transferring two blastocysts, being a top-quality and a poorer-quality one, compared to one top-quality blastocyst transfer?

Summary answer: Transferring a poorer quality embryo with a top-quality one does not increase the pregnancy rate, but leads to high multiple pregnancy rate.

What is known already: Multiple births and its consequences are major adverse effects in IVF treatments. Decreasing the number of embryos transferred (ET) is effective in minimizing those risks. However, transferring more embryos supposing it would increase the chances of pregnancy turns the double embryo transfer (DET) a common worldwide approach. The challenge of single embryo transfer (SET) is the selection of embryos with higher development and pregnancy potential. On the other hand, extended embryo culture until blastocyst stage, associated to morphological classification, has been considered effective to select top-quality (TQ) blastocysts for transfer, leading to higher implantation and pregnancy rates.

Study design, size, duration: This cross-sectional study included fresh IVF cycles with one or two blastocysts transferred, performed from 2014–2016. Cycles with donated oocytes, preimplantacional genetic diagnosis and cleavage embryos transfers were excluded. Three groups were analyzed according to number and quality of blastocyst transferred: 58 cycles with one TQ blastocyst transferred (Group TQ-SET); 176 cycles with two blastocyst transferred - one TQ and one poor-quality (Group Mixed-DET); and 223 cycles with two TQ blastocyst transferred (Group TQ-DET).

Participants/materials, setting, methods: Ovarian stimulation and oocyte retrieval were performed using standard protocols. All case were submitted to ICSI and extended embryos culture was placed as routine. The blastocysts were classified according to Gardner and those containing at least one grade A score, in trophectoderm or inner cell mass, were considered TQ. The fresh blastocyst transfers were done at day 5 following routine protocols, and clinical pregnancy was confirmed by the presence of gestational sac with fetal heartbeat.

Main results and the role of chance: The groups were compared to women age (TQ-SET: 34.6 ± 3.6, MIXED-DET: 34.3 ± 3.1, TQ-DET: 34.3 ± 3.4; p = 0.830), number of oocytes recovered (TQ-SET: 9.0 ± 5.4, MIXED-DET: 10.7 ± 5.2, TQ-DET: 12.5 ± 5.2; p < 0.001), fertilization rate (TQ-SET: 78.5%, MIXED-DET: 80.2%, TQ-DET: 81.8; p = 0.287) and blastocyst formation rate (TQ-SET: 53.5%, MIXED-DET: 58.0%, TQ-DET: 64.6; p < 0.001). Despite the number of oocytes recovered and blastocyst formation rate had been higher in TQ-DET, all groups were considered clinically good ovarian responders and had satisfactory blastocyst formation with at least one TQ blastocyst for transfer. The implantation rates were slight lower in the MIXED-DET group (31.8%) compared to TQ-SET (44.8%; p = 0.079) and TQ-DET (40.5; p = 0.081), and the clinical pregnancy rates were similar between groups TQ-SET (44.8%) and MIXED-DET (49.4%; p = 0.542), but it was higher in the TQ-DET (59.6%) compared with both TQ-SET (p = 0.043) and MIXED-DET (p = 0.042). The multiple pregnancy rates were extremely lower in the TQ-SET (3.8%) compared to MIXED-DET (31.0%; p < 0.001) and TQ-DET (38.9%; p < 0.001). The groups MIXED-DET and TQ-DET were not statistically different for multiple pregnancy rates (p = 0.238).

Limitations, reasons for caution: Besides the group TQ-SET had a reduced sample size, part of patients had just one blastocyst available for transfer and had no choice about transferring one or two embryos. However, all embryos transferred in that group were TQ, which is considered good prognosis.

Wider implications of the findings: When at least one TQ-blastocyst is available, SET should be considered. The presence of a second poorer-quality blastocyst won't improve the clinical pregnancy rate, but will significantly increase multiple gestations. While adding a second TQ-blastocyst will result in slightly higher pregnancy chance, with almost 40% of multiples.

Trial registration number: not applied.

P-225 Pre-clinical validation of the meiotic spindle transfer technique in the mouse model

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Study question: What is the impact of the meiotic spindle transfer technique in the viability of the reconstructed oocytes and their further potential to develop after insemination?

Summary answer: The MST technique does not affect the potential of oocytes to develop up to blastocyst. MST can even ameliorate embryo development in sensitive mouse strains.

What is known already: Mitochondrial replacement techniques have been proposed as a strategy to avoid the inheritance of mutated mitochondrial DNA (mtDNA). Through MST, genomic DNA (structured as a meiotic spindle in MII oocytes) is transferred from an affected oocyte into a previously enucleated healthy one. A recent clinical application of this technique has resulted in the birth of the world's first child born using this technology. However, there are still safety concerns about the usage of this technique, and thus more research is needed to evaluate e.g. the levels of cytoplasmic carry-over during manipulation, as well as oocyte viability to develop after MST.

Study design, size, duration: In a first series of experiments, B6CBAF1 mouse oocytes (n = 214) were subjected to reciprocal MST and inseminated by ICSI. Potential cytoplasmic carryover was estimated by volumetric measurements of karyoplasts. Spindle morphology was evaluated by fluorescence analysis in oocytes after MST and controls.

In a second series of experiments, strains with diverged mitochondrial haplotypes, hybrid B6CBAF1 and NZB, were used to perform MST as a proof-of-concept for this technique (n = 302). After ICSI, embryo development was followed.

Participants/materials, setting, methods: Oocytes were collected from superovulated females (6–10 weeks old). MST was performed in manipulation medium supplemented with cytochalasin B (5 µg/ml). After enucleation, karyoplasts were exposed for 10 seconds to an inactivated sendai virus solution to promote membrane fusion, and immediately placed into the perivitelline space of a previously enucleated donor oocyte. Karyoplast-cytoplast fusion was assessed by 1-2 h, and ICSI performed using Piezo-Drill actuator. Blastocysts were analyzed by fluorescence to calculate their total cell number.

Main results and the role of chance: High survival (95.3%) and karyoplast-cytoplast fusion (98.0%) rates were observed after reciprocal MST among B6CBAF1 oocytes. Survival rates after ICSI were not statistically different

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between reconstructed and control oocytes (91.0% and 95.4%, respectively; p = 0.368). Similarly, blastocyst formation rates were statistically equivalent between MST and control groups (89.0% and 87.1%, respectively; p = 0.800). However, blastocysts (n = 75) derived from reconstructed MST oocytes showed a slightly decreased total number of cells compared to controls (177 ± 28 and 202 ± 26, respectively; p = 0.0007). All analyzed oocytes in control and test group presented a morphologically normal spindle, defined as bipolar, barrel-shaped and with aligned chromosomes in the MII plate.

Embryo development was compared between B6CBAF1 and NZB control groups, after insemination with fresh sperms obtained from the cauda epididymis of B6CBAF1 males. Blastocyst formation rate in control NZB group (n = 76) was significantly lower (5%) when compared to control B6CBAF1 (n = 46) (72%; p < 0.0001). Interestingly, when the meiotic spindle was transferred from NZB oocytes into previously enucleated B6CBAF1 oocytes that were subsequently inseminated by ICSI (n = 106), blastocyst developmental rates significantly increased up to 35% (p < 0.0001). These results suggest that MST could help overcome cytoplasmic-related deficiencies, in this case represented by the NZB control group.

Limitations, reasons for caution: Levels of mtDNA carry-over during MST should be evaluated through molecular analysis. Control and MST blastocysts should be compared in their potential to establish ESCs and develop *in vivo*. To assess whether heteroplasmy levels remain low after MST, these will be compared between ESCs and MST-generated mice in further studies.

Wider implications of the findings: Here, we demonstrate that MST among sibling B6CBAF1 oocytes does not affect oocyte potential to develop *in vitro*. Actually, in the sensitive NZB strain, MST can improve blastocyst development. If proved true in humans, MST could help on overcoming not only mitochondrial-inherited diseases, but also infertility caused by ooplasmic defects.

Trial registration number: Not applicable.

P-226 Morphokinetics analysis and reproductive outcome after Assisted Oocyte Activation with Calcium Ionophore. Multicentric Retrospective Analysis

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Study question: Does ICSI with Assisted oocyte activation (AOA) affect embryo morphokinetics and reproductive outcomes in patients with previous fertilization failure?

Summary answer: Sperm microinjection with Calcium ionophore (ICa) improves reproductive outcomes in patients after fertilization failure and also affects morphokinetics of the early stages of embryo development.

What is known already: AOA with ICa in patients with fertilization failure after ICSI, have shown good results, although the number of reported patients and oocytes studied remain low. On the other hand, the use of morphokinetics parameters for improve embryo selection using algorithms have been widely studied recently, and have offered greater implantation rates when applied in certain groups of patients.

Study design, size, duration: Multicentric retrospective study of 756 oocytes from 93 patients who underwent previous ICSI with fertilization failure (less than 20% normally fertilized oocytes) and severe male factor (ICa group) which were compared to 748 oocytes from 79 patients also with severe male factor (Control Group), without previous fertilization failure between January 2011 and October 2016 in three different clinics

Participants/materials, setting, methods: AOA was performed by injecting the oocytes with spermatozoa in a buffered media with Ica (Ionomicyn from *Streptomyces conglobatus*). Later, they were incubated for twenty minutes with fertilization media and ICa in a 37°C, 6%CO2 atmosphere. Embryo culture was performed in an incubator provided with time-lapse monitoring system under a 37°C, 6%CO2, 5% O2 atmosphere Fertilization, direct cleavage, pregnancy, implantation rates, abortion and embryo morphokinetics, were analysed.

X^2, and ANOVA tests were employed when applicable.

Main results and the role of chance: Statistically significant differences were found in normal fertilization rate, being higher in control group (51.2% vs. 64.7%). Additionally higher triploid proportion and degenerated oocytes were observed in Ica group. No differences among groups were found in the frequencies of direct cleavage events. Similar pregnancy, implantation and abortion were obtained between groups. Timings of cleavages were significantly lower (faster embryos) at the early stages up to 5 cells, from the third cycle and forward, embryos became similar in the development progress. It seems that ICa effect will be observable in early embryo events, and will be diluted later. This morphokinetics parameters should be taken in account for embryo selection. With the use of ICa, the chance of pregnancy, and implantation of patients with severe male factor previous fertilization failure, become similar to the those of patients with severe male factor without fertilization failure.

Limitations, reasons for caution: The embryos herein analysed in ICa were obtained from patients with severe male factor who had a previous complete fertilization failure, and were compared to embryos from severe male factor patients, what may suppose that the conclusions reached may not be applicable for patients with other ethyologies.

Wider implications of the findings: Our findings describe primarily the effect of Ica on embryo development under AOA with Ica and demonstrate the utility of this strategy after fertilization failure.

Trial registration number: 'not applicable'.

P-227 Mechanical vs laser collapse of blastocysts prior to vitrification

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Study question: Is there a difference in embryo survival and clinical outcome of frozen embryo transfer (FET) between blastocysts vitrified after either mechanical or laser collapse?

Summary answer: Despite a trend towards improved outcomes with the use of a laser, no significant differences were found when comparing mechanical vs laser collapse of blastocysts.

What is known already: Artificial collapse of blastocysts prior to vitrification has shown to improve embryo survival rates by reducing the risk of ice crystal formation as a result of fluid evacuation from the blastocoel. Laser collapse of blastocysts has been shown to significantly improve blastocyst survival rate, implantation rate (IR) and clinical pregnancy rate (CPR) compared to non-collapsed controls (Darwash E & Magdi Y, 2016). When comparing laser collapse to mechanical shrinkage of blastocysts, however, there has shown to be no difference in blastocyst survival rate, IR and CPR (Cao S *et al*, 2014).

Study design, size, duration: This was a retrospective analysis of FET treatment cycles undertaken from June 2014 to December 2016. Study Group 1 (n = 193) utilised mechanically collapsed blastocysts vitrified between May 2014 – August 2015. Study Group 2 (n = 137) utilised laser collapsed blastocysts vitrified between September 2015 – August 2016.

Participants/materials, setting, methods: All blastocysts were vitrified using the same methodology: Irvine Scientific VitKit with HSV straws. Mechanical collapse was carried out by inserting an ICSI injection pipette into the blastocoel cavity. Laser collapse was undertaken using the RI Saturn 5™ Laser System. Biochemical pregnancy rate (BPR), IR and CPR was determined for each study group and statistical significance was calculated using the Fisher's exact test.

Main results and the role of chance: There was no difference in the blastocyst survival rate between the two groups (95.0% and 96.9% for mechanical and laser collapse respectively). Although there appeared to be a trend towards better clinical outcomes with the laser group, these did not reach statistical significance (BPR/ET = 43.5% vs 53.3%; IR = 31.0% vs 40.1%; CPR/ET = 32.6% vs 42.3% for mechanical vs laser collapse respectively).

Limitations, reasons for caution: Data for group 1 crossed a broader time period and had a greater number of cycles than group 2. The different techniques for blastocyst collapse were not carried out concurrently.

Wider implications of the findings: This study shows that there is no detriment to using one method of artificial collapse over the other. However, use of

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a laser is more time efficient and, as a result, the time blastocysts are required to remain outside the incubator environment is reduced, minimising additional stress to the embryo.

Trial registration number: not applicable.

P-228 Impact of low versus atmospheric oxygen tension on embryo development at cleavage and blastocyst stages: Intermediate results of a prospective randomized blinded study

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Study question: Is embryo development influenced by oxygen tension applied during culture? If yes, are both cleavage and blastocyst stages vulnerable to oxidative stress?

Summary answer: Hypoxia during embryo culture improves embryo quality and development ability. However, late pre-implantation embryo development seems to be less vulnerable to oxidative stress.

What is known already: In mammals, uterine environment is at low oxygen concentration (2–8% O_2). Then, culturing human embryos under 2–8% O_2 is recommended by ESHRE revised guidelines for good practices in IVF labs. Hence, hypoxia (5% O_2) seems to improve embryo quality at cleavage and blastocyst stages, presumably by reducing damages of oxidative stress. Nevertheless, recent meta-analyses concluded with a very low evidence to a superiority of hypoxia on IVF/ICSI outcomes. Furthermore, a study on mouse embryos suggested an impact of oxidative stress only at cleavage stage. This hypothesis has still never been investigated in humans.

Study design, size, duration: From January to December 2016, 132 IVF/ICSI cycles were included in this prospective randomized blinded study. Each cycle was randomly allocated to: group A (culture from day-0 to day-6 under 20% O_2; n = 42 cycles), group B (culture from day-0 to day-6 under 5% O_2; n = 49 cycles) or group C (culture from day-0 to day-2 under 5% O_2, switched to 20% O_2 from day-2 to day-6; n = 41 cycles).

Participants/materials, setting, methods: Inclusion criteria were: IVF/ICSI using fresh/frozen ejaculate from the male partner; female age < 40 years; absence of hydrosalpinx; ≥8 cumulus-oocyte complexes retrieved. After randomization, oocytes were fertilized and cultured in similar benchtop incubators (G-210, K-Systems®, Denmark), either under 5 or 20% O_2. Fertilization rate, cleavage-stage quality (good-quality at day-2 defined as 3–5 blastomeres, < 20% cytoplasmic fragmentation), blastocyst quality (top-quality: ≥B4AA/AB/BA (Gardner and Schoolcraft's classification)) and implantation rate were compared between groups A, B and C.

Main results and the role of chance: We analyzed our preliminary data. Patients' and cycles characteristics were similar between each group. Culture under hypoxia resulted in significantly higher good-quality embryo rates at day-2 (71.5%) than under atmospheric conditions (63.6%; p = 0.03). Considering blastocyst development, culture under 20% O_2 (group A) yielded a significantly lower top-quality blastocyst rate per cleaved embryo at day 5 (7.6%), when compared with both groups B (18.8%; p < 0.05) and C (15.8%, p < 0.05). Similarly, cumulative top-quality blastocyst rate per embryo at day 5+6 was lower in group A (A: 8.4% *vs.* B: 19.8%, p < 0.05; and *vs.* C: 16.3%, p < 0.05). Interestingly, no difference was observed in terms of blastocyst quality between groups B and C (p = 0.30). Implantation rates were similar in groups A, B and C (39.7% *vs.* 36.3% *vs.* 31.3%, respectively; Global p value = 0.60).

Limitations, reasons for caution: Embryos were not analyzed using morphokinetic parameters, which could have made the interpretation less subjective. Besides, the study being still in progress, these results are preliminary and need to be confirmed and completed with clinical outcomes.

Wider implications of the findings: If confirmed, these results could encourage to systematically culture embryos under hypoxia during early development stages, before embryonic genome activation. However, late preimplantation stage appearing less vulnerable to oxidative stress, atmospheric oxygen tension could be applied from day-2 to day-6 without impairing blastocyst quality.

Trial registration number: ID-RCB: 2015-A02019-40

P-229 Transcriptomic changes derived from Assisted Reproductive Technologies (ART) can be decreased by reproductive fluids in the swine model

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Study question: How extensive are the gene expression changes in preimplantation embryos derived from Assisted Reproductive Technologies and how can we decrease them?

Summary answer: Blastocysts produced by in-vitro fertilization (IVF) with reproductive fluids (Natur-IVF) in the culture media showed fewer differentially expressed genes than embryos obtained by conventional-IVF (C-IVF).

What is known already: In mouse and human, accumulating evidence indicates that the embryo is sensitive to its early environment, and culture media used in ART may have long-lasting consequences. Abnormal phenotypes related to culture system include fetal overgrowth in cattle and sheep, and imprinting syndromes or growth restriction in humans and pigs. Epidemiologic studies in humans suggest that ART-associated procedures can perturb gene expression during early development, although such alterations could also be a consequence of parental characteristics, gamete quality or technique-derived effects. An animal model such as swine, with boars of proven fertility, could allow informative modelling of ART-related disorders in human.

Study design, size, duration: To investigate the basis of increased *birth defects* among *ART*-derived offspring, we analyzed whole transcriptome from individual day 7.5 blastocysts (3 embryos per group) produced *in vivo*, or *in vitro* using a new IVF system (Natur-IVF) or a conventional IVF system (C-IVF).

Participants/materials, setting, methods: A swim-up protocol for selection of porcine spermatozoa and reproductive secretions (follicular, oviductal and uterine fluids) as additives in the culture media were used for embryo production. RNA from single blastocysts was extracted and libraries were generated, further amplified and sequenced 100 bp single end. Raw sequence reads were trimmed to remove adapter contamination and reads with poor quality. Mapping was performed (Tophat software) and data were visualised and quantified (Seqmonk) (Canovas et al., 2017; *eLife*).

Main results and the role of chance: Blastocysts shared 334 genes aberrantly expressed Natur-IVF and C-IVF *vs. In-vivo*. However, 440 genes showed aberrant expression in C-IVF *vs. In-vivo,* but not in Natur-IVF, which could be considered as restored using Natur-IVF system. Amongst these genes were factors related to epigenetic reprogramming (*DNMT3B*, *DNMT1, HDAC5, KDM5A*), embryo development (*CTGF*, *ING2*, *KIT*, *EZH2*, *BMP4*, *TLN1*, *ADAR*), cell growth (*CDCA5*, *SMC1A*, *RB1*, *SMARCA2*), and imprinting (*IGF2BP2, GNAS, DIRAS3*). Ingenuity Pathway Analysis showed 5 networks (score > 30, > 20 factors /network) between these 440 genes. Associated networks functions were: i) RNA post-transcriptional modification/molecular transport/RNA trafficking; ii) Cell morphology, function and maintenance, organ morphology; iii) Cellular development, growth and proliferation, carbohydrate metabolism; iv) Cell-to-cell signalling and interaction, digestive system development and function, embryonic development; v) Cell signalling, post-translational modification, cellular development. Upstream regulators with 10 or more target molecules were HNF4A (53), TP53 (47), ESR1 (32), MYC (28), NR3C1 (27), NUPR1 (15), TCF7L2 (13) and EZH2 (10). Top canonical pathways were Superpathway of cholesterol biosynthesis, NAD Phosphorylation and dephosphorylation, AMPK signalling, tRNA splicing, tRNA charging. Top molecular and cellular functions were Cellular Development (144 factors), Movement (94), Growth and Proliferation (151), Function and Maintenance (122) and Morphology (116).

Limitations, reasons for caution: A limited number of single embryos were analyzed and individual variation between samples from the same group could mask some additional differences between groups.

Wider implications of the findings: Results showed that Natur-ART blastocysts are closer in their gene expression profile to *In-vivo* blastocysts than C-IVF blastocysts, with a number of functionally significant gene expression changes detected only in C-IVF embryos.

Trial registration number: Not applicable.

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P-230 Zona thinning and classic assisted hatching preformed with systematic multipulse laser settings result in significant improvements of clinical outcomes in frozen embryo transfer cycles

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Study question: What is the impact of zona pellucida thinning (ZPT) or classic assisted hatching (AH) techniques in the clinical outcomes of a frozen embryo transfer program?

Summary answer: Both ZPT and AH techniques performed with systematic laser settings on Day3 and Day5/6 embryos can significantly increase clinical outcomes in frozen transfer cycles

What is known already: Improvement of implantation rates is a main goal in human IVF. Intrinsic abnormalities in the blastocyst or ZP can result in implantation failure. The effects of ZP hardening on embryo hatching as a consequence of embryos vitrification/warming may impair their following implantation. Theoretically, ZPT or AH with laser may facilitate this process and subsequently increase implantation rates. However, conflicting reports on the beneficial effects of both techniques have been published possibly due to the lack of consistency on the size of the AH hole or ZP thinned area or on the systematic application of laser settings in the different studies

Study design, size, duration: This study was performed between March 2016 and January 2017. Patients (n = 361) were transferred with vitrified/warmed embryos (from own or donor oocytes) at the cleavage (Day 3) or blastocyst (Day 5/6) stage. Patients were randomized at the time of warming the embryos and assigned to the study or control (non-manipulated) groups. The study groups included: ZPT for Day3 embryos, and either ZPT or AH for Day5/6 blastocysts. In total, 719 warmed embryos were transferred.

Participants/materials, setting, methods: Warmed embryos were treated according to the assigned group. Half of ZP thickness was reduced in a quarter of its perimeter using systematic multipulse laser (*Navilase™*, Octax) settings in all Day3 or Day5/6 embryos included in ZPT groups. In the AH group, a ZP hole (size = 60 µm) was made in the warmed blastocysts. Control embryos were not subjected to laser pulses. Embryo transfers were performed 2–4 h post-warming. Results were compared by *X2* or *Student's t-test.*

Main results and the role of chance: A total of 149 patients were transferred with own Day3 embryos (n = 312) after warming. No differences were found between the control (n = 146) and ZPT group (n = 166) with regards to pregnancy (50.0% *vs* 45.5%, respectively) or implantation (28.1% *vs* 22.9%, respectively) rates. However, patients in ZPT group (37.2 y/o) were significantly older (p = 0.004) than those in control (35.7 y/o) group, which may indicate a beneficial effect of ZPT in this patients' group. This tendency was further confirmed in 70 recipients (age factor excluded) transferred with Day3 (n = 143) warmed embryos (donor oocytes). Pregnancy and implantation rates were significantly higher (p < 0.05) in the ZPT (71.9% and 44.6, respectively) group compared to control (44.7% and 25.6, respectively). Interestingly, clinical pregnancy and implantation rates were equivalent between control (51.5% and 47.5%) and ZPT (48.5% and 30.2%) or AH (58.1% and 40.7%) groups when Day5/6 blastocysts (own oocytes) were transferred into 109 patients (average age was 35.8; 37.8 and 36.4 y/o for the three groups, respectively). In the oocyte donation program, pregnancy and implantation rates with Day5/6 warmed blastocysts were significantly higher (p < 0.05) in the AH (84.6% and 52.0%) compared to the control (69.2% and 47.8%) or ZPT (57.1% and 45.5%) group.

Limitations, reasons for caution: Different publications have shown that the size of the AH hole or ZP thinned area may have an impact in the clinical outcomes of frozen cycles. Laser settings used here were firstly optimized in the mouse model. The study shall continue to increase the statistical power of the data analysis.

Wider implications of the findings: The new generation of lasers developed for ART ensure that multipulse laser settings can be applied in a systematic way in all embryos subjected to ZPT or AH. This allows for more technical reproducibility and consistency in the results in further studies, which should confirm the benefits of both techniques.

Trial registration number: Not applicable for non-clinical trials

P-231 Altered morphokinetics in sequential and single step culture media: a randomized prospective sibling oocyte study

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Study question: Does timing and embryo development differ in sequential vs. single step culture media?

Summary answer: Embryos grown in sequential or single step media have a similar embryological developmental profile but exhibit altered morphokinetics.

What is known already: Various culture conditions, as well as culture media, can influence embryo development and clinical outcome. Before introducing new media and different culture conditions, it is imperative to carry out in-house comparative analyses, ideally on sibling oocytes. It has been shown that embryo morphokinetics are greatly altered between different culture media (sequential vs. single step) and different oxygen concentrations. Moreover, no studies have categorically proven that single step media is better than or equal to sequential media.

Study design, size, duration: A prospective randomize study on sibling oocytes at an academic medical center. Each patient's oocytes were randomly allocated amongst sequential or single step media. All embryos were cultured in time-lapse incubator (Embryoscope, Vitrolife, Sweden) under the same conditions. Embryos were cultured until D6 and ETs were executed on D3 either D5. The best grade embryos were transferred regardless of the culture media used.

Participants/materials, setting, methods: Sequential in-house media C1/C2 and commercial G1/G2 and single step in-house media (C3) and commercial G-TL were used. The study included: Group 1: C1/C2 vs. C3 (n = 123 patients, n = 1579 embryos); Group 2: C1/C2 vs. G1/G2 (n = 20 patients, 298 embryos) and Group 3: C1/C2 vs. G-TL (n = 48 patients, 635 embryos). 2PN; D3 embryo; D3 ET and D5 ET and cryo; blastocyst (BL) rates, and morphokinetic were analyzed. X^2 and Wilcox/Kruskal-Wallis tests were used.

Main results and the role of chance: Fertilization rates between culture media did not differ. Embryos in C1/C2 media had a higher percentage of high grade embryos on D3 compared to C3 or GTL media (p = NS). Embryos selected for D3-ET did not vary among groups and many patients had mix media transfers. On D5, significantly more BL were transferred from C1/C2 vs. C3 (p < 0.05). However, the overall percentage of embryos used (D5-ET + cryo) and having achieved high grade BL did not differ among media. The rate of abnormal division (DUCs) and preliminary ploidy analysis revealed no differences between media.

Embryo kinetic analysis: Group 1. Embryos grew more rapidly in C3 vs. C1/C2 media for the first three divisions and equalized until BL stage (t9 to tEB). Group 2. Embryos in C1/C2 media grew slightly faster during the first two divisions (t2-t4) when compared to G1/G2, without differences in later stages. Group 3. Embryos grown in C1/C2 media had a tendency of growing faster until the morula stage (tM), whereas GTL embryos caught up at morula and early BL stages. No differences were observed in the expanded BL stage. Kinetic studies performed equally if DUCs were removed from the data.

Limitations, reasons for caution: Although this is a randomized sibling oocyte study, retrospective data were used for analysis. Implantation and ploidy couldn't be evaluated due to the mixed media transfers and a small sample of PGS cycles. Additional commercial media and a higher number of sibling oocyte cycles are needed to confirm this finding.

Wider implications of the findings: The timing of embryo development is influenced by culture media composition. This should be taken into account when creating embryo selection models. Sibling oocyte studies represent an optimal approach to evaluate different media. Media specific morphokinetics should be applied when creating embryo selection models for D3 or D5 ET.

Trial registration number: N/A.

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P-232 Blastocysts from abnormally-fertilized zygotes can be euploid/diploid and are reproductively competent: live-births from preimplantation genetic diagnosis of aneuploidy/polyploidy in embryos with abnormal pronuclear morphology

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Study question: Is it possible to rescue abnormally fertilized zygote (AFZs) if the risk of polyploidy is evaluated by a novel combined protocol for simultaneous aneuploidy/polyploidy assessment?

Summary answer: Polyploidy assessment in AFZ-derived blastocysts is an effective approach to rescue diploid embryos from monopronuclear/tripronuclear-derived blastocysts and may improve the live-birth rate in IVF cycles.

What is known already: Approximately 10% of inseminated oocytes fertilized abnormally and the embryos derived from them are discarded all over the world in the absence of a reliable approach for the monitoring of the genetic risk for polyploidy. Here, we report the development and application of an improved comprehensive chromosomal diagnosis protocol for the detection of 24-chromosome aneuploidy and parallel polyploidy assessment. The combined test was implemented clinically, with the aim of rescuing AFZs in poor prognosis patients to maximize live-birth rate per IVF cycle.

Study design, size, duration: Longitudinal cohort study performed between January2015-September2016 involving 553 patients (39.2 ± 3.2 yr) and 719 consecutive PGD-A cycles. Blastocyst PGD-A was offered to patients of advanced female age (> 35 years), a history of unsuccessful IVF (> two failed IVF cycles) or previous miscarriages (> two). The combined genetic analysis was performed when a blastocyst from monopronuclear(1PN) or tripronuclear(3.1PN) zygote was obtained. Euploid-diploid embryos obtained from AFZs were considered suitable for replacement if others from normally-fertilized zygotes (NFZs) were not available.

Participants/materials, setting, methods: Injected oocytes were individually cultured and fertilization was assessed between 16–18 hours. 1PN zygotes were defined when a single pronucleus was present. Zygotes showing one extra smaller pronucleus on the top of two evenly sized pronuclei were defined as 3.1PN. Blinded ploidy assessment using single nucleotide polymorphism allele ratios was conducted on trophectoderm biopsies from AFZ-derived blastocysts by targeted-NGS (1:1 = diploid; 2:1 = triploid; loss of heterozygosity = haploid). Samples of known ploidy were included as controls

Main results and the role of chance: For validation, a blinded analysis of previously-established triploid and diploid cells was performed. The allele ratio was 2.12 ± 0.12(1.97–2.31) and 1.29 ± 0.15(1.18–1.50) for triploidy and diploidy, respectively($p < 0.01$). Furthermore, the DNA products from one triploid miscarried blastocyst and two sibling unknown-ploidy vitrified euploid blastocysts were blindly submitted to ploidy assessment. The results correctly identified the triploid blastocyst.

In the clinical phase, 3785 zygotes (200 1PN-AFZs;27 3.1PN-AFZs;3558 2PN-NFZs) from 719 PGD-A cycles were assessed. In 25.2% (n = 181/719) and 3.6% (n = 26/719) of the cycles AFZs and AFZ-derived blastocysts were observed, respectively. Significantly less blastocysts were obtained from 1PN-AFZs (n = 13/200, 6.5%) with respect to both 3.1PN-AFZs and 2PN-NFZs (n = 14/27, 51.9%; n = 1667/3558, 46.9%; $p < 0.05$), mainly due to a lower cleavage rate at the first cell division after fertilization.

Ploidy analysis identified 23.1% (n = 3/13), 69.2%(n = 9/13) and 7.7% (n = 1/13) of the 1PN-AFZ-derived blastocysts as haploid, diploid and triploid, respectively. A normal diploid constitution was observed in 85.7% (n = 12/14) of the 3.1PN-AFZ-derived blastocysts, while 2 (3%) were triploid.

In 0.8% of the cycles (n = 6/719) AFZ-derived euploid/diploid blastocysts were identified. Four of them were transferred and resulted in 2 healthy pregnancies (one from a 1PN-AFZ and one from a 3.1PN-AFZ).

Limitations, reasons for caution: Gestational and neonatal outcomes of AFZ-derived pregnancies still need to be investigated in a larger sample size. Only 1/3 of the pronuclei check on day1 was performed in a time lapse system. However,AFZs were re-evaluated within 4 hours from the first check by an independent embryologist to confirm the pronuclear abnormality

Wider implications of the findings: Overall, AFZs account for ~10% of the zygotes in IVF and are generally discarded. Here, we report the development and validation of a reliable protocol for simultaneous aneuploidy and ploidy assessment from a single trophectoderm biopsy and the first clinical experience that resulted in two healthy pregnancies from AFZs.

Trial registration number: none.

P-233 Impact of endometrial decidualization on human blastocyst growth and cytokine secretion

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Study question: We hypothesize that the decidualization status of the endometrium has a positive impact on human blastocyst development.

Summary answer: Exposure of human blastocysts to secretome/conditioned media (CM) of decidual cells does not compromise the blastocyst growth and results in a blastocyst-specific secretion of CXCL8/IL8.

What is known already: Human endometrial decidualization is the transformation of endometrial stromal cells (EnSC) into decidual cells (DEC). It is a sequential process characterized initially by a transient pro-inflammatory response, which spans the window of implantation. This is followed by an anti-inflammatory response and acquisition of a mature decidual phenotype. Exposure of murine uterus to the pro-inflammatory secretome of human DEC promotes implantation of transferred murine embryos. However, exposure to secreted factors from EnSC or DEC of anti-inflammatory phase leads to embryonic demise prior or soon after implantation.

Study design, size, duration: Endometrial biopsies were obtained from ten patients undergoing infertility treatment. EnSC were isolated and pooled into one *in vitro* cell culture. Forty-one high quality blastocysts (thirty patients) that became available for research after a legally determined storage period were warmed. Thirty-one of the warmed blastocysts had good survival and were included in the experiment. Both biopsies and embryos were used with informed consent of the patients and following approval of the relevant ethical committees.

Participants/materials, setting, methods: Isolated EnSC were decidualized *in vitro* to DEC in the pro- and anti-inflammatory phase (4 and 10 days *in vitro* decidualization, respectively). CM of DEC (Day 4 and 10) and CM of their EnSC-controls were collected. Next, six to eight day 5 human blastocysts were cultured individually for 24 h in microdroplets of the different CM. Blastocyst growth was scored and 45 cytokines in microdroplets were quantified by multiplex suspension bead immunoassay (controls = empty CM microdroplets).

Main results and the role of chance: Analysis of the blastocyst growth rates demonstrated that the CM of EnSC compromised blastocyst development, exemplified by a growth score 0 (indicative of poor development) in 8/17 (47%) embryos. By contrast, the growth rates were not compromised when blastocysts were incubated in CM of DEC [growth score 0 in 1/14 (7.1%) embryos (Chi-square test: $P = 0.02$)], irrespective of the phase of decidualization. In addition, cytokine analysis revealed a blastocyst-specific response following culture in DEC versus EnSC CM, which was most pronounced in DEC CM of the pro-inflammatory phase. Taking into account the cytokine concentration in the control droplets, two cytokines were identified as potentially important mediators of the blastocyst response. CXCL8/IL8 was secreted by the blastocysts in response to signals from DEC and not EnSC (Two-way ANOVA test: $P < 0.0001$). On the contrary, CCL2/MCP-1 was only secreted to signals from Day 4 EnSC (Two-way ANOVA test: $P < 0.0001$).

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Limitations, reasons for caution: This study is limited by the use of endometrial biopsies from patients undergoing infertility treatment. Culture of blastocyst with their autologous EnSC was not possible. Nevertheless, EnSC from the ten patient biopsies were pooled together into one *in vitro* cell culture to reduce the patient-to-patient variability across the experiments.

Wider implications of the findings: Understanding the endometrium-embryo crosstalk during human embryo implantation will advance the development of new strategies to improve the IVF outcome, for example optimized embryo culture medium and timing of embryo transfer.

Further experiments on the effect of the blastocyst-secreted cytokines on endometrial cells are ongoing.

Trial registration number: None.

P-234 Culturing human embryos in benchtop/topload incubators improves clinical outcomes compared with large-box conventional incubation systems

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Study question: Does culture in individual small incubator chambers improve good quality embryo (GQE) rates and clinical in vitro fertilization-embryo transfer (IVF-ET) outcomes in comparison with culture in a conventional system?

Summary answer: Despite similar GQE rates at early cleavage stages on days 2/3, culturing embryos in benchtop/topload incubators increases good quality blastocyst, implantation and clinical pregnancy rates.

What is known already: New generation of benchtop/topload incubators with individual small chambers, aiming to ensure continuous and reliable temperatures and gas mix while providing optimal environment for the embryos, have rapidly emerged in laboratories of reproductive biology worldwide. However, despite expected improvement in terms of embryo development and pregnancy rates, objective data is still lacking.

Study design, size, duration: This single-center study included retrospectively 1424 IVF-ET cycles from September 2013 to December 2015. After transvaginal ultrasound-guided retrival, oocytes were allocated for culture either in conventional large-box (n = 732) or benchtop/topload incubators (n = 692). In both groups, top (TQE) or good (GQE) quality embryo rates at early cleavage stages, implantation (IR) and clinical pregnancy (CPR) rates were analyzed. In case of extended culture, blastulation and good quality blastocyst (GQB) rates were also compared.

Participants/materials, setting, methods: Overall, 1424 cycles, with at least one mature oocyte recovered, were enrolled in the present investigation. Oocytes were fertilized either using standard IVF or ICSI procedures. Embryos were cultured using appropriate media at 37°C under 6% CO_2, 21% O_2 atmosphere, for 2, 3, or 5 days either in conventional large-box incubator or in benchtop/topload incubators

Main results and the role of chance: Overall, mean day 2 or 3 TQE (38.4 ± 30.6 *vs.* 39.1 ± 30.2%; 23.3 ± 22.0 *vs.* 24.1 ± 22.7%, respectively) and GQE (53.0 ± 31.0 *vs.* 53.7 ± 31.0%; 47.3 ± 28.7 *vs.* 49.9 ± 28.0%, respectively) rates did not differ between conventional and benchtop/topload incubators groups. However, IR (21.6 *vs.* 16.7%; p = 0.01) and CPR per embryo transferred (32.7 *vs.* 26.3%, *p = 0.04*) were significantly increased after embryo culture in individual small incubator chambers. When extended culture was decided, both blastulation (89.1 ± 25.2 *vs.* 82.0 ± 30.0%, *p = 0.03*) and GQB (37.7 ± 34.0 *vs.* 29.7 ± 28.5%, *p = 0.04*) rates were significantly higher in benchtop/topload incubators (n = 197 cycles) when compared with a large-box conventional system (n = 103 cycles)

Limitations, reasons for caution: The major limitation of the present investigation is the lack of randomization.

Wider implications of the findings: The present study, based on the analysis of embryo morphology, constitutes the first demonstration of the superiority of small individual incubator chambers over conventional large-box incubators for optimizing embryo viability, blastulation and GQB rates following culture. As a result, these benchtop/topload incubators should be preferred to others in ART laboratories

Trial registration number: Not applicable.

P-235 Comparison of vitrified-warmed blastocyst transfer in natural and artificially prepared menstrual cycle

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Study question: Is the reproductive outcome of vitrified-warmed blastocyst transfer in natural cycle comparable to artificial cycle protocol?

Summary answer: The clinical pregnancy rate is comparable in both protocols for endometrium preparation. The live-birth rate was higher in natural cycle due to lower miscarriage rate.

What is known already: In recent years, frozen embryo transfer (FET) has become a very important part of in vitro fertilization program. Still, it is not clear what is the optimal method of endometrium preparation. To date, meta-analyses comparing artificial cycle (prepared by oestrogen and progesterone) and natural cycle showed no difference in terms of clinical pregnancy rate (CPR) and live-birth rate (LBR). Nevertheless, some investigators have reported higher CPR and lower miscarriage rates in natural cycles. Excessive oestrogen environment or suboptimal ratio between progesterone and oestradiol has been suggested as a possible cause of less favourable reproductive outcome in artificial cycles.

Study design, size, duration: All vitrified-warmed blastocyst transfers (n = 1,865) performed during a period of 3 years (2013–2015) at the Department for reproductive medicine, University Medical Centre Maribor were included in this retrospective study. In women with regular ovulatory cycles, vitrified-warmed blastocyst transfer was performed in natural cycle without hCG triggering (NC). Endometrium preparation by oestrogen and progesterone without GnRH agonist suppression (AC) was used in women with unpredictable or irregular cycles.

Participants/materials, setting, methods: Patients' characteristics and clinical data were collected from our software database. Reproductive outcomes between AC and NC were compared using Pearson's Chi-squared test. Univariate and multivariate logistic regression models were constructed to identify the factors that were significantly associated with the LBR and clinical pregnancy loss.

Main results and the role of chance: In the study period, there were 1,317 vitrified-warmed blastocyst transfers performed using NC and 528 using AC. The clinical pregnancy rate was comparable in both groups (37.0% vs. 36.6%), but there was statistically significant higher live-birth rate (32.8% vs. 26.7%, p = 0.01) and lower clinical miscarriage rate (13.7% vs. 27.3%, p < 0.001) in NC compared to AC group. In the multivariate logistic regression model, women's age (OR: 0.94, 95% CI: 0.91–0.98), transfer of morphologically optimal blastocysts (OR: 1.72, 95% CI: 1.28–2.31) and the type of endometrium preparation (OR: 0.63, 95% CI: 0.45–0.88) were important independent prognostic factors for live birth. The only independent factors that were significantly associated with pregnancy loss were women's age (OR: 1.07, 95% CI: 1.02–1.13) and the type of endometrium preparation (OR: 2.26, 95% CI: 1.39–3.68). Endometrial thickness, number of blastocysts transferred, cause of infertility, cycle irregularity, assisted hatching and prior birth after fresh transfer were not found to be associated with reproductive outcome.

Limitations, reasons for caution: This is a single-centre retrospective analysis and despite robust methodological approach, the presence of potential bias cannot be excluded.

Wider implications of the findings: Results of our study suggest that in women with regular cycles FET in natural cycle should be recommended. Larger prospective randomized multicentric studies are needed to confirm these findings. There is a need for further basic studies to optimise endometrial preparation with hormone supplementation.

Trial registration number: None.

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P-236 Analysis of embryo morphokinetics as a probable predictive variable of euploid embryos by time-lapse monitoring technique

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Study question: Does time-lapse monitoring (TLM) analysis of blastocysts has a predictive value in depicting aneuploid embryos?

Summary answer: There is a different morphokinetic behavior observed on TLM between euploid and aneuploid embryos after next generation sequencing (NGS) testing.

What is known already: Embryo morphokinetics have been observed to be different between euploid and aneuploid embryos. In 2014, Basile et al introduced a proposed algorithm as a predictive tool to classify embryos in order to increase the probability of noninvasively selecting chromosomally normal embryos after arrayCGH. Although the safety of trophectoderm biopsy on embryo survival has been validated, the request for a non-invasive method for aneuploidy prediction is required. There has been an ongoing debate on the reduced implantation potential of biopsied embryos and therefore development of a model based on morphokinetics is valuable.

Study design, size, duration: 161 blastocysts generated after intracytoplasmic sperm injection (ICSI) from 46 patients were included in this study. The time period of this retrospective observational cohort study is between January 2016 to January of 2017. The patient data was divided into different age groups (≤35, 36–39 and ≥40 years old). The blastocysts were divided into two groups: euploid and aneuploid embryos after performing NGS.

Participants/materials, setting, methods: Patients for preimplantation genetic screening (PGS) were included. The conditions for PGS were recurrent implantation failure, recurrent pregnancy loss and advanced maternal age. The embryo development was retrospectively analyzed using a time-lapse imaging system (Embryoscope, Unisense Fertilitech). Different kinetic parameters were considered in this study calculated in hours post-insemination (t2, t3, t4, t5, tM, tsB, tB, cc2, cc3 and s2). The Student's t-test was used for the statistical analysis of the data.

Main results and the role of chance: We observed that the time from insemination to blastocyst formation (tB) was statistically significant in all age groups when comparing euploid and aneuploid embryos. In the ≤ 35 year-old group, the tB was 97.1 ± 9.6 hours for euploid embryos versus 106.9 ± 8.9 hours for aneuploid embryos (p value: 0.017); in the 36–39 year-old group, tB was 103.5 ± 7.8 hours versus 108.7 ± 9.9 hours respectively (p value: 0.015) and in the ≥40 year-old group, tB was 97.1 ± 6.7 hours versus 107.1 ± 9.9 hours (p value: 0.005). The delay in blastocyst formation in aneuploid embryos is particularly noted in the advanced maternal age group and the role of chance is that in ≥ 40 year-old patients, we require more blastocysts (ratio 1:8) to detect one euploid embryo. This is the first study that has observed this morphokinetic difference in comparison to aneuploidy rates using NGS technique.

Limitations, reasons for caution: Larger studies with an increased number of blastocysts are needed to confirm our findings.

Wider implications of the findings: The morphokinetic analysis of the embryo can be used as a non invasive tool for selecting euploid embryos.

Trial registration number: Not applicable.

P-237 The influence of clinical and laboratory factors on the formation of monopronucleated zygotes after intracytoplasmic sperm injection (ICSI)

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Study question: Do clinical or laboratory factors affect the development of monopronucleated zygotes (1PN-2PB) after ICSI?

Summary answer: 1PN-2PB zygote formation is associated with impaired sperm motility, higher number of oocytes retrieved, higher number of MII and longer time to injection.

What is known already: The formation of 1PN-2PN zygotes is an unexpected complication of ICSI. It has been suggested that they can be the product of asynchrony during pronuclear formation, result of syngamy or of oocyte parthenogenetic activation. However, recent data from time-laps monitoring have not confirmed any of these theories: recent reports show that 1PN-2PB ICSI are zygotes with maternal and paternal genomes combined together and surrounded by an irregular membrane, most of which are chromosomally abnormal even if they develop up to the blastocyst stage. However, none of these reports investigate any possible relation between clinical or laboratory factors and 1PN formation.

Study design, size, duration: This was a retrospective comparative study. ICSI cycles were reviewed and analyzed according to the occurrence of 1PN-2PB zygotes at fertilization after ICSI and divided into two groups: cycles with no 1PN-2PB (control group, A) and cycles with one or more 1PN-2PB (study group, B). Adjusted odds ratios were calculated by multivariable logistic regression.

Participants/materials, setting, methods: A total of 341 ICSI cycles performed at FertiClinic -Villa Margherita from January 2012 to December 2016 were enrolled in the study. Group A included 240 cycles with no 1PN-2PB while the Group B included 101 cycles with 1 or more 1PN-2PB. Age, stimulation protocol, infertility factor, amount of gonadotrophin administered, peak estradiol levels, number of follicles, oocytes retrieved and mature oocytes, time between retrieval and injection and sperm characteristics were compared between groups.

Main results and the role of chance: Compared to patients in group A, those in group B showed a higher number of astenozoospermic male patients, 28.7% (68/237) vs. 45.3% (43/95) respectively, ($p = 0.004$). The two groups differed also in the median(Q1-Q3) number of oocytes retrieved at pick-up, 5(3–8) for group A vs. 11(7–15) for group B ($p < 0.001$) and in the median number of MII, 4(2–7) for group A vs. 9(6–12) for group B, ($p < 0.001$). A statistically significant difference was observed also in the length of time interval between hCG administration and ICSI, specifically, group B showed a longer time interval 39 h(39-40 h) with respect to group A, 38 h(38-39 h), $p < 0.001$. The final logistic multivariable model confirmed all the associations: group B showed a higher incidence of astenozoospermia (OR 2.15, 95% CI = 1.20–3.86, $p < 0.010$), a higher number of oocytes retrieved (OR 1.12, 95% CI = 1.07–1.17, $p < 0.001$), higher number of mature oocytes (OR 1.18, 95% CI = 1.11–1.25, $p < 0.001$) and longer time to injection (OR 2.13, 95% CI = 1.50–3.03 $p < 0.001$) compared to group A.

Limitations, reasons for caution: This was a retrospective observational study. A prospective study where the influence of 1PN-2PB zygotes on clinical outcomes is examined should be conducted also to understand if their occurrence has a prognostic value to predict the fate of the remaining cohort of zygotes and the final outcome of the cycle.

Wider implications of the findings: This study demonstrate that 1PN occurrence can be associated also with clinical or laboratory factors, thus, clinicians and embryologists should be respectively aware that enhancing ovarian stimulation in order to increase the number of CCOCs retrieved or delaying the time of injection can raise the probability of 1PN formation.

Trial registration number: none.

P-238 When should cleavage stage embryos be biopsied?

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Study question: To evaluate the precise biopsy timing during the 8 cell stage on day 3 for which the embryos will have the best implantation potential.

Summary answer: Our results demonstrate that biopsying fast-cleaving embryos during 15–20 h post t8, has the least effect on their further development.

What is known already: Blastomere biopsy at the cleavage stage is routinely used for women with a genetic indication who undergo PGD. Embryo biopsy is

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usually performed on the morning of day 3 when the embryos are at the 6- to 8-cell stage. Human embryos usually remain at the 8-cell stage for a relatively long "arrest phase" in which cells grow, duplicate their DNA and synthesize various proteins in preparation for the subsequent division.

Study design, size, duration: We studied 195 embryos that underwent blastomere biopsy for PGD between 9/2012–6/2014 and were cultured in the EmbryoScope™. The control group included 115 embryos from ICSI cycles (not biopsied) performed during the same period and cultured until day 5, resulting in good-quality blastocysts (3AA, 3AB, 3BA 4AA, 4AB, and 4BA). Time points of key embryonic events were annotated and analyzed with an EmbryoViewer.

Participants/materials, setting, methods: Biopsy during the 8-cell stage was divided into 4 quarters: during the first 5 h post-t8 (Q1), at 5–10 h post-t8 (Q2), at 10–15 h post-t8 (Q3) and at 15–20 h post-t8 (Q4). The non-biopsied control embryos were divided into 4 equivalent quarters. The study embryos were evaluated for timing of developmental events following biopsy, and those were compared between PGD and control embryos as well as with 56 PGD implanted embryos with known implantation data (KID positive).

Main results and the role of chance: Embryos that were biopsied during Q3 were delayed in all 3 subsequent developmental events, including first cleavage after biopsy, compaction (tM) and start of blastulation (tSB). In contrast, these events in embryos that were biopsied during Q1, Q2 or Q4 of the 8-cell stage occurred exactly at the same time as in the control group.

The results also show that embryos that were biopsied during Q1, Q2 or Q3 of the 8-cell stage demonstrated a significant delay compared to the biopsied implanted embryos already in t8 as well as in tM and tSB. However, embryos that were biopsied during Q4 demonstrated dynamics similar to those of the biopsied implanted embryos in t8 and tM, with only a delay observed in tSB.

Limitations, reasons for caution: This is a retrospective study that is limited to the timing of biopsy routinely performed in IVF. A prospective study in which biopsies are performed at a desired timing is needed in order to differentiate between the effect of the biopsy per-se and the effect of its natural cleavage rate.

Wider implications of the findings: When selecting biopsied embryos for transfer, it may be preferable to choose the fast-cleaving embryos that were biopsied during Q4 of the 8-cell stage. Those embryos are probably less affected by the biopsy procedure, and their implantation potential is better than that of embryos biopsied earlier during the 8-cell stage.

Trial registration number: not applicable.

P-239 Embryological outcomes in patients with previous developmental problems: a sibling study using Ca2+ ionophore

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Study question: Can Ca2+ ionophore improve embryological outcomes in patients with previous developmental problems?

Summary answer: Although statistically not significant, the rate of usable embryos was higher in the group of oocytes treated with Ca2+ ionophore.

What is known already: The use of calcium ionophore in IVF was initially recommended for couples with severe male infertility, previous history of fertilization failure or major morphological sperm defects like globozoospermia. Recently, a prospective multicenter study including patients with complete embryo developmental arrest in a previous cycle, complete developmental delay or reduced blastocyst formation on day 5 showed that application of Ca2+ ionophore leads to increased rates of cleavage to 2-cell stage, blastocyst formation and clinical pregnancy/live birth (Ebner et al., 2015). In this study, preceding cycles of the same patients constituted the control group.

Study design, size, duration: This retrospective study comprised a two-year period (2015-2016). Patients with previous poor embryo development (n = 73) and without severe male factor were included in the study. In the treatment cycles, half of the metaphase II (MII) oocytes were exposed to a commercially available ready-to-use ionophore for 15 min immediately after ICSI. After a three-step washing procedure, in vitro culture was performed in low oxygen tension using a single-step culture medium as for the control sibling oocytes.

Participants/materials, setting, methods: The mean age of patients was 31.2. The mean number of cumulus oocyte complexes was 14.2. A total of 5.1 MII oocytes were allocated for treatment with Ca2+ ionophore. The mean number of control sibling oocytes was 6.6.

Main results and the role of chance: The fertilization rate was 68.9% and 66.8% for the oocytes with and without ionophore treatment, respectively. The rate of day 3 grade 1 embryo rate was higher in the absence of treatment (30.4% and 25.2%), although not reaching statistical significance ($p = 0.19$). On day 4, the grade 1 embryo rate was 24.4% and 22.9% for the oocytes with and without ionophore treatment, respectively. Similarly, the rate of blastocyst formation was slightly higher for the group subjected to Ca2+ ionophore (43.7% vs. 42.2%; $p = 0.6$). The good quality blastocyst rate was of 21.7% and of 20.1% for the oocytes with and without ionophore treatment, respectively ($p = 0.68$). Finally, the usable embryo rate (including transferred and frozen embryos) was superior when Ca2+ ionophore was used (34.9% vs. 29.2%).

Limitations, reasons for caution: Although the sibling approach allowed a realistic comparison of embryo development, statistical significance could not be reached.

Wider implications of the findings: Ionophore treatment can benefit to patients with compromised embryo development. However, the effect on pregnancy outcomes needs to be addressed in prospective randomized trials.

Trial registration number: Not applicable.

P-240 What is the effect of follicle flushing on oocyte yield and quality in ART cycles of poor responders?

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Study question: Is there any role of sequential follicle flushing on oocyte number and quality in poor responders?

Summary answer: By sequential flushing, number of retrieved oocytes were increased additional to no detrimental effect on quality.

What is known already: Although previous studies have shown no beneficial effect of follicular flushing in normal responders, it may result in lower implantation and clinical pregnancy rates in poor responders. However, no study have demonstrated the net effect of sequential aspiration-flushing on oocyte quality. Due to the limited number of studies in poor responder patients, it is still not so clear whether flushing may increase the oocyte retrieval or not.

Study design, size, duration: This preliminary prospective cohort study was aimed to determine whether sequential follicular aspiration-flushing increases oocyte number and to evaluate the effect of follicle flushing on oocyte quality, in 45 poor responder patient according to Bolognia criterias who underwent standard Antagonist protocol. 17-gauge double lumen needle was used to aspirate the follicles. After each aspiration if cumulus-oocyte-corona complexes (COC) was not obtained, 2-3 ml aspiration medium was reinjected and aspirated three times maximally.

Participants/materials, setting, methods: Each oocyte was investigated according to 6 criteria for quality: morphology, cytoplasm, perivitelline space, zona pellucida, polar body and oocyte size, so establishing an average total oocyte score (TOS) as offered by Lazzaroni-Tealdi-et al. 2015. Average TOS values for oocytes obtained by directly/non-flushing group (group 0) compared with oocytes obtained by flushing 1 (group 1), flushing 2 (group 2), flushing 3 (group 3).

Main results and the role of chance: Average age for all patients was 37.5 ± 5.3 years and mean oocyte yield was 3.27 ± 2.29. 50 (35.2%), 34 (23.9%), 38 (26.8%) and 20 (14.1%) oocytes were aspirated in group 0, 1, 2, and 3. There was no significant difference between Median TOS values of group 0: 3 (range -2 to 5) compared to group 1: 2.5 (range -1 to 5); group 2: 3

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(range -2 to 6); group 3: 3 (range -2 to 5) and average value for all oocytes obtained by flushing: 3 (range -2 to 6).

Limitations, reasons for caution: According to this preliminary study, we reported the results of 142 separately evaluated oocytes. Implantation rates should also be evaluated after the investigation of more oocytes.

Wider implications of the findings: This was the first preliminary study in literature that evaluated the effect of aspiration-flushing on oocyte yield and quality follicle by follicle. We demonstrated an increase in the number of oocytes retrieved by sequential flushing. Additionally, serial flushing had no negative impact on the oocyte quality.

Trial registration number: -.

P-241 Single or group embryo culture in an oocyte donation programme: a prospective, randomized sibling-oocyte study

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Study question: Is there a difference in embryo development between single and group embryo culture by terms of blastocyst rate and top quality blastocyst rate?

Summary answer: Group culture embryos are characterized by a trend for higher, not statistically significant, top-quality blastocyst formation rate.

What is known already: Obtaining the optimal culture conditions that improve embryo development and IVF outcomes, is still an issue of great importance. Studies in mice and bovine models suggested that group culture ameliorates blastocyst formation. However, even though group culture of human embryos has not been reported to be detrimental, it is controversial if it has an impact on embryo development in reference to blastocyst formation, overall blastocyst quality and pregnancy rate. Embryo density and droplet volume are determining variables for the secretion of autocrine factors/accumulation of paracrine byproducts but few studies have evaluated them.

Study design, size, duration: A prospective, randomized, comparative study was performed from May 2016 to December 2016. All cases were participating in oocyte donation program of Iakentro IVF Center and followed ICSI. For each case, the injected oocytes were randomly allocated in Control Group and Study Group and cultured individually and in group, respectively.

Participants/materials, setting, methods: A total of 1915 sibling oocytes were used for the study, 1008 in the Control Group and 907 in the Study Group. The Control Group droplet was 30 µl while for the Study Group were used two different volume droplets, a 30 µl droplet or a 60 µl droplet. Blastocyst formation rate and top quality blastocyst rate were the main outcomes.

Main results and the role of chance: On day-5, no statistically significant difference was observed in the blastocyst formation rate between Control and Study Group, in both 30 µl (58.6% vs. 61.1% respectively, $p = 0.29$) and 60 µl (57.8% vs. 58.5% respectively, $p = 0.3$) Study Group droplets. However, a tendency for top quality blastocysts was observed in both 30 µl (28.5% in Control Group vs. 32.5% in Study Group) and 60 µl (30.5% in Control Group vs. 35% in Study Group) Study Group droplet even though no statistical significant difference was detected ($p = 0.08$ and $p = 0.09$ respectively). As it refers to pregnancy rates, the opposite trends were observed. In the oocytes of the 30 µl Study Group droplet we observed a reduction in pregnancy rate (68% in Control Group vs. 53% in Study Group, $p = 0.3$) which is the opposite from what observed in the oocytes of the 60 µl Study Group droplet (66% in Control Group vs. 75% in Study Group, $p = 0.7$).

Limitations, reasons for caution: Being a study with oocytes from a donation program, the results may not be reproducible in cases of older patients. Moreover, the number of cases should be increased in order to confirm the above data and extend the clinical outcomes.

Wider implications of the findings: We speculate that the decrease in pregnancy rate that is observed in the 30 µl Study Group droplet is due to the restricted amount of nutrients per embryo and the higher accumulation of embryo byproducts when many embryos are cultured together in small volume of media.

Trial registration number: None.

P-242 Efficiency of a closed vitrification system for the cryopreservation of human oocytes: a case series study

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Study question: Can a closed-aseptic vitrification system be compared in efficiency to the widely adopted but under criticism, open systems for the cryopreservation of oocytes?

Summary answer: The closed vitrification system applied can be as efficient as widely adopted open ones in terms of survival, fertilization, blastocyst formation, pregnancy and implantation rates.

What is known already: Many vitrification procedures have been described that differ from each other in many parameters and most fundamentally, in the type of cooling and storage device. These either permit or not the direct contact of the biological material with the liquid nitrogen and are characterized as open and closed vitrification systems, respectively. Nevertheless, a controversy about the potential risk of the sample from its direct contact with the liquid nitrogen still exists. For this purpose, closed vitrification systems have been proposed and validated as equally effective but yet not widely adopted.

Study design, size, duration: A case series study was performed, including a total of 300 oocytes, retrieved from 6 stimulated cycles of patients undergoing IVF treatment and of 24 donors participating in our Unit's (Newlife IVF-Greece) oocyte donation program, between December 2015 and November 2016.

Participants/materials, setting, methods: All retrieved oocytes after denudation underwent vitrification using FertiPro Vitrification kit (FertiVit Cooling kit). After their exposure to the equilibration and vitrification solutions, they were loaded on the gutter of the vitrification carrier (VitriSafe), inserted into a 0.3 ml straw (CBS), thermo sealed and submerged in liquid nitrogen. Warming was performed using Fertipro warming Kit (FertiVit Warming kit). All survived oocytes were fertilized by ICSI.

Main results and the role of chance: Regarding the homologous cycles, a total of 48 oocytes underwent vitrification. The survival rate after warming was 87,5 % (42/48) and the fertilization rate after ICSI was 85,7 % (36/42). The blastocyst formation rate was 33,3 %. Four out of 6 had a positive βHCG (clinical pregnancy rate 66,6 %). Regarding the donor cycles, a total of 252 oocytes underwent vitrification. The survival rate after warming was 92,8 % (234/252) and the fertilization rate was 68,3 % (160/234). The blastocyst formation rate was 40% (64/160). Eighteen out of 24 cases had a positive βHCG (clinical pregnancy rate 75%). In 6 of these cases a severe male factor was detected and sperm from a testicular biopsy was used. Four out of 6 had a positive βHCG. In total, from these pregnancies, 14 are still ongoing while 8 had led to full term delivery giving birth to 10 healthy babies.

Limitations, reasons for caution: Small sample size of the study participants is due to the rarity of such cases. Larger prospective trials with an increased number of patients are needed to confirm our findings.

Wider implications of the findings: Closed vitrification system could be efficiently applied for oocyte cryopreservation in cases of urgent fertility preservation or due to male incapability to produce semen on the day of oocyte retrieval. Furthermore, could be applied as a standard procedure in oocyte banks cryopreservation programs increasing the total safety conditions.

Trial registration number: n/a.

P-243 Autologous Endometrial Cell Co-culture (AECC) significantly improves human embryo quality

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Study question: Does Autologous Endometrial Cell Co-culture (AECC) clinically improve IVF treatment outcomes?

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Summary answer: AECC increases the number and quality of blastocysts, as well as their survival post vitrification, compared to conventional culture medium.

What is known already: Studies have demonstrated the benefits of AECC in terms of embryo maturation and pregnancy outcome. However, the majority of these studies were conducted in specific clinical cases of infertility, such as poor ovarian reserve and repeated implantation failure. Our group focused on demonstrating the potential beneficial effects of AECC in patients predicted to be good responders to IVF treatments.

Study design, size, duration: This interventional study took place at **clinique ovo** from April 2013 to March 2015, as a monocentric, randomized, double-blind controlled trial. A total of 1398 mature ovocytes from 136 couples undergoing IVF were analysed: 326 embryos from 63 patients were cultured on an autologous co-culture system of endometrial cells (EC) (study group) and 573 embryos from 73 patients in conventional culture medium (control group); 475 ovocytes, unsuccessfully fertilized, were excluded from the current analysis.

Participants/materials, setting, methods: A biopsy of the endometrium was performed during the luteal phase of the cycle before IVF treatment. EC isolated from biopsies were put in culture the day following ovulation triggering. According to randomization group, embryos were either placed at day 2 (D2) on AECC, or in conventional culture medium. All IVF cycles were then pursued following clinic standards.

Main results and the role of chance: Our results demonstrate that the use of a co-culture system for embryo development increases the number of available top quality blastocysts (TQB) per cycle.

Growth on AECC was found to significantly increase the number of useful embryos compared to conventional culture medium (46% vs. 35%, respectfully; $p < 0.05$), as well as significantly improved blastulation rate (42.6% vs. 28.8%; $p < 0.001$). Predictors of blastulation [OR(95%CI)], identified using logistic regression models, were AECC cohort [4.819(3.599, 6.452); $p < 0.001$], and higher number of mature oocytes [1.046 (1.022, 1.071); $p < 0.001$].

In addition, blastocyst survival rates showed improvement after AECC, with a rate of 85.4% in the AECC group, compared to 73.8% in the control group. Following frozen embryo transfers, pregnancy outcomes, assessed using live birth rate, were higher after AECC (34.1%) than following conventional medium culture (25.6%), for all stages of embryo growth. Although not found to be statistically significant, these improvements in survival and live birth rates are nonetheless of clinical relevance in the context of IVF.

Limitations, reasons for caution: N/A

Wider implications of the findings: AECC utilisation increases the number of available embryos per cycle, thus improving IVF treatments, Moreover, the quality blastocysts obtained from AECC show improved survival and pregnancy rates after frozen embryo transfer.

Trial registration number: NCT01886118.

P-244 Differential human blastocyst vitrification: A randomized comparative trial assessing solution and device treatments under varying laboratory conditions

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Study question: How is human blastocyst vitrification influenced by different solutions in an open or closed device system using a crossover re-vitrification (rVTF) experimental model?

Summary answer: Human blastocysts rVTF success proved vulnerable to a technicians' device and solution familiarity and may be adversely influenced by total exposure to different cryoprotective agents.

What is known already: Human blastocysts vitrification has evolved into a highly reliable and efficient process within most laboratories. Slower cooling, closed aseptic vitrification devices have proven to be equally effective to ultra-rapid cooling open methods. Yet, variation between laboratories in the type of cryoprotective agent or device system used has created some concerns. Less toxic, potentially unstable vitrification solutions (e.g., 30%EG/DMSO) are used widely in open device systems, while a more concentrated, metastable solution (e.g., Glycerol/EG) may be more appropriate with a closed device. There have been no comparative trials contrasting the potential advantages or disadvantages of these commonly used clinical systems.

Study design, size, duration: Research consented, discard blastocysts at 2 laboratories (A/B; n = 40 and 41 embryos, respectively; 4 technicians/lab) were randomly assigned to a 2×2 factorial design: comparing open-Cryolock (CL) devices to a closed-microSecure (μS) vitrification system using either a EG/DMSO (15/15% solution) or a Glycerol/EG (G/EG;≥7.9 M) vitrification solution. Differences in recovery rate, %survival and %24hr development were statistically compared by Chi-square analysis ($p < 0.05$). Additionally, a series of objective survey questions pertaining to potential device usage differences were contrast.

Participants/materials, setting, methods: Vitrified blastocysts (AA to BB quality) were obtained from patients consenting to research discard usage. Standard rapid warming/sucrose dilution protocols were applied consistent with the device-solution treatment combinations. Embryos were allowed to equilibrate in culture before being randomly selected for rVTF treatment, and subsequently warmed, re-equilibrated and cultured in a humidified, tri-gas atmosphere (37°C). Embryo survival assessments, including photo documentation, was performed at 0-2hr and +24hr (i.e.,continued development) post-warming across control and crossover treatments.

Main results and the role of chance: Laboratory A has previously shown that human blastocysts are highly cryotolerant to rVTF (up to 5 times) in their control μS-VTF/G-EG system. However, in this study Lab B (a control CL-EG/DMSO user) experienced decreased post-warming survival/development in μS-G/EG treated blastocysts (64% vs their 89%control) and a further decrease in the crossover treatments (30–36%). In contrast, Lab A showed no difference between control groups (100% survival and development) and only a 10–20% developmental decline in the CL-G/EG or μS-EG/DMSO groups. Overall, mean survival/ development in the CL-EG/DMSO control (100%/94.7%) was higher ($p < 0.05$) than CL-G/EG (81%/66.7%) or μS-EG/DMSO (75%/65%), but not different than μS-G/EG controls (81%/81%). Interestingly, survey responses reflected a laboratory bias toward their control treatments, however all technicians agree that μS-VTF was safer than open-blade devices (e.g.,CL) in terms of LN_2 handling procedures or potential embryo-pathogen interaction risk factors. As an aseptic closed device, μS-VTF offers greater long-term security and tamperproof labeling, as well as an appreciable annual cost-savings as a non-commercialized VTF system. In summary, technician familiarity and experience to vitrification devices and solutions had a greater effect than anticipated, revealing that a learning curve for the μS device and G/EG solution treatments is needed to optimize post-warming viability.

Limitations, reasons for caution: Additional trials, increased sample sizes and an additional collaborating laboratory are needed to fully discern the treatment effects of differential cryoprotective agent exposures causing damaging osmotic stress to vulnerable blastocysts. It is also possible that differences in blastocyst quality/cryo-stress vulnerability between laboratories may have contributed to rVTF survivability.

Wider implications of the findings: To reduce inter-laboratory variability and make continued improvements in global human blastocyst vitrification success, we must continue asking questions and challenge existing methodologies to fully understand the complexity of outcome data. This study has demonstrated the value and importance of implementing continuing education/quality control studies, even among well-trained Embryologists.

Trial registration number: None.

P-245 The association between vitamin A, E, D and B6 concentrations in follicular fluid with embryomorphokinetics, embryo quality and pregnancy rates in assisted reproductive techniques

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Study question: Is there any association between vitamin A, E, D and B6 levels in follicular fluid with embryomorphokinetics, embryo quality and clinical pregnancy rates?

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Summary answer: High follicular fluid vitamin A and B6 levels were significantly associated with high embryo quality and optimal embryo morphokinetics.

What is known already: There is a paucity of information regarding the roles of antioxidant vitamins (vitamin A, E, D and B6) in early embryogenesis, embryomorphokinetics and clinical pregnancy rates.

Study design, size, duration: Fifty eight patients with unexplained infertility who were admitted to the IVF center of Izmir University Medical Park Hospital between March 2016 and June 2016 were included in this prospective clinical study.

Participants/materials, setting, methods: For each patient vitamin A, E, D and B6 levels of follicular fluid was assayed by high performance liquid chromatography system. After ICSI of each oocyte the relation between level of vitamin A, E, D and B6 content of each follicular fluid and subsequent embryo quality, embryo morphokinetics and clinical pregnancy rates were investigated. Embryos were classified as grade 1,2,3,4 according to morphokinetic parameters using t5-t2 and t5-t3 (cc3)

Main results and the role of chance: Follicular fluid vitamin A and B6 levels were significantly higher in Grade 1-2 embryos than grade3, 4 embryos ($p < 0.05$). There were no significant relation between follicular fluid vit E and D levels and embryo quality. There were no significant relation between vitamin A, E, D and B6 levels in follicular fluid and clinical pregnancy rates.

Limitations, reasons for caution: Relatively small sample size might be a limitation of our study.

Wider implications of the findings: Our results support the role of vitamin A and B6 in early embryogenesis and embryomorphokinetics. Vitamin A and B6 supplementation might be beneficial to improve embryo quality. Further research is necessary to find out the clinical effects of these vitamins in in vitro fertilization outcomes.

Trial registration number: none.

P-246 Embryo selection: are we looking at the full picture?

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Study question: Is day-3 (D3) embryo selection improved by examining all embryos simultaneously in a cohort compared to individually evaluating and selecting them based on morphology measurements and/or morphokinetic parameters?

Summary answer: D3 embryo cohort selection improves identification of embryos most likely to develop into high-quality blastocysts when compared to using standard morphology and morphokinetic selection methods.

What is known already: Recent attention has been focused on the ability of non-invasive computer-automated morphokinetic measurements to predict blastocyst formation with the ultimate goal of successful single cleavage-stage embryo transfer. Studies have shown that adjunctive use of standard morphology scoring plus morphokinetic parameters improves embryo selection by enabling the deduction of embryos unlikely to develop to the blastocysts. In traditional methods of embryo grading, embryos have always been evaluated individually and ranked accordingly for transfer. However, simultaneous evaluation of patients' entire embryo cohort at a single time-point to predict high-quality blastocysts has never been looked at.

Study design, size, duration: Study Design: Retrospective case-control study.

Size: 50 infertile women with their entire cohort of embryos imaged in the EmbryoScope from Day 1 to Day 5 and who had at least one blastocyst on day-5 of development.

Duration: September 2014 - December 2015.

Participants/materials, setting, methods: Top quality embryos from each patient cohort were selected based on: 1) D3 morphology (D3M), 2) D3M and validated morphokinetic parameters (P2 and P3 time-lapse scoring) (TLM), 3) D3 embryo cohort selection performed individually by 3 embryologists, blinded to D3M and TLM results, who selected the top quality embryo from a single image taken at 68hrs of all embryos in the cohort (CM).

Statistics: Chi-square test. $P < 0.05$ was considered statistically significant.

Main results and the role of chance: The overall blastocyst formation prediction rate was higher with the CM when compared to D3M and TLM (95% vs 86% and 89%; $p = 0.06$ and $p = 0.21$, respectively). Similarly, predicting high quality blastocyst formation (expanded blastocysts with good ICM and trophectoderm) was higher with the CM when compared to both D3M and TLM (75% vs 66% and 67%; $p = 0.21$ and 0.33, respectively). The prediction rate for overall and high quality blastocyst formation between D3M and TLM was similar.

Limitations, reasons for caution: Limitations include:

- retrospective design
- the completion at a single academic center with higher blastocyst prediction rates using D3M than that reported in the literature, indicating the need for larger numbers to prove potential statistical significance in the differences seen
- only one time point (D3) was evaluated, potentially masking other important time-points

Wider implications of the findings: This improved method of identifying embryos with the highest potential to form a blastocyst, may not only improve success of cleavage-stage transfers in a cost-effective manner but also aid in the development of deep learning models designed to identify top quality embryos from a patient's entire embryo cohort.

Trial registration number: Not applicable.

P-247 Impact of morphological parameters at high magnification, nuclear structure and ultrastructure of spermatozoa on ART outcomes

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Study question: Whether morphological parameters at high magnification, nuclear structure and ultra-structure of spermatozoa can effect on ART outcome in patients with male infertility.

Summary answer: Head size of Spermatozoa and large nuclear vacuoles are significant relation with ART outcome. TEM assessments revealed that origin of vacuoles are mainly nuclear.

What is known already: Sperm morphology is one of the important characteristics in semen analysis for evaluation of male fertility. Emerging high magnification evaluation of sperm morphology has improved some aspects of the infertility treatment in assisted reproductive technology such as repeated implantation failure.

Study design, size, duration: This study was performed as a prospective observational study. 113 men diagnosed with male infertility were randomly selected for the study from March 2013 to July 2015 at Yazd Research and Clinical Center for Infertility.

Participants/materials, setting, methods: Men undergone ICSI treatment were randomly selected, of whom 99 cases met inclusion criteria. Smears of CMA3 were prepared and examined after density gradient sperm washing and preparation. Suspended spermatozoa introduced 3 µl of PVP micro-droplets were then evaluated at high magnification (> 6000×), upon MSOME parameters including: Cassuto-Barak criterion, presence of vacuoles, size and number of vacuoles, measurement of the head length, head shape, presence of cytoplasmic droplet and shape of acrosome.

Main results and the role of chance: In terms of vacuole presence, only 8% of spermatozoa had no vacuoles and mix vacuoles allocated the highest rate. Moreover, around 40% of spermatozoa had normal size, 8% showed normal shape, 51% illustrated normal acrosome and 40% with cytoplasmic droplets. There were no correlation between different types of vacuole and embryo

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quality and pregnancy rate; while, spermatozoa having large vacuole revealed an inverse correlation with fertilization rate (p = 0.05). On the other hand, pregnancy rate showed a direct relationship with normal size of spermatozoa (p = 0.045), though no significant correlations were found with other MSOME parameters such as shape of head, presence of cytoplasmic droplet and shape of acrosome. There was no relevance between these parameters and quality of embryo. In addition, there was a significant correlation between spermatozoa having no vacuole and parameters of normal head size, normal shape and normal acrosome rates (p = 0.012, p < 0001 and p < 000, respectively).Regarding chromatin packaging, $CMA3^+$ showed a direct significant relationship with parameters of mix vacuole and sperm count (p = 0.007 and p = 0.012, respectively) and an inverse correlation with small vacuole and presence of cytoplasmic droplet (p = 0.027 and p = 0.012, respectively).

Limitations, reasons for caution: Quality of high magnification microscope still is not enough good and need to be better.

Wider implications of the findings: Our data showed, there were no significant correlation between Cassuto-Barak classes of spermatozoa with fertilization and quality of embryos rates.Similarly, there were no correlation between different types of vacuole and embryo quality and pregnancy rate. Some nuclear vacuoles appears to be a membranous folding of sperm head at TEM assessments.

Trial registration number: not applicable.

P-248 Antioxidants in IVF medium significantly improve embryo development

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Study question: What is the effect combined antioxidants Acetyl-L-Carnitine (ALC), N-Acetyl-L-Cysteine (NAC) and α-Lipoic Acid (ALA) on embryo development, when present in the IVF medium?

Summary answer: Combined antioxidants resulted in a significant increase in blastocyst cell number and faster developmental times from 5- cell stage through to expanded blastocyst stage.

What is known already: The combination of the antioxidants ALC, NAC and ALA in IVF media and/or culture media has a significant beneficial effect on embryo development.

Study design, size, duration: Morphokinetics of mouse embryos were quantitated using time-lapse imaging. Blastocysts underwent differential nuclear staining for inner cell mass (ICM) and trophectoderm (TE).

Participants/materials, setting, methods: IVF was conducted in atmospheric oxygen in the presence or absence of antioxidants (10 μM ALC / 10 μM NAC /5 μM ALA). Gamete collection and fertilisation was conducted in IVF medium with and without antioxidants and subsequent embryos cultured in 20% oxygen. Embryo development was analysed through time-lapse microscopy followed by differential nuclear staining to determine cell allocation in the blastocyst. Controls were gametes and embryos that were not exposed to antioxidants in the medium.

Main results and the role of chance: IVF was conducted with antioxidants present or absent in oocyte and/or sperm IVF medium and subsequent embryos cultured in medium without antioxidants. Embryos with antioxidants in both oocyte and sperm IVF medium developed faster to 2 cell cleavage stage (14.8 ± 0.1 h vs. 15.2 ± 0.1 h; $P < 0.05$) which continued to hatching blastocysts (85.9 ± 0.9 h vs. 89.1 ± 0.8 h; $P < 0.05$), and had higher TE cell numbers (49.8 ± 2.1 vs. 36.6 ± 2.0; $P < 0.01$) and total blastocyst cell numbers (61.9 ± 2.6 vs. 47.2 ± 2.4; $P < 0.001$), when compared to controls that had no antioxidants in oocyte and sperm IVF medium. Embryos with antioxidants solely in oocyte IVF medium had faster times to 2 cell cleavage stage (14.8 ± 0.1 h; $P < 0.01$) which continued to expanded blastocysts (81.2 ± 0.6 h; $P < 0.01$) and had increased TE cell numbers (46.5 ± 2.1; $P < 0.01$) and total blastocyst cell numbers (58.5 ± 2.5; $P < 0.05$) when compared to controls. Embryos with antioxidants only in the sperm IVF medium had no differences in developmental times and blastocyst total cell numbers (51.3 ± 2.2) compared to controls. There were no differences in fertilisation or blastocyst development rates between all groups.

Limitations, reasons for caution: Embryo development was only examined in the mouse.

Wider implications of the findings: The presence of antioxidants during IVF imparts significant benefits on embryo development rate and cell numbers. This indicates that supplementation of antioxidants to the IVF medium, especially during oocyte collection and fertilisation will further assist in maintaining the viability of human embryos in ART, through the reduction of oxidative stress.

Trial registration number: not applicable

POSTER VIEWING SESSION
ENDOMETRIOSIS AND ENDOMETRIAL DISORDERS

P-249 Endometriosis and adverse pregnancy outcomes: Systematic review and meta-analysis

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Study question: Are adverse pregnancy outcomes higher for spontaneous pregnancy in endometriosis-affected patients compared to general population?

Summary answer: In spontaneous pregnancies, endometriosis appears to be a risk factor of adverse pregnancy outcomes and especially of miscarriages, small-for-gestational-age fetuses and preterm births.

What is known already: The association between endometriosis and adverse pregnancy outcomes in spontaneous pregnancy has long been debated without reaching a consensus.

Study design, size, duration: We searched the Cochrane Library, MEDLINE and reference lists of eligible studies from inception to December 2015, without any restriction. We selected studies that compared endometriosis-affected pregnant women to disease-free pregnant women. To ensure the quality of the methodology, the PRISMA criteria have been met at all stages of the development of this meta-analysis.

Participants/materials, setting, methods: The primary adverse pregnancy outcomes studied were placenta-mediated outcomes, which include: pre-eclampsia, gestational hypertension, intra uterine growth restriction (IUGR), miscarriage, stillbirth, and placenta praevia. The secondary outcomes we considered were others adverse pregnancy outcomes including small-for-gestational-age new born (SGA), gestational diabetes, antenatal bleeding incidence, preterm birth and caesarian birth. Two reviewers independently extracted the studies' characteristics and outcome data. Finally, estimates were assessed using random effects models and sensitivity analyses.

Main results and the role of chance: Of 424 identified abstracts, 9 primary studies (8 cohorts, 1 case control) met our inclusion criteria by comparing spontaneous pregnant patients with endometriosis to disease-free women. Compared to the general population, endometriosis pregnant women experienced a higher incidence of SGA age new born (OR 1,39 [CI 95%; 1.07–1.80]). The risk of preterm birth was higher in the OSIS group (OR 1.40 [CI 95%; 1.22–1.62]), along with caesarian section (OR 1.75 [IC 95%; 1,58–1.95]) and antenatal bleeding (OR 1,95 [IC 95%; 1,07– 3,54]). Fetal loss was also increased in the OSIS group due to a higher miscarriage rate (OR 1,77 [CI 95% 1.13 – 2,78]). The hypertensive outcomes, gestational diabetes, IUGR and placenta praevia risks did not show a significant statistical difference between the two groups.

Limitations, reasons for caution: The main criticism of the analysis of available evidence is the heterogeneity in exposure categorizations, disease phenotypes and criteria for endometriosis diagnosis in studies included.

Wider implications of the findings: Further prospective studies or an individual patient-level meta-analysis are needed to confirm these results and to establish the exact link between endometriosis and adverse pregnancy outcomes in spontaneous pregnancy.

Trial registration number: No trial registration number.

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P-250 The efficacy of medroxyprogesterone acetate (MPA) in women with endometriosis undergoing controlled ovarian hyperstimulation for in vitro fertilization

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Study question: This study investigated using MPA for patients with advanced endometriosis and tubal infertility during COH and to compare cycle characteristics and pregnancy outcomes.

Summary answer: MPA is effective for women with endometriosis and normal ovarian function undergoing controlled ovary hyperstimulation.

What is known already: Progestins, synthetic progestational agents, have been used in endometriosis therapy for more than 40 years, and it is believed that they act as progesterone receptor agonists. Our previous work has shown that progesterone can prevent moderate/severe ovarian hyperstimulation syndrome (OHSS) in controlled ovarian stimulation. Subsequently, we have further found that MPA is an effective oral alternative for the prevention of a premature LH surge in woman undergoing COH, and the pregnancy outcomes from frozen-thawed embryo transfer (FET) indicated that the embryos originating from MPA co-treatment with human menopausal gonadotrophin (hMG) regimen had similar developmental potential as the short protocol.

Study design, size, duration: A retrospective case control study of 147 advanced endometriosis patients and 147 age-matched tubal infertility patients from November 2015 to October 2016. Patients were allocated to three groups: the surgery group (62 patients) were diagnosed with ovarian endometriomas by laparoscopy or laparotomy underwent 71 IVF/ICSI; the aspiration group (85 patients) had ovarian "chocolate" cysts (> 3 cm) that were aspirated underwent 90 IVF/ICSI; the control group (147 patients) with tubal infertility underwent 168 IVF/ICSI.

Participants/materials, setting, methods: This study investigated using MPA for patients with advanced endometriosis who have normal ovarian function and tubal infertility during controlled ovarian hyperstimulation and to compare cycle characteristics and pregnancy outcomes in subsequent frozen-thawed ET (FET) cycles. Human menopausal gonadotropin (hMG) and MPA were administered simultaneously beginning on cycle day 3 in tertiary-care academic medical center. Response to gonadotropins, fertilization, cleavage, implantation, and clinical pregnancy outcomes were recorded.

Main results and the role of chance: The mature oocyte rate and the fertilization rate were not significantly different between the two endometriosis groups and the control group. The cleavage rate was higher in the surgery group compared with the control group, but there was no significant difference between the aspiration and control groups. This might be because: 1) the endometriosis itself is unlikely to affect the quality of oocytes; 2) it is associated with a small sample size; 3) the application of progesterone may improve the egg or embryo quality of women with endometriosis. Viable embryo rate referred to number of high-quality embryos and blastocysts that were finally frozen, which did not show difference compared with the number of high-quality embryos frozen on D3. This may be because that P can improve the abdominal and ovarian microenvironment in patients with endometriosis, so as to improve the inflammatory reaction, thereby improving quality of oocytes and embryos. At the same time, in terms of pregnancy outcome, there were no significant differences in implantation rate and clinical pregnancy among the three groups. This again proved that for endometriosis patients with normal ovarian function, a high-P ovulation regimen is a very appropriate choice.

Limitations, reasons for caution: A major limitation is that it is a retrospective analysis; a well-designed, prospective, randomized study in severe advanced endometriosis patients undergoing FET and embryos derived from long-term pituitary downregulation should be performed. Another probable bias was the effect of granulosa cells and various immune factors during the MPA protocol.

Wider implications of the findings: The new treatment provides a novel insight into an alternative to the ultra-GnRH agonist regimen. Progestogens are generally well-tolerated, and may be used repeatedly. The role of MPA appears to be promising although many questions remain to be answered,the possible influence on embryo development potential and the hormonal milieu.

Trial registration number: none.

P-251 Intrafollicular inflammatory cytokines but not steroid hormone concentrations are increased in naturally matured follicles of women with proven endometriosis

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Study question: To assess whether the intrafollicular cytokine profile in naturally developed follicles is different in women with endometriosis, possibly explaining the lower reproductive outcome in endometriosis patients.

Summary answer: Intrafollicular inflammatory cytokines are increased in naturally matured follicles of women with proven endometriosis, which may contribute to the reproductive dysfunction in endometriosis.

What is known already: Altered levels of inflammatory cytokines have been found in the follicular fluid (FF) of endometriosis patients undergoing conventional, gonadotropin stimulated IVF (cIVF) and the hormonal milieu was altered in the FF of patients with endometriosis. A dysregulated intrafollicular hormone milieu and an abnormal intrafollicular cytokine profile might be a cause of reduced fertility in endometriosis patients. However, these findings were mostly obtained from cIVF in which the exogenous gonadotropins considerably affect the intrafollicular immune system and hormonal milieu. Natural cycle-IVF (NC-IVF) seems to be an adequate model for a physiological follicle and closely represent natural follicles.

Study design, size, duration: A matched case-control study was conducted at a University based infertility and endometriosis centre. The study population included 17 patients with laparoscopically and histologically confirmed endometriosis (rAFS II-IV), each undergoing one Natural cycle IVF (NC-IVF) treatment cycle between 2013 and 2015, and 17 age matched NC-IVF women without diagnosed endometriosis (control group).

Participants/materials, setting, methods: Follicular fluid and serum was collected at the time of follicle aspiration. The concentrations of inflammatory cytokines (IL-1β, IL-6, IL-8, IL-15, IL-18, TNF-α) and hormones (testosterone, estradiol, AMH) were determined in follicular fluid and serum by single or multiplexed immunoassay and compared between both groups.

Main results and the role of chance: In the FF, IL-1β and IL-6 showed significantly higher median concentrations in the endometriosis than in the control group ($P < 0.001$ and 0.010 respectively, $N = 17$). IL-8 and IL-18 were not significantly different. Moreover, IL-1β and IL-6 showed a tendency towards a dependence on the severity of the disease (rAFS stage) while no such trend was observed for the FF concentrations of IL-8 and IL-18. IL-15 and TNF-α could not be detected in the FF.

The four cytokines which were measurable in FF were also investigated in the serum. IL-1β and IL-6 could not be detected in the matching serum samples collected on the day of oocyte recovery, except for one result for IL-1β. The concentrations of all four interleukins were significantly higher ($p < 0.01$) in FF than in serum over the entire study population.

Regarding the hormones in FF, E_2 concentrations were lower in severe than in mild cases of endometriosis (without statistical significance) and in control women ($P = 0.036$). Median follicular AMH concentration was slightly increased with severe endometriosis (without reaching statistical significance), while testosterone did not differ between groups. In FF, hormone and cytokine concentrations did not correlate with the different endometriosis stages.

Limitations, reasons for caution: We did not prove that women of the control group were free of endometriosis as laparoscopy without any signs of endometriosis is not indicated and therefore ethically not acceptable.

Wider implications of the findings: In women with moderate and severe endometriosis some intrafollicular inflammatory cytokines are upregulated and not correlated with intrafollicular hormone concentrations. This might be due to the inflammatory microenvironment in endometriosis women, affecting follicular function and thereby possibly contributing to the reproductive dysfunction in endometriosis.

Trial registration number: No trial registration number.

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P-252 Clinical outcomes of infertility treatment for women with adenomyosis

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Study question: Does adenomyosis have negative impact on female fertility? Dose pre-treatment including medication and conservative surgery improve reproductive outcome?

Summary answer: The pregnancy rate of women with adenomyosis is about 40%. There was no data suggesting that medication or surgery increased the pregnancy rate.

What is known already: The relationship between adenomyosis and female fertility is still unclear. Adenomyosis can be classified into two categories, focal type and diffuse type. The involvement of the type of adenomyosis in female fertility is also obscure. Although successful pregnancies after prolonged down-regulation with GnRH-agonist and conservative surgery have been reported, there is no agreement on the most appropriate therapeutic methods for managing infertile patients with adenomyosis. Since available data on the relationship between adenomyosis and infertility is still scant and limited to small-scale cases, it is difficult to evaluate the accurate impact of adenomyosis on female fertility.

Study design, size, duration: We conducted a nationwide survey to evaluate the effect of adenomyosis on infertility treatment and pregnancy outcomes as an official project of the Japan Society of Obstetrics and Gynecology (JSOG). A retrospective survey was performed using questionnaires sent to 1149 Japanese medical facilities, including 725 institutes authorized as a training facilities by the JSOG and 582 institutes registered to the JSOG for assisted reproductive technology (ART).

Participants/materials, setting, methods: Questionnaire 1 (Q1): management policy of infertile women with adenomyosis, Questionnaire 2 (Q2): outcomes of infertility treatment in women with adenomyosis. Q2 involved methods of the diagnosis, size, type, localization, infertility treatment, outcome of infertility treatments, and any pre-treatments for adenomyosis before infertility treatment. Patients with leiomyoma and endometriosis were excluded to eliminate the influence of these diseases on fertility. This survey was approved by the Ethical Committee of Yamaguchi University Graduate School of Medicine.

Main results and the role of chance: Q1: Out of the 155 facilities providing the management policy, infertility treatment was performed without any pre-treatment for adenomyosis in 26 facilities (16.8%), after medication 9 facilities (5.8%), after operation in 4 facilities (2.6%). Management policies were not established in 105 facilities (67.1%). Q2 was obtained from 190 facilities with data on 535 infertile women with adenomyosis. The total pregnancy rate was 41.7% and the miscarriage rate was 29.8%. The pregnancy rate and miscarriage rate by the usual infertility treatment without ART were 37.5% (90/240) and 21.1% (20/90), whereas the rate was 44.4% (131/295) and 34.3% (54/157) by ART. Eighty-five patients received medications (GnRH agonist 67, LEP 12, Danazol 7, Dienogest 4) and 89 patients underwent surgery (laparoscopic operation 24, laparotomy 65) as pre-treatment before infertility treatment, while 361 patients had no pre-treatment. Pregnancy rate (no treatment: 41.3%, medication: 43.5%, operation: 41.5%) and miscarriage rate (no treatment: 30.3%, medication: 31.7%, operation: 26.1%) were not affected by pre-treatment for adenomyosis. Of 535 patients, 162 patients represented the focal type and 336 patients diffuse type. The pregnancy rate and miscarriage rate in focal type (43.2% and 24.7%) and in diffuse type (42.9% and 33.1%) was not affected by the type of adenomyosis.

Limitations, reasons for caution: The study was nationwide questionnaire survey to investigate the current clinical status of adenomyosis without control. The survey was a retrospective analysis based on the clinical records of each facility, the diagnosis of adenomyosis was made by the gynecologist of each facility.

Wider implications of the findings: There were no data suggesting that medication or surgery as a pre-treatment for adenomyosis improved the reproductive outcome, and the type of adenomyosis showed any association with the reproductive outcomes. However, a prospective and well-conducted randomized study is required to evaluate the true effect of adenomyosis on fertility outcomes.

Trial registration number: NO.

P-253 Oocytes retrieval difficulties in women with ovarian endometriomas

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Study question: What is the impact of the presence of ovarian endometriomas on the technique of oocytes retrieval?

Summary answer: The presence of ovarian endometriomas is associated with increased technical difficulties during oocytes retrieval.

What is known already: Considering that ovarian reserve may be injured following surgery for ovarian endometriomas, conservative management has grown in recent years. There is now an increasing conformity that endometriomas, in particular those with a mean diameter below 4 cm, should not be systematically removed before IVF. However, conservative management is not without potential drawbacks and risks. A neglected issue here is the possibility that the presence of these cysts may interfere with oocytes retrieval. In particular, one may hypothesize that the procedure may be more difficult and dangerous.

Study design, size, duration: Prospective cross-sectional study. All women undergoing oocytes retrieval between January and December 2015 in a single institution were recruited. Cases were women with an ultrasound diagnosis of one or more endometriomas. Controls were women without a diagnosis of endometriosis. Women could be included only for their first treatment cycle.

Participants/materials, setting, methods: We recruited 56 women with endometriomas and 227 unaffected controls. Each clinician was requested to complete a questionnaire regarding the technical difficulties encountered during the oocytes retrieval. Moreover, patients were interviewed two weeks after the procedure to assess complications occurring after they were discharged. A binomial distribution model was used to calculate the 95% Confidence Interval (95%CI) of the most relevant proportions.

Main results and the role of chance: The endometrioma had to be transfixed during oocytes retrieval in 8 cases (14%, 95%CI: 7–25%). Follicular fluid contamination with the endometrioma content occurred in 9 cases (16%, 95% CI: 8–27%). The number of retrieved oocytes was significantly inferior in cases compared to controls (5.6 ± 4.4 and 7.2 ± 5.2, respectively), (p = 003). However, the proportion of retrieved oocytes per aspired follicles did not differ. The median (interquartile range) was 61% (40–80%) and 67% (50–87%), respectively (p = 0.31). The procedure of oocytes retrieval was scored as difficult or very difficult in 5 (9%) cases and 8 (4%) controls (p = 0.14). The aspiration of follicles was not complete (some follicles were not punctured because of technical difficulties) in 8 cases (14%) and 10 controls (4%) (p = 0.01). The corresponding Odds Ratio (OR) was 3.6 (95%CI: 1.4–9.6). Finally, four women with endometriomas (7%) and four controls (2%) referred to the hospital during the two weeks period after oocytes retrieval because of pain (p = 0.05), corresponding to an OR of 4.3 (95%CI: 1.0–17.8). None of these cases was diagnosed with clinically relevant complications and all recovered with pain killers. No ovarian abscess occurred (0%, 95%CI: 0–5%).

Limitations, reasons for caution: Personnel was aware of the condition of the patients and study purposes. It cannot thus be totally excluded that differences could have been inflated for some of the outcomes. Type II errors cannot be fully excluded in some outcomes due to the relatively small samples size.

Wider implications of the findings: The presence of ovarian endometriomas exposes women to higher risks. However, the magnitude of the observed differences is modest and we did not observe any clinically demanding complication. Advocating systematic surgery before IVF based on this evidence is thus not justified.

Trial registration number: Not applicable.

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P-254 Freeze-all strategy in endometriosis patients – a new indication?

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Study question: To compare IVF outcome in women with moderate/severe endometriosis undergoing fresh versus subsequent embryo transfer after elective freezing of oocytes/embryos.

Summary answer: A freeze-all strategy does not improve IVF outcome in women with moderate/severe endometriosis.

What is known already: The strategy of freeze-all is gaining popularity among physicians and patients due to the concept of a more natural environment and its implication on endometrial receptivity if embryos are transferred in an unstimulated cycle. This could be of particular interest in women with endometriosis, whose endometrial receptivity is still poorly understood as there is a pro-inflammatory status that could be enhanced by ovarian stimulation. Data of freeze-all strategy in this particular groups of patients is scarce and not clear.

Study design, size, duration: Retrospective analysis conducted in a University-affiliated IVF unit – infertile women undergoing IVF/ICSI between January 2014 and December 2015. Endometriosis was diagnosed either by histologically proven biopsy in those patients who had previous surgery or by published imaging criteria using transvaginal ultrasound.

Participants/materials, setting, methods: Infertile women diagnosed with moderate or severe endometriosis –early stages were not considered- underwent ovarian stimulation for IVF/ICSI. One group of patients underwent fresh embryo transfer in the stimulated cycle whereas the other group opted for elective freezing of oocytes/embryos and embryo transfer was deferred in a subsequent cycle, under hormonal replacement therapy.

Main results and the role of chance: 1674 women were included in the study. Patients received similar doses of gonadotropins (UI), 1805 ± 64 vs. 1745 ± 99, p = 0.336 of recombinant FSH and 1310 ± 62 vs, 1158 ± 100, p = 0.013 of HP-hMG for fresh and frozen embryo transfer respectively.

Patients who underwent elective freezing showed significant higher estradiol levels compared with the fresh group at the time of triggering (1591 ± 90 vs. 2858 ± 287 pg/mL, p < 0.001), higher progesterone levels (0.7 ± 0.1 vs. 1.9 ± 0.5 ng/ml, p = 0.048), and more metaphase II oocytes (8.5 ± 0.2 vs 11.4 ± 0.8, p < 0.001). Finally, clinical results were significant better with fresh embryo transfer compared with elective freezing for implantation rate (41.7% vs. 34.5%, p = 0.016), miscarriage rate (15.8% vs. 20.7%, p = 0.040) and ongoing pregnancy rate (38.0% vs. 32.1%, p = 0.046).

When we analyzed the data based on whether or not patients had operated on endometriosis prior to initiating the reproductive treatment, we did not find statistical differences in ovarian response parameters or clinical outcomes, although patients with previous ovarian surgery showed lower estradiol levels but differences were non-significant (1800 ± 186 vs. 2081 ± 88 pg/ml, p = 0.052).

Limitations, reasons for caution: Since it is a retrospective study, not all pertinent risk factors are likely to have been identified and subsequently recorded. So only association, and not causation, can be inferred from the results. The study design could be improved in a prospective, randomized controlled trial.

Wider implications of the findings: From our study, it does not seem that women with endometriosis may benefit from a freeze-all strategy, as it may not only increase time to pregnancy but also add unnecessary interventions such as oocyte/embryo freezing.

Trial registration number: 1701-MAD-005-JG

P-255 Increased interleukin-6 and interleukin-8 expression via hypoxia-mediated down-regulation of dual specificity phosphatase-2 in endometriosis

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Study question: How does hypoxia-mediated down-regulation of dual specificity phosphatase-2 (DUSP2) affect the development of endometriosis?

Summary answer: It demonstrated the angiogenic role of interleukin-8 (IL-8) and the anti-apoptosis function of interleukin-6 (IL-6) in loss-of-DUSP2 endometriotic stromal cells.

What is known already: DUSP2 is down-regulated in endometriotic stromal cells in a hypoxia-dependent manner. Down-regulation of DUSP2 may contribute to the pathological process of endometriosis.

Study design, size, duration: This study recruited 20 patients of reproductive age with endometriosis treated with laparoscopy at the University Hospital. In addition, a transplant-induced mouse model of endometriosis using 13 mice in a 28-day treatment was also included.

Participants/materials, setting, methods: Eutopic endometrial and ectopic endometriotic stromal cells from patients with endometriosis were isolated for primary culture and for various experimental treatments. The microarray analysis in DUSP2-overexpressed cells was aimed to identify genes regulated by DUSP2 in endometriosis. Molecular and cellular techniques were employed to assess effects of hypoxia-mediated DUSP2 downregulation, including quantitative RT-PCR, promoter activity assay, Western blot, tube formation assay, proliferation assay. The transplant-induced mouse model was used to elucidate the angiogenic function.

Main results and the role of chance: Quantitative RT-PCR results showed that levels of IL-6 and IL-8 were elevated in endometriotic stromal cells than that in eutopic stromal cells, which is inversely correlated with the expression of DUSP2. The increased levels of IL-6 and IL-8 were recapitulated by hypoxic treatment or knockdown of DUSP2 in eutopic stromal cells. Treatment with IL-8 receptor inhibitor significantly blocked the tube formation of human umbilical vein endothelial cells induced by conditioned medium collected from hypoxia-treated stromal cells, demonstrating the critical role of IL-8 in angiogenesis. Nevertheless, DUSP2-induced IL-6 induced the phosphorylation of signal transducer and activator of transcription 3 (STAT3) in eutopic stromal cells, which promoted cell proliferation and protected eutopic stromal cells from apoptosis.

Limitations, reasons for caution: This study was conducted in primary human cell cultures and a mouse model, which may not fully reflect the in vivo situation.

Wider implications of the findings: Results from this study demonstrate that loss-of-DUSP2 in endometriotic stromal cells causes the upregulation of IL-6 and IL-8, which ultimately results in increased angiogenic capacity, cell survival and cell proliferation.

It suggests an alternative mechanism via hypoxia which may enhance the development of endometriosis.

Trial registration number: nil.

P-256 Vitamin D and endometriosis: facts or fancies

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Study question: Is there any difference in serum 25-hydroxyvitamin-D_3 [25 (OH)D] between women with and without endometriosis?

Summary answer: Serum levels of 25(OH)D do not differ between women with and without endometriosis.

What is known already: A growing amount of evidence suggests a potential role of vitamin D in the pathogenesis and progression of numerous diseases, including gynecological ones, due to its immune-modulatory, anti-proliferative and anti-inflammatory properties. In addition, vitamin D receptor is expressed in ovarian tissue, endometrium and fallopian epithelial cells. In the last decade, both molecular and animal studies supported a possible role of vitamin D in the pathogenesis of endometriosis. However, studies investigating serum 25(OH)D (the form of the vitamin D reflecting human body reserve) in affected women are scanty and inconsistent.

Study design, size, duration: Case-control study recruiting Caucasian Italian women with endometriosis between 2014–2016. Controls were asymptomatic

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women referring for periodical gynecological care and without a previous diagnosis of endometriosis. They were matched to cases for age, parity and study period. Exclusion criteria were a recent significant sun exposure, assumption of supplementary vitamin D, a diagnosis of uterine fibroids, unexplained infertility or multiple sclerosis and a history of cancer. Recruitment was limited to the autumn and winter seasons.

Participants/materials, setting, methods: 282 women with endometriosis ($n = 113$ with deep endometriosis (DIE), and $n = 169$ with ovarian endometriomas) and 282 controls were enrolled. The diagnosis of endometriosis was based on standard histologic criteria and, in case of rectovaginal forms, on vaginal and rectal examination, presence of visible endometriotic lesions, transvaginal and transrectal ultrasonography, and biopsy of the posterior fornix. Vitamin D insufficiency and deficiency corresponded to serum levels of 25(OH) D < 20 and < 10 ng/ml, respectively.

Main results and the role of chance: The mean ± SD serum 25(OH)D in women with and without endometriosis was 18.9 ± 7.1 and 18.1 ± 7.3 ng/ml, respectively (p = 0.18). The number (%) of women with vitamin D insufficiency was 170 (60%) and 185 (66%), respectively (p = 0.22). The number (%) of women with vitamin D deficiency was 34 (12%) and 38 (13%), respectively (p = 0.70). Moreover, no statistical difference was identified when comparing the two endometriosis sub-groups: the mean ± SD serum 25(OH)D was 19.0 ± 7.0 ng/ml in women with DIE and 18.8 ± 7.2 ng/ml in those with ovarian endometriosis (p = 0.20). The number (%) of women with vitamin D insufficiency was 68 (60%) in DIE and 103 (61%) in ovarian endometriosis group (p = 0.90). The number (%) of women with vitamin D deficiency was 13 (12%) in DIE and 21 (12%) in ovarian endometriosis group (p = 0.85).

Limitations, reasons for caution: Vitamin D status is fluctuating and can be influenced by several external factors such as seasonality, sun exposure, age, parity, diet and BMI. Restriction of the study period to autumn-winter and the strict selection criteria adopted should have reduced the possible impact of confounders but could not completely eliminate them.

Wider implications of the findings: Our findings do not support a crucial role of vitamin D deficiency in the pathogenesis of endometriosis. Systematic testing and supplementation with vitamin D products with the objective of preventing and treating endometriosis seems unsubstantiated.

Trial registration number: not applicable.

P-257 Comparison of oocyte quality in IVF/ICSI cycles between patients with histologically proven endometriosis and patients where endometriosis has been excluded by laparoscopy

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Study question: Does endometriosis affect oocyte quality? Are oocytes of endometrioses patients different in quality and appearance compared to oocytes of patients without endometriosis?

Summary answer: In operated patients with histologically proven endometriosis, lower-quality oocytes are present. Endometrioma surgically treated before IVF/ICSI has no impact on number and quality of oocytes.

What is known already: Endometriosis, a highly prevalent gynecological disease is often associated with infertility. However, no consensus has been established about this interrelation, and the possible mechanisms involved have not been completely elucidated. Controversial studies have suggested that impaired oocyte quality may be involved in the pathogenesis of endometriosis-related infertility. Oxidative stress events associated with alterations in the peritoneal, serum or follicular microenvironments might result in poor oocyte quality and compromise the reproductive potential. Up to now, no study has evaluated the effect of oocyte quality with a zygote score from infertile women with endometriosis in comparison to women excluded endometriosis.

Study design, size, duration: In this experimental study, 413 zygotes from 39 women with histologically proven endometriosis were examined. 22 of them had mild and 17 severe endometrioses graded according to the revised American Society for Reproductive Medicine guidelines of 1997. 13 had additional surgery of endometrioma. Controls were 309 zygotes from 37 women, where endometriosis has been excluded by laparoscopy and who were matched to cases by age and type of cycle.

Participants/materials, setting, methods: IVF and ICSI procedures were carried out using standard protocols. The morphology of the zygotes was rated on using an inverted microscope at magnification of ×400 including size, number and alignment of pronuclei and nucleoli, cytoplasmic halo effect, the presence of vacuoles and granularity of ooplasm. This established PN-scoring system allows selection of those zygotes with favourable developmental potential for further embryo culture. Serum total protein and albumin were measured out of the follicular fluids.

Main results and the role of chance: There were no significant differences in the number of retrieved oocytes between patients with endometriosis and the control group (8.4 ± 5.0 vs. 10.4 ± 9.9). However patients with endometriosis had a lower quality of zygote, showing significantly higher mean zygote scores (18.1 ± 2.3 vs. 16.8 ± 1.8, p = 0,002) with often strong cytoplasmic vacuolization and an extreme or no halo effect. Comparison between severe endometriosis and mild endometriosis revealed a significantly lower rate of the mean number of mature oocytes in patients with severe endometriosis (12.9 ± 11.7 vs. 6.5 ± 5.4, p = 0,019). No difference was observed with regard to the mean zygote number. Ovarian responsiveness and oocyte quality did not significantly differ between patients with a history of laparoscopic excision of endometriom and patients without. Moreover, there was no difference in the serum total protein and albumin in the follicular fluid between patients with endometriosis and endometrioma and the control group.

Limitations, reasons for caution: This study had a small sample size and thus further properly designed studies using a large cohort of patients are needed to confirm these results. Also, we cannot exclude that our group of infertile women present associated other causes of infertility.

Wider implications of the findings: Endometriosis patients, in particular those with severe endometriosis, present a lower ovarian responsiveness and quality of oocytes. Further studies are necessary to understand mechanisms of endometriosis-associated infertility to improve the chances of a successful IVF/ICSI in endometriosis patients.

Trial registration number: None.

P-258 The incidence of polycystic ovarian syndrome is not decreased in women suffering from endometriosis

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Study question: To determine whether there is a reduced incidence of polycystic ovarian syndrome (PCOS) in endometriosis – an ovulation associated disorder.

Summary answer: The incidence of PCOS is comparable in women with endometriosis and non-affected controls. In addition, no differences were observed according to the endometriosis phenotype.

What is known already: Endometriosis is an enigmatic disorder of unknown origin affecting up to 10% of women of reproductive age and 40–50% of infertile women. PCOS is the most common ovulatory disorder encountered in young women, affecting 5–10% of women. No information currently exists on the incidence of PCOS in endometriosis women. Because endometriosis appears fueled by ovulation, we queried whether due to its oligo-anovulation feature, PCOS might be rarer in endometriosis.

Study design, size, duration: This cross-sectional study, carried out in a tertiary care university-based clinic, used data prospectively collected in non-pregnant < 42-year-old patients who were surgically explored for a benign gynecological condition between 2004 and 2016. For each patient, a structured questionnaire was completed by the surgeon before surgery and AMH levels were measured in serum samples during the previous month.

Participants/materials, setting, methods: Two groups were compared: a group made up of women with histologically proven endometriosis and no prior

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endometriosis surgery (Endometriosis group) and a control group having no endometriosis.

Endometriosis women were phenotyped according to the surgical classification of endometriosis in superficial endometriosis (SUP), endometrioma (OMA) and deep endometriosis (DIE). PCOS in this study was arbitrarily defined as AMH > 4.9 ng/ml (surrogate for multi-cystic ovaries) associated with oligo-anovulation.

Main results and the role of chance: During the study period, 2607 women had surgery of whom 2465 signed the informed consent. Serum AMH was available in 991 of them. After thorough surgical examination of the abdomino-pelvic cavity, 354 women were allocated to the endometriosis group (superficial endometriosis (n = 66), endometrioma (n = 113) and deep endometriosis (n = 175)) and 474 non-affected women constituted the controls.

Mean serum AMH was not different in endometriosis and controls, at 3.83 ± 3.12 and 4.10 ± 3.49 ng/mL, p = 0.506, respectively. Likewise, the frequency of oligo-anovulation was not different with 53 cases (15%) in endometriosis and 53 (11.2%) in controls (p = 0.106).

The incidence of PCOS defined as AMH > 4.9 ng/mL associated with oligo-anovulation was not different in endometriosis and controls, with 26(7.3%) and 31(6.5%), p = 0.651, respectively. In endometriosis women, the incidence of PCOS was comparable in all 3 endometriosis phenotypes with 7(10.6%), 6 (5.3%) and 17 (7.4%) cases (p = 0.566), for SUP, OMA, DIE, respectively.

Additional analyses also showed no differences between endometriosis and controls when different AMH cuts-off of 4.2 ng/mL and 5.6 ng/mL are taken.

Limitations, reasons for caution: PCOS defined by the Rotterdam consensus takes the antral follicle count (AFC) as one of the diagnostic criteria. For our study, we used an AMH cut-off as surrogate for AFC in the diagnosis of PCOS. Moreover, androgen excess (clinical or biological) was not taken into account.

Wider implications of the findings: This study indicates that the presence of PCOS should not lessen our efforts to diagnose endometriosis on the wrongful assumption that this association might be rare.

Trial registration number: NA.

P-259 Endometrioma Related Reduced Ovarian Reserve: Preliminary Results of the ERROR Study

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Study question: Do the existence of endometriomas are related with diminished ovarian reserve and naturally induce ovarian damage as evaluated by consecutive serum anti-Müllerian hormone (AMH) levels?

Summary answer: Endometriomas cause a significant reduction in consecutive serum AMH levels, assessed in 6 to 8 months interval. Decline was even more highlighted for bilateral endometriomas.

What is known already: It is controversial whether existence of endometrioma itself affects ovarian reserve. There has been no prior data evalauting followed-up ovarian reserve markes in a cohort of endometrioma patients. Previous studies indirectly evaluated ovarian reserve in endometrioma patients by response to ovarian stimulation in IVF cycles. Due to lack of sufficient knowledge, we aimed to determine effect of endometriomas on ovarian reserve by consecutive serum AMH levels, in the same patient cohort with endometriomas.

Study design, size, duration: A prospective cohort study including 60 women of reproductive age who had an ultrasonographic diagnosis of endometrioma and age matched women, without diagnosis of ovarian pathology as the healthy control group.

Participants/materials, setting, methods: Women who had diagnosis of endometrioma ≥3 cm and who followed up for 6–8 months period, without surgical or medical intervention which could affect ovarian reserve. Serum samples for AMH levels were examined at the first visit. After follow up period, control AMH levels were examined for comparison. Same assessments done for the healthy control group. Statistical analyses were performed as correlation analyses to detect factors of endometrioma related alteration in ovarian reserve.

Main results and the role of chance: From 60 women with endometrioma, 39 (65 %) had unilateral and 21 (35 %) had bilateral endometriomas, with a median diameter of 6 cm (3 – 8). Overall serum AMH values were significantly decreased after 6–8 months follow up period, compared to initial, in endometrioma patients (3.6 ± 3.2 ng/ml versus 3.08 ± 2.6 ng/ml, p = 0.004). Acording to laterality; decline in serum AMH levels was more remarkable for bilateral endometrioma patients (4.1 ± 3.4 versus 2.9 ± 2.1, p = 0.016) than unilateral endometrioma patients.

Limitations, reasons for caution: As preliminary results of our study, because of ongoing patient recruitment, the absence of control groups' (defined as non-endometiroma group) consecutive AMH levels results prevents precision of the comment for the endometrioma related decline in ovarian reserve.

Wider implications of the findings: Our findings suggest that existence of endometrioma can be related with diminished ovarian reserve. The present study is the first to show that endometriomas themself induces ovarian damage in naturally. However, it should also be acknowledged that additional studies are required to conclude definitive results for the prominent issue.

Trial registration number: NCT02438735.

P-260 Metabolomics analysis of follicular fluid in women with ovarian endometriosis undergoing in vitro fertilization

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Study question: Is there any difference between the follicular fluid metabolomics profile of women with ovarian endometriosis and controls when undergoing in vitro fertilization

Summary answer: Follicular fluid metabolomics profile has altered in infertile women with ovarian endometriosis

What is known already: Previous studies have shown alterations in the content of follicular fluid from women with endometriosis. However, to date no study has investigated the metabolomics of follicular fluid from infertile women with endometriosis.

Study design, size, duration: This study was conducted between January 2015- May 2016. Twelve (12) women with ovarian endometriosis (age < 40 and BMI < 30 kg/m^2) and 12 age-BMI matched controls (women with infertility as a result of pure male factor) who underwent ovarian stimulation for intracytoplasmic sperm injection enrolled in this study.

Participants/materials, setting, methods: Follicular fluid samples were collected from both of the groups at the time of oocyte retrieval for ICSI. Next, ^{1}H NMR spectroscopy of the collected follicular fluids was performed. Metabolic compositions of follicular fluids were then compared with the help of univariate and multivariate statistical analysis of NMR data.

Main results and the role of chance: Univariate and multivariate statistical analysis of NMR data showed that, metabolomics profiles of follicular fluids obtained from the women with ovarian endometriosis were distinctly different from the control group.

With the univariate statistical analysis, it is found that follicular fluid of women with endometrioma contain higher levels of lactate (2.75 mM vs. 1.6 mM, p = 0.05, respectively); β-glucose (1.25 mM vs 0.83 mM, p = 0.03, respectively); pyruvate (0.19 mM vs. 0.14 mM, p = 0.03, respectively) and valine (0.17 mM vs. 0.12 mM, p = 0.02 respectively) than controls.

Limitations, reasons for caution: This study has a small sample size. An investigation with a larger group of patients would certainly enhance the study.

Wider implications of the findings: This study provides a new perspective on the etiopathogenic mechanism of infertility related to ovarian endometrioma. Identifying and understanding the underlying molecular mechanism responsible for endometrioma-associated infertility provide novel diagnostic and therapeutic approaches for endometriosis related infertility.

Trial registration number: None.

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P-261 Prognostic markers in women with primary idiopathic infertility

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Study question: Endometrial Natural killer cells and soluble molecules in the uterine flushing of idiopathic primary infertile women can be considered as potential "co- factors"of female infertility?

Summary answer: Endometrial NK cells, molecular components (HLA-G, HLA-E, cytokines) and viral infections create an unfavorable condition for the embryo implantation in primary idiopathic infertile women.

What is known already: A coordinated complex of biochemical and structural events is responsible for the so-called "window of uterine receptivity", during the mid-luteal phase of each menstrual cycle. It is believed that the impairment of endometrial receptivity can be a major cause of primary idiopathic infertility, a phenomenon that occurs with the failure of implantation. The reduction of endometrial Natural killer cell frequency, the presence of a herpesvirus concurrent viremia and changes in the expression of Human leukocyte antigen (HLA)-G and HLA-E molecules,Th17Th2/Th17 cytokines and leukemia inhibitory factor (LIF) have been associated by some authors to the "sterility" condition.

Study design, size, duration: The project has enrolled 103 women sudivided into 67 primary infertile and 36 secondary infertile women, during two years recruitment. Forty control women with proven fertility were also enrolled. Uterine flushing samples were collected and stored as required by the study protocol.

Participants/materials, setting, methods: This is a prospective observational case-control study. The samples were collected by the OU Gynecology (University of Ferrara) and the Center of Reproductive Medicine (Hospital of Bruneck) and were analyzed as follow: Analysis of NK cells: flow cytometry with anti-CD56, CD16, CD9, CD49a, KIRs, CD94, CD107a

Herpesvirus (HSV-1,-2, EBV, CMV, HHV-6, HHV-7, VZV, HHV-8) DNA detection: PCR

Analysis sHLA-G: ELISA (MEM-G9 and anti-beta2-microglobulin HRP)

Analysis sHLA-E: ELISA (anti-HLA-E (3D12) and anti-beta2-microglobulin HRP)

Main results and the role of chance: The analysis of NK cells as a prognostic marker for primary idiopathic infertility has led to the identification of an endometrial NK phenotype (CD56bright CD16-) which has lower frequencies in patients with primary infertility (2.8% vs 12%, $p < 0.0001$). These results suggest a possible implication of eNK cells in primary idiopathic infertile condition. The infection with HHV-6 A was detected in 40% of women with primary infertility and correlated with a decreased percentage of eNK CD56posCD16neg cells (6.3% vs 18.9%; $p = 0.001$), suggesting a role of this infection in the endometrial immunological status. We observed average levels of sHLA-G and sHLA-E significantly lower ($p = 0.001$) in uterine flushings of women with primary idiopathic infertility, who also have higher levels of IFN-gamma, IL-1b and IL-6 ($p = 0.001$).

Limitations, reasons for caution: The main limitation of this project is the number of enrolled subjects. To confim these data it will be necessary to validate them in a new cohort of subjects.

Wider implications of the findings: Although our data do not lead to a definitive solution to the idiopathic infertile condition, they sustain the implication of immunological and microbiological mechanisms, suggesting that this is probably the correct way to go to reach the solution of these healthcare problem.

Trial registration number: NA.

P-262 EFI or r-AFS: the non-IVF-ET pregnancy in endometriosis-associated infertility

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Study question: To discuss the predictive value of endometriosis fertility index and r-AFS score to the pregnancy outcome in endometriosis-associated infertility after laparoscopic surgery.

Summary answer: The EFI has clinical guiding significance for the pregnancy outcome of endometriosis-associated infertility after laparoscopic surgery while r-AFS score doesn't have.

What is known already: Endometriosis (EMT) is a disease associated with infertility. The current EMT staging criteria is revised American Fertility Society (r-AFS) in 1996.The r-AFS have large predictive value of postoperative recurrence, but the predictive value of postoperative pregnancy outcome is insufficient. In 2010, Adamson and Pasta proposed endometriosis fertility index (EFI) as a new stage standard of endometriosis. The standard is based on total historical factors and total surgical factors, including age, years infertile, prior pregnancy, AFS endometriosis score, AFS total score, and puts forward the least function score (LF) to predict postoperative pregnancy rate.

Study design, size, duration: A retrospective cohort study including 516 patients with infertility and diagnosed endometriosis after laparoscopic surgery from January 1st 2010 to June 30th 2014. And 87.40% of the patients got followed up.

Participants/materials, setting, methods: We followed-up all patients through outpatient medical records and telephone after the operation, collected the condition about postoperative pregnancy including natural pregnancy and artificial insemination pregnancy from after operation, till the longest to 3 years after operation. Then the non IVF-ET pregnancy rate were compared among 9-10 EFI points, 7-8 points, 4–6 points and 0–3 points patients, and between mild group with stage I-II and severe group with stage III-IV according to r-AFS staging.

Main results and the role of chance: The non-IVF-ET pregnancy rate showed a downward trend with EFI ($P < 0.05$) in 6months, 12months, 18months, 24months, 36months after laparoscopic surgery ($P < 0.05$); and compared in pairs, the non-IVF-ET pregnancy rate showed significant difference between 4–6 score group and 9-10 score group or 7-8 score group ($P < 0.05$), but no significant difference between 9-10 score group and 7-8 score group ($P > 0.05$) in each period. What's more, the non-IVF-ET pregnancy rate had no significant difference between I-II and III-IV r-AFS score ($P > 0.05$).

Limitations, reasons for caution: The couples who suffered endometriosis-associated infertility might not try to get pregnant actively, and turned to get IVF in three years, even though they would succeed if they kept trying.That may infect the non-IVF-ET pregnancy rate.

Wider implications of the findings: Our reach verified previous literature in larger number of subjects and different periods of time from 6months, 12months, 18months, 24months till 36months after surgery.

Trial registration number: none.

P-263 Evaluation of an experimental mouse model of endometriosis, Role of Estrus cycle and transplantation period

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Study question: influence of transplantation period and estrus cycle of uterine tissue samples sutured to the abdominal wall on the establishment and maintenance of endometriotic lesions in an autologous endometriosis mouse model.

Summary answer: the endometriotic lesions at metestrus/diestrus phase after six to eight weeks of implantation are more likely to develop relative to other cycles or periods.

What is known already: In the majority of previous animal studies, either the endometrium or uterine tissues are implanted at proestrus phase and in some other studies authors have not mentioned the estrus cycle. Furthermore, there are different methods for inducing the endometriosis in rodents, including homologous or heterologous methods. These models have different limitations such as the lack of consistency with respect to estrogen supplementation, different types of tissue used for implantation, low and variable (30–50%) or unreported peritoneal implant take rates as well as model take rate.

Study design, size, duration: experimental design

Participants/materials, setting, methods: An autologous endometriosis mouse model was induced in 52 female *NMRI* mice by suturing the uterine tissue samples of the left uterine horn to the right and left sides of the peritoneum in the abdominal wall. To induce the endometriosis model, some mice were transplanted at proestrus or estrus cycles and others at metestrus or diestrus cycles. The mice were also randomly divided into four groups based on transplantation duration

Main results and the role of chance: The mice were also randomly divided into four groups based on transplantation duration and were sacrificed as follows: group 1 (two weeks, n = 13), group 2 (four weeks, n = 13), group 3 (six weeks, n = 13), and group 4 (eight weeks, n = 13). Six and eight weeks after transplantation, the incidence rate was 100% at metestrus or diestrus mice. However, in proestrus/estrus mice, the highest model take rate was observed after four and six weeks of transplantation (71.4%) and decreased to (42.9%) after eight weeks of transplantation. The expression of Caspase3, ki67, and CD31 proteins was different among four time transplantation periods. These results show that the endometriotic lesions best develop at the metestrus/diestrus phase and after six to eight weeks of transplantation.

Limitations, reasons for caution: The NMRI strain was chosen because of its low price and easily handling. Only female mice were suitable for inducing of endometriosis model.

Wider implications of the findings: In conclusion, these results show that the estrous cycle and implantation period affect the change of ectopic endometriosis. It seems that the endometriotic lesions at metestrus/diestrus phase after six to eight weeks of implantation are more likely to develop relative to other cycles or periods.

Trial registration number: —.

P-264 Long term pretreatment with GnRH agonist before standard GnRH agonist protocol for adenomyosis in IVF/ICSI fresh embryo transfer cycles

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Study question: Can long term pretreatment with GnRH agonist before standard GnRH agonist protocol improve pregnancy outcome of IVF/ICSI fresh embryo transfer cycles in adenomyosis?

Summary answer: Uterine size on hCG day, but not control ovarian stimulation protocol is associated with live birth rate of IVF/ICSI fresh embryo transfer cycles in adenomyosis.

What is known already: Although adverse effect of adenomyosis on implantation in IVF is controversial, present evidences indicate that adenomyosis is associated with higher miscarriage rate in IVF. GnRH agonist can cause hypoestrogenism therefore reduce uterine size. Long term use of GnRH agonist is shown to improve pregnancy outcomes in women with adenomyosis in frozen embryo transfer cycle. Standard GnRH agonist protocol can cause supra-physical elevation of estrogen level and enlargement of uterus, therefore may counteract with shrinkage effect of long term pretreatment with GnRH agonist on uterus in IVF/ICSI fresh embryo transfer cycles.

Study design, size, duration: This is a retrospective cohort study; patients were categorized into two groups (standard GnRH agonist protocol with or without long term pretreatment with GnRH agonist); Fresh embryo transfer live birth rate (LBR) were evaluated by group. Binary logistic regression was used to assess the association between control ovarian stimulation (COH) protocol and LBR after adjusting for confounding factors that were identified as significant in the univariate analysis.

Participants/materials, setting, methods: A total of 95 women diagnosed as adenomyosis who underwent standard GnRH agonist protocol with or without long term pretreatment with GnRH agonist in IVF/ICSI fresh embryo transfer cycles at our hospital from 2012 to 2015 were identified and reviewed. Associations between COH protocol and LBR were analyzed.

Main results and the role of chance: There was no significant difference in the clinical pregnancy rate and live birth rate in two groups as well as the other baseline characteristics as female age, insemination method, basal FSH, progesterone level and uterine anteroposterior diameter on hCG day, number and type of transferred embryo, et al. The LBR is associated with uterine anteroposterior diameter on hCG day (OR 0.565, 95% CI 0.329–0.970, P = 0.038), but not COH protocol (long term pretreatment group OR 2.707, 95% CI 0.662–11.069, P = 0.166)after adjusted for female age, basal FSH, complication with endometriosis, progesterone level on hCG day, number of retrieved oocytes, metaphase II oocytes, normally fertilized oocytes, transferrable embryo, transferred embryo, high quality embryo and type of embryo.

Limitations, reasons for caution: As a retrospective study, our analysis depends on previously recorded data; therefore, certain variables such as serum CA125 level could not be collected.

Wider implications of the findings: COH with long term pretreatment with GnRH agonist may benefit pregnancy outcome of IVF/ICSI fresh embryo transfer in adenomyosis when uterine size on hCG day is controlled and suitable for embryo transfer. However, suitable uterine size for embryo transfer is still to be explored.

Trial registration number: none.

P-265 Pain activations in patients with Dysmenorrhea – An fMRI Study

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Study question: Patients with dysmenorrhea/ endometriosis have an altered neural processing of interoceptive pain.

Summary answer: Patients with dysmenorrhea/ endometriosis did not show altered cerebral processing of interoceptive pain stimuli compared to healthy controls.

What is known already: Brain imaging studies have shown certain characteristics in dysmenorrhea patients such as structural alterations in gray matter volume or an abnormal cerebral metabolism. Former studies especially in patients with irritable bowel syndrome- another chronic pelvic pain condition- revealed altered cerebral processing of interoceptive pain stimuli. In dysmenorrhea patients no studies with visceral pain stimuli in combination with fMRI (functional magnetic resonance imaging) exist.

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Study design, size, duration: This case-control study was designed with female patients suffering from dysmenorrhea/ endometriosis in comparison to a healthy control group. On the first visit informed consent was given and inclusion and exclusion criteria were analyzed. On the second visit within day 1–5 of the menstrual cycle the fMRI measurement with an interoceptive pain stimulus was performed. The aim was to include 20 subjects in each group over a period of two years.

Participants/materials, setting, methods: 23 female patients with primary/secondary dysmenorrhea and 23 healthy women underwent fMRI measurements in a 3 T scanner (Verio, Siemens) at a university hospital. A visceral pain stimulus was applied by rectal distensions using a MRI-compatible BAROSTAT device. Sub- and suprathreshold pain stimuli (distensions) were applied in a pseudo-randomized paradigm. FMRI data analysis was performed with SPM12 in the framework of the general linear model.

Main results and the role of chance: After excluding 7 patients with motion artefacts and handling errors 19 dysmenorrhea patients and 20 healthy controls were analysed. The contrast of pain vs. baseline and pain vs. no-pain in both groups revealed typical areas involved in pain processing with activations in the insular cortex, prefrontal, orbitofrontal and somatosensory cortices and cingulate cortex ($p < 0.05$ FEW-corrected). Nevertheless, no significant differences between the groups were found.

Limitations, reasons for caution: Study limitations are the small sample size and a single measurement only. Therefore no comparison between different cycle phases and in the patient group under spontaneous pain and no pain within one person is possible. Possibly other interventional stimuli than rectal are applicable in this patient group.

Wider implications of the findings: As chronic pelvic pain leads to an altered central pain processing cyclic chronic pain might induce other mechanisms of adaption to the pain.

Dysmenorrhea and endometriosis deserve attention as a chronic pain condition as they have an enormous impact on the patients´ daily quality of life.

Trial registration number: AN 4940 321/ 4.16 at Innsbruck Medical University.

P-266 Gene expression of human endometrial L-selectin ligand (LSL) in relation to the phases of the natural menstrual cycle

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Study question: Little information is available on the types of LSL components and gene expression patterns at different phases of the natural cycle in human endometrium.

Summary answer: Five peptide components of LSL were detected: podocalyxin, endomucin, nepmucin, GlyCAM-1, and CD34. Their gene expressions showed differential patterns throughout the natural cycle.

What is known already: LSLs in high endothelial venules of lymph nodes are glycoproteins that serve as addressin for homing and support of lymphocyte extravasation. The ligands include CD34, podocalyxin, GlyCAM-1, endomucin, and nepmucin in mouse. All have mucin-like polypeptide structures with sulfated O-linked glycans. In human, the expression of LSLs is associated with endometrial receptivity. However, there are no studies exploring the gene expression patterns of LSLs in human endometrium throughout the menstrual cycle. It is unclear whether the types of LSL components in human endometrium are the same as that in mouse lymph nodes.

Study design, size, duration: This is a prospective, cross sectional study. We recruited 41 endometrial samples from reproductive-aged women with leiomyoma undergoing hysterectomy from Aug 2008 to July 2009. Eleven from the proliferative phase (days 7 to 14), 9 from the early-secretory phase (days 15 to 19), 10 from the mid-secretory phase (days 20 to 24), and 11 from the late-secretory phase (days $\geqq$ 25). Eleven samples were obtained as controls from menopausal women undergoing vaginal hysterectomy.

Participants/materials, setting, methods: The inclusion criteria of the participants were: (1) age ranging from 35 to 50 years; (2) regular menstrual cycle; (3) body mass index < 28; (4) no hormone therapy at least 2 months before surgery; (5) no known gynecological diseases; (6) no sexually transmitted diseases. Immunohistochemistry and RT-PCR were performed in the study. Nonparametric Kruskal-Wallis one-way analysis of variance with multiple comparisons was performed to examine differences between the phases of the menstrual cycle.

Main results and the role of chance: The mean age and BMI of the patients were not significantly different between phases ($P > 0.05$). Immunohistochemistry showed that L-selectin ligands were expressed in the luminal and glandular epithelium of the endometrium in all four phases. The MECA-79 intensity of L-selectin ligands varied between phases and was strongly expressed at the early and mid-secretory phases. Interestingly, weak expression of L-selectin ligands was also found in menopausal endometrium.

Five types of L-selectin ligand components were detected in the reproductive and menopausal endometrium by RT-PCR: podocalyxin, endomucin, nepmucin, GlyCAM-1, and CD34. The expression patterns of L-selectin ligand genes revealed variation among the four phases. The relative fold changes of endomucin, nepmucin, and CD34 are higher in the proliferative phase. The relative fold changes in endomucin are significantly different between the proliferative and early-secretory phases ($P < 0.05$).

The gene expressions of CHST2 and CHST4, which are the key enzymes for the generation of L-selectin ligand epitopes, were steady among the four phases. Estrogen receptor α expression was also relatively stable without significant differences among the four phases. However, the gene expression of progesterone receptor showed a significant, gradual decrease from the proliferative to the late-secretory phase ($P < 0.05$).

Limitations, reasons for caution: The small number of samples could limit the power of the study. The heterogeneity of the samples could impact the results. Finally, because of ethical considerations, we used the endometrial samples of uteruses with leiomyoma instead of normal uteruses, which could not completely represent the actual natural cycle.

Wider implications of the findings: Five types of L-selectin ligand components were detected in human endometrium: podocalyxin, endomucin, nepmucin, GlyCAM-1, and CD34. The expression patterns of L-selectin ligand genes revealed variation among the four phases. The significant gene expression of endomucin between the proliferative and early-secretory phases might highlight its potential role in endometrial receptivity.

Trial registration number: none.

P-267 In vitro and in vivo synergistic effects of MK2206 and chloroquine combination therapy on endometriosis

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Study question: Is MK2206 (an AKT inhibitor) and chloroquine (anti-malarial, autophagy inhibitor) combination therapy effective treatment in endometriosis?

Summary answer: MK2206 and chloroquine combination therapy significantly decreased the size of endometriotic implants in a xenograft model of endometriosis compared with treatment with either drug alone.

What is known already: We previously showed that the combined treatment with U0126 (a MEK inhibitor) and MK2206 synergistically inhibited cell proliferation of deep endometriotic stromal cells (DES) in vitro. However, cell proliferation of DES after drug discontinuation was high. Our previous findings suggest that MK2206 treatment may induce autophagy, which may inhibit cell death, resulting in cell survival from combined treatment with U0126 and MK2206 and subsequent cell proliferation. A recent study showed that hydroxychloroquine, an autophagy inhibitor, could decrease lesion numbers and disrupt lesion histopathology in a mouse model of endometriosis.

Study design, size, duration: A laboratory study. Forty patients (20 with and 20 without endometriosis) of reproductive age with normal menstrual cycles were recruited. A total of 40 nude mice received a single injection of

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proliferative endometrial fragments on day 0. Then, mice were randomized into four experimental groups. Each group of mice was given vehicle, chloroquine, MK2206 or chloroquine with MK2206 for 6 days. Treatment was started on day 15 and mice were sacrificed on day 21.

Participants/materials, setting, methods: We evaluated the effects of MK2206 and autophagy inhibition on cell proliferation of DES in vitro. Autophagy was inhibited by knockdown of the Beclin 1 gene, and/or pharmacologic agents (chloroquine, bafilomycin A1 and/or 3-methyalanine) treatment. Clonogenic assay was performed to evaluate the effects of MK2206 and chloroquine on long-term survival of DES. Additionally, the effects of MK2206 and chloroquine treatment on endometriotic implants were evaluated in a xenograft model of endometriosis in immunodeficient nude mice.

Main results and the role of chance: MK2206 and chloroquine synergistically inhibit proliferation of DES in vitro. A combination of MK2206 (9 μM) and chloroquine (50 μM) significantly more inhibited cell proliferation of DES (83.0 % ± 11.0, mean ± SD, $p < 0.01$) compared with those treated with either drug alone (chloroquine: 17.5 % ± 12.0, MK2206: 26.1 % ± 8.3). Clonogenic assay confirmed that this combined treatment dramatically suppressed long-term cell survival of DES. In a xenograft mouse model, combined treatment with MK2206 and chloroquine significantly decreased the size of endometriotic implants (median size reduction –79.5%, range -51 to -87.1, $p < 0.01$) compared with those treated with either drug alone. However, treatment with either drug alone did not significantly affect the size of endometriotic implants compared with those treated with vehicle alone. The TUNEL assay demonstrated that endometriotic implants treated with a combination of MK2206 and chloroquine, exhibited a significant increase in apoptotic cells (48.2% ± 12.6, $p < 0.0001$) when compared with those treated with either drug alone (chloroquine: 4.3 % ± 2.8, MK2206:7.5 % ± 5.8).

Limitations, reasons for caution: The present mouse model of endometriosis does not mimic human endometriosis. In the present mouse model, it is not possible to evaluate whether re-growth of endometriotic implants could occur after drug discontinuation.

Wider implications of the findings: The combination therapy of MK2206 and chloroquine may be effective for treatment in patients with endometriosis.

Trial registration number: N.A.

P-268 Differential epithelial-mesenchymal transition status between types of endometriosis and adenomyosis

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Study question: Is there any difference in epithelial-mesenchymal transition (EMT) status between endometriosis, adenomyosis and normal endometrium?

Summary answer: The results suggested the differential EMT status in each endometriotic lesion and the potential of ZEB1 as an indicator of invasiveness or severity of endometriosis.

What is known already: Although endometriosis is a benign disease, it shares some features with cancers, such as invasiveness and the potential to metastasize. It has been reported that EMT plays a critical role in invasion and subsequent metastasis of cancers. In recent years, several reports suggested that EMT might be involved in the pathogenesis of endometriosis and adenomyosis. However, there is no report about ZEB1 (Zinc finger E-box-binding homeobox 1, an EMT-associated transcriptional factor) expression on endometriosis and adenomyosis, and EMT status have not yet been investigated on each type of endometriosis and adenomyosis.

Study design, size, duration: Ten endometriosis patients and nine adenomyosis patients undergoing laparoscopic excision of lesions were recruited. Twelve control women without endometriosis undergoing surgery for other benign diseases were also recruited. Excised lesions of endometriosis, adenomyosis and normal endometria were subjected to immunohistochemistry.

Participants/materials, setting, methods: We evaluated the expression of E-cadherin, N-cadherin, vimentin and EMT-related transcriptional factors (Snail and ZEB1) in epithelial cells of various endometriotic lesions, adenomyosis and normal endometria by immunohistochemical analysis on the paraffin sections prepared from all excised lesions. The relationship between ZEB1 expression and serum level of CA125 was also investigated.

Main results and the role of chance: Immunohistochemical scoring revealed that E-cadherin, N-cadherin, Snail, and vimentin were expressed in epithelia of all types of endometriosis and adenomyosis as well as normal endometria. However, most markers were differently expressed between the types of samples. Notably, ZEB1 expression was only expressed in epithelia of endometriosis and adenomyosis, not in normal endometira. Additionally, ZEB1 was more frequently observed in epithelial cells of deep infiltrating endometriosis and adenomyosis than the other types of endometriosis. Furthermore, the patients with high serum CA125 level were more likely to have ZEB1-positive lesions.

Limitations, reasons for caution: The sample size of this study is relatively small.

Wider implications of the findings: We here for the first time demonstrate the differential EMT status, especially ZEB1 expression, in each endometriotic lesion and adenomyosis. The preferential expression of ZEB1 in deep infiltrating endometriosis and adenomyosis raises a possibility that ZEB1 might be a switch to endow endometriosis with invasive properties through EMT.

Trial registration number: Not applicable.

P-269 P450-aromatase pathway is altered in granulosa cells from women with endometriosis

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Study question: To evaluate the molecular mechanisms by which endometriosis can negatively influence growth, steroidogenesis and function of granulosa cells (GCs).

Summary answer: GCs from women with endometriosis show a dysregulation of the steroidogenesis pathway involving P450-aromatase and ERK1/2 signaling pathway potentially affecting oocyte development

What is known already: Main functions of GCs are nurture the oocyte and, via hormonal influence, guide it through the series of events necessary for achieving cytoplasmic and nuclear competence. Previous evidence would indicate that P450-aromatase mRNA expression is lower in GCs from women with endometriosis. This could influence estradiol production and could be the cause of some of the detrimental effects found in the local intrafollicular environment of endometriosis patients. The molecular mechanisms by which endometriosis can negatively influence growth, steroidogenesis and function of GCs are still lacking.

Study design, size, duration: Human GCs were obtained by ultrasound-guided follicle aspiration from women receiving assisted reproduction treatment at Centro Scienze Natalità of San Raffaele Hospital (Milan, Italy). Patients with a laparoscopic diagnosis of endometriosis with stage III-IV endometriosis ($n = 28$) and women undergoing assisted reproduction procedures for male infertility (control group $n = 41$) were studied

Participants/materials, setting, methods: GCs were isolated from the follicular fluids after density gradient centrifugation on Ficoll-Paque and removal of contaminating adherent macrophages. The P450-aromatase and 3β-hydroxysteroid dehydrogenase mRNA expression levels of GCs were determined by qPCR. Furthermore, protein levels of phosphorylated ERK1/2 were assessed using *Western Blotting* analysis.

Main results and the role of chance: GCs from women with endometriosis patients had significantly lower transcripts of 3β-hydroxysteroid dehydrogenase and P450-aromatase compared to controls (0.37 ± 0.07 *vs* 0.76 ± 0.14 relative

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units; p = 0.04 and 0.03 ± 0.009 *vs* 0.27 ± 0.06 relative units p = 0.01, respectively); both key enzymes are involved in the estradiol production. Indeed, we found a higher estradiol content in the culture media from GCs of control women compared with those from GCs of women with endometriosis. We focused our study on the P450-aromatase enzyme and found for the first time that not only the enzyme transcript but also the protein content were reduced in GCs from women with endometriosis. Since it has been shown that a sustained activation of ERK1/2 signaling cascade may have an inhibitory effect to the steroidogenesis pathway, we decided to test the activation of this pathway through the analysis of the phosphorylation levels of ERK1/2 in these cells. Phosphorylation levels of ERK1/2 were higher in GCs from women with endometriosis compared to those from controls. In order to analyze whether the up-regulation of phospho-ERK1/2 signaling correlated with the lower expression of P450-aromatase in GCs cells, we *in vitro* treated GCs with the ERK1/2 inhibitor U0126 resulting in an increase of aromatase protein.

Limitations, reasons for caution: Future analyses need to be performed in order to correlate these data with oocyte quality and IVF outcomes. Furthermore, the analysis should be extended to a higher number of cases and controls

Wider implications of the findings: These data suggest a dysregulation of the steroidogenesis pathway in GCs from patients with endometriosis. This alteration may negatively alter the follicular environment underlying an adequate development of the oocyte and may be one of the cause of the worse performance of assisted reproduction procedures in this population.

Trial registration number: n.a.

P-270 IVF in patients with recurrent endometrioma and low ovarian reserve: original treatment technology improves outcomes

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Study question: If the original treatment technology of recurrent ovarian endometrioma prior to treatment with ART affects IVF-ICSI outcome in low ovarian reserve patients?

Summary answer: Original treatment technology do not reduce ovarian reserve (OR), increases number of eggs, embryos quantity and quality, IVF-ICSI pregnancy rate with own oocytes.

What is known already: Endometriosis is considered one of the main challenges in the ART. Evidence drawn from studies on oocyte recipient cycles demonstrated worse reproductive outcomes in those who received oocytes from women with endometriosis, suggested that the oocyte quality of women with endometriosis is compromised. Ovarian endometrioma is another challenge. On the one hand, there is no evidence that cystectomy prior to treatment with ART improves pregnancy rates. Moreover, surgery prior to treatment with ART can reduce ovarian reserve especially in the women with previous ovarian surgery. On the other hand, surgery can be needed to improve the accessibility of follicules.

Study design, size, duration: During 2010–2016 thirty eight infertile women with endometriosis and low ovarian reserve due to previous ovarian surgery were admitted to the Reproductive Health Institution for ART treatment with recurrent endometrioma (> 3 cm). Among them, in 17 women laparoscopic cystectomy was done prior to admission for ART (the Control). After informed consent 21 women were enrolled in the study aimed to test effect of original technology for recurrent ovarian endometrioma treatment on ART outcome (the Case).

Participants/materials, setting, methods: There was no difference in mean age (33.11 ± 0.90 vs 33.2 ± 1.07 years old), infertility duration (6.34 ± 0.61 vs 7.41 ± 0.87 years), endometriosis history (6.76 ± 0.58 vs 6.18 ± 0.76 years) between the groups. Original technology included Dienogest for two months prescription with aspiration of endometrioma after desensitization by GnRH agonists. Ovarian reserve tests: AMH, antral follicle count (AFC), tumor markers (CA125; HE4) were studied before and after either laparoscopic surgery or transvaginal echo guide endometrioma

Main results and the role of chance: There were no difference in AFC (4.57 ± 0.32 vs 4.06 ± 0.5) between the Groups before enrolment. No difference was in AMH among the Cases before and after aspiration of the endometrioma (0,63 ± 0.07 vs 0.7 ± 0.13 ng/ml), whereas in the Controls decline was significant (0.74 ± 0.08 vs 0,35 ± 0.07 ng/ml, p < 0.05). Both Groups demonstrated decrease in CA 125 (55.73 ± 4.45 to 36.53 ± 3.47 in the Case and 50.61 ± 3.93 to 29.35 ± 3.8 U/ml in the Control, p < 0.05) after intervention.

Controlled ovarian stimulation was done in long agonists GnRH (0,1) protocol. Duration of stimulation was 12.72 ± 0.3 and 13.18 ± 0.36 days, respectively. Cancelled cycle: 1 in the Case and 6 in the Control. Embryological data: oocytes 4.24 ± 0.87 in the Case and 2.18 ± 0.49 in the Control (p < 0.05); oocytes M2 – 3.32 ± 0,71 and 1.53 ± 0,34 (p < 0,05); zygotes 2PN - 2.68 ± 0.55 and 1.18 ± 0.27 respectively, p < 0,05. Embryos transferred (ET) were 1.87 ± 0.11 and 1.82 ± 0.15. Embryo criopreservation were done to 13 women in the Case and 1 – in the Control. There were 14 pregnancies (66.67%) in the Case and 3 (17.65%) in the Control. Subsequent Cryo ET increased total pregnancy rate in the Case to 71.43% and in Control group 23,53% (p < 0,05).

Limitations, reasons for caution: Because there is no difference in the basic characteristics of the patients, results obtained can be associated with original treatment technology proposed for recurrent ovarian endometrioma. These results are to be treated carefully because patients were not randomly assigned for the treatment.

Wider implications of the findings: Original treatment technology can be considered as the only option for those needed in ART with recurrent ovarian endometrioma and low ovarian reserve to improve the accessibility of follicles and not affect ovarian reserve. Clinical trial is needed to prove our findings.

Trial registration number: Not trial. This study was approved by Ethics Committee of the Reproductive Health Institution.

P-271 Untargeted metabolomics reveals altered metabolite profile in tissue and serum of women with endometriosis

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Study question: Does any correlation exist between serum and eutopic endometrial tissue metabolic profile of women with endometriosis?

Summary answer: Our study indicates that there is correlation between the tissue and serum metabolic profile of endometriosis women.

What is known already: Preliminary studies suggest that the serum metabolic profile in endometriosis patients is altered compared to controls.

Study design, size, duration:

^{1}H NMR was performed on tissue and serum samples collected from endometriosis and controls over a period of three years.

Participants/materials, setting, methods: Eutopic endometrial tissue and serum samples were collected from two tertiary referral hospitals, Institute of Reproductive Medicine and Institute of Post-Graduate Medical Education and Research, Kolkata, India. The experiments, data analysis and interpretation were performed in the School of Medical Science and Technology, IIT Kharagpur, India. Endometriosis cases (n = 126) with Stage I (n = 20), Stage II

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(n = 16), Stage III (n = 45) and Stage IV (n = 45) were recruited. Fifty seven cases undergoing interval tubectomy were used as controls.

Main results and the role of chance: Several metabolites such as proline, alanine, leucine, lysine, glucose, phenylalanine, glutamine, glutamate, acetate, tyrosine, formate, taurine, myo-inositol and glycine were found to be characteristic for different stages of endometriosis. While alanine ($p < 0.0001$), lysine ($p = 0.0017$), phenylalanine ($p = 0.005$) and leucine ($p = 0.01$) showed negative correlation, proline (0.001) showed positive correlation between serum and tissue levels of early stage endometriosis patients.

Limitations, reasons for caution: The present study focuses only on the secretary phase. For robust assessment of these markers, the candidate metabolites' expression in the proliferative phase also needs investigation.

Wider implications of the findings: Since the current gold standard for diagnosis of endometriosis is a surgical procedure, laparoscopy, identification of a panel of metabolite markers is expected to be useful in early prediction of the disease in a minimally invasive manner.

Trial registration number: Not applicable.

P-272 DNA microarray analysis of gene expression in eutopic endometrium from patients with endometriosis

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Study question: Does endometrial gene expression in late secretory phase endometrium differ between patients with and without endometriosis?

Summary answer: The eutopic endometrium from patients with advanced-stage endometriosis has distinct gene expression profile from eutopic endometrium of control without endometriosis.

What is known already: Endometriosis, a condition in which the uterus lining tissue appears and flourishes outside the uterus, is a progressive disease that causes diverse symptoms such as pelvic pain, menstrual pain, and infertility. However, the exact molecular mechanism of the development of endometriosis has not been elucidated. It has recently been proposed that there are differences between the endometrial tissues of women with endometriosis and those of normal women, and these may be the cause of infertility in these patients.

Study design, size, duration: This was prospective laboratory study. Five patients with laparoscopically proven advanced-stage endometriosis and 5 controls underwent endometrial biopsy in the late secretory phase.

Participants/materials, setting, methods: Analysis of eutopic endometrial gene expression was performed using Affymetrix gene arrays and differentially expressed genes were assigned to gene ontology groups based on overrepresented analysis using DAVID software. Microarray data were obtained for 5 control samples and 3 samples from the patients with endometriosis. However, during Affymetrix GeneChip® analysis, the array data of two patients that may be biological outliers were excluded. Expression patterns of selected five genes were validated by quantitative RT-PCR.

Main results and the role of chance: Four hundred sixty two genes were identified as up-regulated such as matrix metalloproteinase 10 (MMP10), cytochrome P450 family 24 subfamily A polypeptide 1 (CYP24A1), matrix metalloproteinase 3 (MMP3), chemokine (C-C motif) ligand 20 (CCL20), Rho family GTPase 1 (RND1), interleukin 1-beta (IL1B), and insulin-like growth factor binding protein 1 (IGFBP1), while 643 genes were down-regulated in all endometriotic samples. A lot of genes related with metabolic process, cellular ketone metabolic process and ncRNA metabolic processing were included.

Limitations, reasons for caution: The question still remains whether the interactions or differences between genes that show increased or decreased expression is the cause or result of endometriosis, and so studies are required to compare the genetic expression in each subdivided phase of menstrual cycle and which use more diverse bioinformatics.

Wider implications of the findings: The confirmation of the existence of pathophysiologically meaningful genes in patients with endometriosis through microarray analysis both broadens our understanding of the pathogenesis of endometriosis and assists the development of noninvasive diagnosis techniques and new approaches to therapy.

Trial registration number: N/A.

P-273 Gamma secretase inhibition reveals a possible crosstalk of LIFR, SOX2 and PODXL with the Notch-Msi1 signaling pathway in primary endometriotic cells

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Study question: Which functional properties and stemness-associated molecular pathways are influenced by inhibition of notch signaling in primary endometriotic cells?

Summary answer: Gamma secretase inhibition of primary and immortalised endometriotic cells induces apoptosis and results in downregulation of SOX2, LIFR, IFITM1 and PODXL.

What is known already: Expression of Msi1, a modulator of the stemness-associated notch signaling pathway, is upregulated in endometriosis and endometrial carcinoma. Interference with Msi1 function leads to a downregulation of notch-1 and to an induction of apoptosis in endometrial carcinoma cells.

Study design, size, duration: In vitro cell culture study (control vs gamma secretase inhibitor treatment) on the imortalized endometriotic cell line 12Z and primary stroma cells derived from endometriotic lesions of 7 patients.

Participants/materials, setting, methods: Stroma cells were isolated from biopsies of endometriotic lesions of 7 patients and cultured in vitro. The immortalied endometriotic epithelial cell line 12Z and primary endometriotic stroma cells were subjected to gamma secretase inhibitor treatment for 24 h and analyzed for changes in gene expression by TaqMan low density arrays and qPCR. The impact on stem cell properties was investigated by flow cytometric aldehyde dehydrogenase activity assays, cell cycle analysis and annexin V apoptosis assay.

Main results and the role of chance: qPCR analysis demonstrated expression of the notch pathway components notch 1–4, Msi1-2, numb, DLL1,3,4, Hes1, Hey1 and the presence of an ALDH+ cell pool, a surrogate marker of stem cell activity in 12Z cells. GSI treatment lead to a reduction of ALDH+ cells ($p < 0.05$), reduced cell viability in MTT assays ($p < 0.05$), and increased apoptosis ($p < 0.05$). In 12Z cells, the cell cycle shifted from the S- to the G2 phase after GSI-treatment. TaqMan Low density array analysis followed by qPCR confirmation in 12Z and primary cells revealed a significant downregulation of the pluripotency-associated transcription factor SOX2, previously shown to be associated with endometriosis, of the LIF receptor (confirmed by flow cytometry), IFITM1, a regulator of primordial germ cell function, and the stemness-associated factor PODXL (all $p < 0.05$). Expression of Msi1 and the notch antagonist numb was upregulated by GSI, while treatment of 12Z cells with recombinant notch-1 induced transcriptional downregulation of Msi1, numb, and the notch ligands DLL1 and DLL4 (all $p < 0.05$).

Limitations, reasons for caution: This in vitro study needs to be confirmed in more complex in vivo assays to assess possible side effects. The study relies on one pharmacological inhibitor and should be confirmed using other modes of notch pathway inhibition.

Wider implications of the findings: Our data suggest that pharmacological interference with the notch signaling pathway may be a worthwhile approach in the treatment of endometriosis that warrants further investigation. Inhibition of stemness-associated functions in endometriosis may reduce pathogenesis-associated parameters such as unlimited proliferative potential and developmental plasticity, thus reducing lesion persistence.

Trial registration number: not applicable.

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P-274 IL-10 secreted by plasmacytoid dendritic cell contributing to the pathogenesis of endometriosis by triggering angiogenic growth factors

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Study question: We propose that altered IL-10 expression may contribute to the immune suppression as well as angiogenesis in the development of endometriosis

Summary answer: The plasmacytoid dendritic cells can regulate the angiogenic activity of IL-10, which was mediated by VEGF in vitro and contributes to endometriosis development.

What is known already: Accumulating evidence suggest that angiogenesis and immune dysregulation may play crucial roles in the pathogenesis and development of endometriosis. Our previous study has shown that anti-inflammatory cytokine IL-10 over-expression may enhance the growth of endometrial tissue through suppressing the anti-endometrial immunity.

Study design, size, duration: After treating cytokine IL-10 and anti-IL10R in HUVEC cells and ectopic endometrial cells, the regulated angiogenic and migratory activity were investigated. Whether IL-10 expressing stimulated by pDCs promote endometriosis development was investigated in a surgery-induced mice model. The dermal biopsy punches of uterine horns were sutured to the peritoneal walls in female C57BL/6 mice

Participants/materials, setting, methods: HUVEC cells and ectopic endometrial cells were used to determine the angiogenic activity of IL-10 using tube formation assay and transwell migratory assay. Purified pDCs ($2x10^4$) from C57BL/6 mice or PBS as control were intravenously transferred into syngeneic female mice. Purified pDCs from IL-10 +/+ and IL-10 -/- mice were adoptively transferred into C57BL/6 mice

Main results and the role of chance: We found that IL-10 promote the tube formation and migratory activity of HUVEC cells, while anti-IL-10 R blocking antibody significantly suppress these two angiogenic activities of HUVEC cells. Furthermore, the soluble factor(s) released by IL-10-treated ectopic stromal cells also enhanced the tube formation and migratory activity of HUVEC cells using transwell assay. We showed that the angiogenic activity of IL-10, at least in part, was mediated by VEGF in vitro, and plasmoid dendrite cell secretes IL-10 can promote endometriosis in vivo.

Limitations, reasons for caution: The main limitation of this study was that it was limited to cell and animal experiment, further validation should be investigated in clinical patients.

Wider implications of the findings: The final goal of this research is to ultimately define the mechanistic link between immune suppression and angiogenesis, and to provide a rational basis for the development of novel therapeutic approaches that will have the direct and significant impact on endometriosis and endometriosis-associated cancers.

Trial registration number: Not applicable.

P-275 Novel endometrial receptivity markers detected in large-scale RNA-sequencing study combining samples from different populations

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Study question: Can we find novel, robust and reproducible endometrial receptivity markers using RNA-sequencing and comprehensive analytical design in a study group combining different populations?

Summary answer: In healthy women, 3,591 potential transcriptome-based endometrial receptivity biomarkers were identified. Comparison with recurrent IVF failure patients proposed novel mechanisms for endometrial-origin implantation failure.

What is known already: Endometrial transcriptome studies have reported numerous differentially expressed genes during the transition from pre-receptive to receptive state. Additionally, transcriptome dysregulation leading to problems with endometrial receptivity has been proposed as a potential mechanism behind repeated IVF failure in some patients. However, due to small between-study overlap, we still have only a handful of robust clinical biomarkers for diagnosing problems with endometrial receptivity or selecting the optimal receptivity window for embryo transfer in IVF.

Study design, size, duration: Cohort study; paired samples from pre-receptive (n = 35) and receptive (n = 35) endometrium in healthy fertile women (aged 23–36) and receptive phase samples (n = 39) from women with repeated IVF failure (aged 26–49) were collected in Estonia and Spain.

Participants/materials, setting, methods: Endometrial biopsies were analysed using Illumina paired-end RNA-sequencing. In each cohort, paired pre-receptive phase samples were compared to receptive phase samples from the same healthy women, while receptive phase samples from women with repeated IVF failure were compared to those of healthy women. For cohort-level differential expression analyses, edgeR software was used, and meta-analysis was performed using METAL software. g:profiler was used for enrichment analyses. Results were selectively validated using qPCR.

Main results and the role of chance: A total of 17,490 mRNA transcripts were evaluated. After Bonferroni multiple testing correction, 3,591 differentially expressed transcripts (1,799 up-regulated, 1,791 down-regulated) were identified in the pre-receptive vs receptive endometrium analysis of healthy women, with consistent effects in all studied groups. Up-regulated transcripts were enriched for immune system and cellular adhesion terms, while cell-cycle and DNA repair-related processes were down-regulated. In addition, differentially expressed genes were enriched for E2F, ETF, ZF5 and Sp1 transcription factor binding sites ($p < 5 \times 10^{-45}$), proposing their central role in regulating endometrial function. Comparison of receptive phase samples from healthy women and repeated IVF failure patients resulted in 49 transcripts with significantly different expression levels after multiple testing correction, and similar effects in both Estonian and Spanish datasets. These transcripts were clearly enriched for cellular adhesion and immune response, including several HLA molecules.

Limitations, reasons for caution: Rigorous analytical design can result in the loss of some potentially relevant markers. Not all differentially expressed transcripts have been assessed for their suitability as biomarkers.

Wider implications of the findings: Differentially expressed transcripts identified in our study have consistent effects in different populations and therefore have the potential for being evaluated as robust and efficient markers for endometrial receptivity in the clinical setting. Comparison between healthy women and IVF patients provides novel information on potential causes of repeated IVF failure.

Trial registration number: NA.

P-276 History of surgery for endometriosis and Assisted-Reproductive Technology outcomes in women with deep infiltrating endometriosis

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Study question: To evaluate assisted reproductive technology (ART) outcomes in a series of 222 deep infiltrating endometriosis (DIE) patients and to assess the impact of previous surgery for endometriosis

Summary answer: In a population of DIE patients, a history of previous surgery for endometriosis is associated with poor assisted-reproductive technology outcomes.

What is known already: Surgical removal of endometriosis (OSIS) lesions is one of the options to manage OSIS-related infertility. However, surgery results remain controversial, with some authors showing that excision of endometriosis lesions improves in vitro fertilization (IVF) outcomes and others describing a negative impact on ART pregnancy rate. Surgery may also result in severe complications particularly in DIE, requiring significant expertise. Conversely, reverting to IVF may expose women to some risks, as the ovarian stimulation may accelerate the growth and progression of the lesions. In this context of discordant ART results after surgery, there is still no consensus about managing infertile-DIE patients.

Study design, size, duration: Retrospective observational cohort study, including 222 consecutive DIE patients undergoing in vitro fertilization or intracytoplasmic sperm injection, between June 2005 and February 2013 at a University Hospital. The diagnosis of DIE was based on imaging criteria (transvaginal ultrasound (TVS), magnetic resonance imaging (MRI)); histological proof confirmed the diagnosis in women with a history of surgery for endometriosis.

Participants/materials, setting, methods: ART outcomes were studied in the whole population, and then compared between patients with a history of surgery and those without. Main outcome measures were clinical pregnancy rates and live birth rates per cycle and per embryo transfer. Cumulative pregnancy rates were also calculated. Prognostic factors were identified by comparing women who became pregnant and those who did not, using univariate and adjusted multiple logistic regression models.

Main results and the role of chance: Two hundred and twenty-two DIE patients underwent 440 assisted reproductive technology cycles. Ninety (40.5%) patients became pregnant, and 70 (31.5%) had a live birth. The clinical pregnancy rate and the live birth rate per embryo transfer was 27.6% and 20.1% respectively. Patients with a history of previous surgery for endometriosis had significantly lower implantation rates ($p < 0.001$), clinical pregnancy rates ($p < 0.001$) and live birth rates per embryo transfer ($p = 0.002$), than patients who never had endometriosis surgery. They also had lower cumulative live birth rates compared to non-operated women (26% versus 51.3% after 4 cycles, $p < 0.001$). After multivariate analysis, previous history of surgery for endometriosis ($p = 0.001$) or past surgery for endometrioma ($p = 0.005$) were independent factors associated with lower pregnancy rates. Age > 35 years ($p = 0.018$), AMH levels < 2 ng/mL ($p = 0.018$), antral follicle count < 10 ($p = 0.005$) and multifocality of DIE lesions ($p = 0.002$) were also associated with negative assisted reproductive technology outcomes.

Limitations, reasons for caution: In 30.2% of patients there was no histological proof of endometriosis. However, endometriosis was diagnosed using previously published imaging criteria using TVS and MRI. This limitation – lack of surgical/histological confirmation of diagnosis – is one that affects most studies on OSIS and ART and is therefore not entirely avoidable.

Wider implications of the findings: These findings need to be confirmed by further prospective studies taking into account the type of surgery. Yet, it brings a new insight in the complex task of dealing with infertile DIE patients and might help the physicians to choose between reverting to ART or surgery.

Trial registration number: NA.

P-277 Influence of ovarian endometriosis on ovarian-responsiveness to hyperstimulation: An AMH-matched controlled study

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Study question: To evaluate ovarian responsiveness to hyperstimulation and assisted reproductive technology (ART) outcomes in women suffering from ovarian endometriosis (OMA), as compared to disease-free controls.

Summary answer: Presence of OMA decrease ovarian responsiveness to hyperstimulation but not pregnancy chances. Previous history of OMA surgery is a main risk-factor of poor ovarian response.

What is known already: Reverting to ART is a therapeutic option for many endometriosis-affected infertile women. However, the influence of ovarian endometriosis (OMA) on ovarian responsiveness to hyperstimulation is a matter of debate.

Study design, size, duration: We conducted a large retrospective observational cohort study in a tertiary care university hospital between 01/10/2010 and 31/12/2015. After matching by age and AMH level, 201 infertile women suffering from OMA (OMA group) and 402 disease-free women (control group) undertaking ART procedure were included in the study. Study endpoints were number of oocytes retrieved and live birth rate.

Participants/materials, setting, methods: All endometriosis-affected women have undergone a pre-ART work-up, in order to precise diagnosis and staging of endometriosis. OMA Diagnosis was based on published imaging criteria (transvaginal sonography or magnetic resonance imaging) or on histology for patients with a previous history of endometriosis surgery. Poor ovarian response to hyperstimulation was defined as a reduced number of retrieved oocytes (≤3 oocytes retrieved). Statistical analyses were conducted using univariate and multivariate logistic regression models.

Main results and the role of chance: The number of oocytes retrieved (7.5 ± 5.4 in OMA group versus 9.4 ± 6.1 in control group; $p < 0.01$) and the number of mature oocytes (6.4 ± 4.8 in OMA group versus 7.6 ± 5.0 in control group; $p < 0.01$) were significantly lower in OMA group as compared to control group. An embryo transfer was performed in 151/201 (75.12%) and 324/402 (80.59%) women in OMA and control group respectively. No significant differences were found between OMA and control groups in term of clinical pregnancy rate [53/151 (35%) versus 134/324(41.3%) respectively; $p = 0.23$] and live birth rate [39/151 (25.8%) versus 99/324(30.5%) respectively; $p = 0.33$]. After a multivariate logistic regression analysis, independent risk factors for poor ovarian response to hyperstimulation are the following: Previous history of OMA surgery [OR = 2.21; 95% CI:1.141-4.260], women's age (> 35y.o) [OR = 1.73; 95% CI: 1.161-2.575] and pre-operative AMH serum level (< 2 ng/ml) [OR = 3.49; 95% CI: 2.346-5.193].

Limitations, reasons for caution: The fact that all women did not have an endoscopic exploration to phenotype or exclude the endometriosis status could constitute a possible bias. However, all included women underwent an appropriate pre-ART evaluation with a careful clinical examination and pelvic imaging to determine the endometriosis status.

Wider implications of the findings: Our results suggest that previous history of OMA surgery is linked to poor ovarian response to hyperstimulation. In some case where surgery appears essential, a primary fertility preservation could be proposed.

Trial registration number: NA.

P-278 Decreased autophagy of human endometrial stromal cells (HESCs) impairs decidualization via PI3K-Akt-mTOR pathway in adenomyosis

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Study question: To explore whether maladjusted autophagy impacts the regulation of human endometrial decidualization for patients with adenomyosis.

Summary answer: The lower decidualization response of eutopic HESCs from patients with adenomyosis is associated with its reduced autophagy induced by mTOR pathway.

What is known already: A defective decidualization response has been associated with reproductive disorders such as adenomyosis, endometriosis and

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recurrent pregnancy loss. Common benign gynecological disorder as adenomyosis is, studies revealed its blunted decidualization, even in eutopic stromal cells, which has a negative effect on implantation rates and increase the miscarriage risk. Autophagy is the natural, regulated, destructive mechanism of the cell that disassembles unnecessary or dysfunctional components. It was reported that some autophagy-related genes was abnormally expressed in eutopic endometria, which was supposed to affect the proliferation and migration of endometrial cells. Whether autophagy defect is envloved in decidualization process is still unkown.

Study design, size, duration: Immortal human endometrial stromal cell line T-HESCs and primary secretory phase HESCs are applied in *in vitro* experiments. Medroxyprogesterone acetate (MPA) and 8-Br-cAMP are uesd to induce decidualization, with PRL and IGFBP-1 as markers of decidualization. Rapamycin and M3-methyladenine are uesd to inhibit or activate mTOR or autophagy.

Participants/materials, setting, methods: Primary HESCs are isolated from eutopic endometrial biopsy of infertile patients with simple fallopian tube problems (n = 7) and patients with adenomyosis (n = 5) by diagnostic uterine curettage during secretory phase. Decidual samples (n = 7) are obtain though abortion operation of early pregancy. The expression of LC3A/B, Beclin-1, ATG5 and ATG12 are analyzed by western blot. QPCR and ELFA was performed to measure secretion of PRL and IGFBP-1.

Main results and the role of chance: LC3A/B, Beclin-1, ATG5 and ATG12 are up-regulated in a time-dependent manner in HESCs when stimulated with both 8-Br-cAMP and MPA for 24 h. Correspondingly, decidua (n = 7) of early pregnant women demonstrated higher expression of LC3A/B ($P < 0.01$), Beclin-1 ($P < 0.01$), ATG5 ($P < 0.05$) and ATG12 ($P < 0.05$) compared with secretory phase endometria (n = 7) from women with normal menstrual cycle. M3-methyladenine, the inhibitor of PI3K, significantly decreased PRL and IGFBP-1 ($P < 0.001$) secretion from decidual T-HESCs. LC3A/B($P < 0.001$), Beclin-1($P < 0.01$), ATG5($P < 0.05$) and ATG12($P < 0.01$) are all decreased expressed in edometria from patients with adenomyosis (n = 5) compared with the control groups (n = 7). The lower secretion of PRL and IGFBP-1 of decidual HESCS from adenomyosis patients endometria was recovered by mTOR inhibitor Rapamycin to a relatively normal level.

Limitations, reasons for caution: This study was only performed *in vitro*, animal experiments and autophagy relevant mechanism exploration are demanded in the further study.

Wider implications of the findings: We provide convinced evidences that autophagy is induced druing decidualization and the inhibitor of PI3K impairs T-HESCs decidualization. Except for adenomyosis, endometrial autophay disorder has been discovered in endometriosis, PCOS, and obesity, which are all impressionable for decidualization defect, indicating that autophay may be a new target for endometrium diseases.

Trial registration number: None.

P-279 Dysregulated immune genes and pathways likely contribute to endometriosis-associated infertility

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Study question: Are immune genes and pathways dysregulated in the eutopic endometrium of women with endometriosis?

Summary answer: In the eutopic endometrium of women with endometriosis, immune genes and pathways are upregulated contributing to infertility in women with endometriosis.

What is known already: Endometriosis is a pro-inflammatory disease leading to infertility in women with endometriosis. In endometriosis, impaired immune surveillance contributes to increased inflammation as well as ectopic invasion of tissue. Alteration in the immune response may lead to infertility.

Study design, size, duration: A prospective case-control study was conducted on 16 women with endometriosis and 13 without the disease between 1 April 2015 and 30 March 2016.

Participants/materials, setting, methods: Eutopic endometrial biopsies were obtained by Pipelle curette. Paralleled gene expression profiling using high density oligonucleotide microarrays was applied to investigate dysregulation of genes and pathways in eutopic endometrium of women with endometriosis and compared to that of women without the disease. Data analysis was conducted by GeneSpring version 4.0.4. T-test using P value < 0.05 and a fold-change expression > 1.5 was applied to assess statistical significance. Dysregulated genes were randomly chosen and validated by RT-PCR.

Main results and the role of chance: In the eutopic endometrium of women with endometriosis, genes involved in regulating immune response as well as the pathways involved in maintaining immune surveillance were significantly upregulated when compared to that from women without endometriosis.

Limitations, reasons for caution: The limitation of the study may be the sample size.

Wider implications of the findings: Upregulation of genes and pathways involved in regulating immune response likely contributes to increased inflammation by promoting production of cytokines, growth factors and oxidative stress. These may further contribute to impaired embryo attachment and apoptosis during the window of implantation leading to decreased pregnancy rates in women with endometriosis.

Trial registration number: Nil.

P-280 Identification of markers of endometrial epithelial cells (EEC's) contributes to the characterisation of EEC subtypes

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Study question: Are there specific markers to define human endometrial epithelial cell (EEC) subtypes?

Summary answer: The expression pattern of specific markers in the 3 different anatomical areas of the endometrium suggests that different EEC subtypes exist.

What is known already: The EECs reside in three different anatomical locations in the endometrium: the luminal epithelium, the functionalis glands, and the basalis glands. These three areas respond differentially to the same circulating steroid hormones, suggesting the presence of functionally distinct EEC's. There is detailed understanding of the epithelial cell subtypes in other organs with similar regenerative capacity to the endometrium, such as the intestine and fallopian tube. This has facilitated cell specific research, which has improved the understanding of epithelial associated disease. This detailed knowledge is still lacking in the endometrium.

Study design, size, duration: This is a prospective observational study, analysing full-thickness, healthy human endometrial samples from a total of 21 women (n = 16 pre-menopausal and n = 5 post-menopausal).

Participants/materials, setting, methods: Endometrium was obtained from women undergoing hysterectomy for benign conditions. The premenopausal samples were from proliferative (n = 7), secretory (n = 6) and menstrual (n = 3) phases of the menstrual cycle. The expression of AKR1C3, CD133, CK5/6, PODXL, MUC-1, Sialylated-SSEA-1, SSEA-1, and proliferative marker, Ki-67, was examined with immunohistochemistry. We also examined the differential expression of the genes coding these proteins by interrogating the published microarray datasets using a system biology approach.

Main results and the role of chance: The epithelial-specific marker CK5/6 was expressed in all 3 EEC subtypes, with greatest expression in the secretory phase luminal epithelium ($P < 0.05$). The hormone-metabolising enzyme AKR1C3 was highly expressed in the functionalis EEC's during the proliferative phase ($P < 0.01$). The transmembrane protein PODXL was found to express strong immunoreactivity in the cytoplasm, most notably in the basalis, and in post-menopausal endometrium. This pattern was distinct from the expected apical expression seen in the majority of EEC's, and provides evidence that basalis EEC's could be functionally different. The surface marker sialylated-SSEA-1 was highly expressed almost exclusively on the basalis EEC's. The embryonic stem cell surface marker, SSEA-1, marked the basalis EEC's, where expression was significantly greater than in the proliferative phase functionalis

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($P = < 0.05$). CD133 expression was weakest in luminal epithelium throughout the menstrual cycle, and strongest in the basalis, specifically in the postmenopausal endometrium. The microarray data generally confirmed the expression pattern specific to the functionalis of the genes coding for the proteins studied, highlighting the difficulties when all EEC's are considered together. This has provided a snapshot of differences in protein expression in EECs across the endometrium, suggesting functional dissimilarities, and the presence of EEC subtypes.

Limitations, reasons for caution: This is a descriptive study with a small sample size, and only protein expression of possible EEC markers analysed.

Wider implications of the findings: The differential expression of these EEC markers has provided preliminary evidence of the presence of different EEC subtypes. This warrants further investigation; with the aim of identifying the EEC subtypes that predispose to conditions such as endometriosis and endometrial carcinogenesis, and developing methods of isolation.

Trial registration number: Not applicable.

P-281 Usefulness of hematological parameters for differential diagnosis of endometriomas in younger and older women

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Study question: Does evaluation of hematological parameters has clinical significance in the differential diagnosis of endometriomas from other benign adnexal cysts in younger and older women?

Summary answer: Hematological markers have poor diagnostic value for distinguishing endometriomas from other benign adnexal cysts in both younger and older reproductive age women.

What is known already: Numerous biochemical markers have been evaluated to confirm the presence of the endometriosis. The most extensively used marker is CA-125, but it has limited diagnostic performance in detecting endometriosis. The available evidence suggests that inflammatory responses are involved in the etiopathogenesis of endometriosis. Previous studies have investigated the use of inflammatory markers including white blood cell (WBC) count, neutrophil- to- lymphocyte ratio (NLR), platelet indices, and platelet- to -lymphocyte ratio (PLR) for determining endometriosis. But, controversy remains regarding the clinical value of these complete blood count parameters in the differential diagnosis of endometriomas.

Study design, size, duration: This was a retrospective chart review study included five hundred and two patients during the period between January 2013 and December 2016.

Participants/materials, setting, methods: The study comprised a total of 502 patients who underwent laparoscopic surgery at Zekai Tahir BurakWomen's Health Research and Education Hospital. Of these, 267 had histologically proven endometriomas (endometrioma group) and 235 had other benign adnexal cysts (control group). Hematological parameters including WBC count, NLR, platelet indices, PLR, and serum CA-125 levels were compared between the groups. Furthermore, subgroup analysis was undertaken based on age (younger (< 25 years) and older (≥25 years) women).

Main results and the role of chance: There were no statistically significant differences in total and differential WBC counts, NLR, mean platelet volume (MPV) and platelet distribution width (PDW) levels among the endometrioma and control groups, The mean levels of hemoglobin,platelet count, PLR, plateletcrit (PCT) and CA-125 levels were significantly higher in the endometrioma group than the control group ($p = 0.001$). In subgroup analysis, these results were also not differ significantly in both younger and older women. The area under the curve (AUC) value for CA-125 was 0.854 (95% CI, 0.820-0.887) at the optimal cut-off value of 24.8 IU/mL with 78.7% sensitivity and 82.1% specificity ($p = 0.001$) for detecting endometriomas. But, platelet count, PLR and PCT showed poor discriminative ability.

Limitations, reasons for caution: The main limitation of our study is that the subgroup analysis based on age was done with limited number of women. Futher larger studies are needed to investigate the clinical usefulness of hematological parameters as simple and inexpensive markers for differential diagnosis of endometriomas.

Wider implications of the findings: Hematological markers are not sufficient for distinguishing endometriomas from other benign cysts in both younger and older women. It seems that, CA-125 remains more accurate biomarker for the differential diagnosis of endometriomas.

Trial registration number: NA.

P-282 In Vitro study of two natural compounds for Endometriosis treatment: Ellagic and Retinoic acid

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Study question: In this work we propose to assess the in vitro effect of two natural compounds: ellagic acid (EA) and retinoic acid (RA), on human endometrial cell adhesion.

Summary answer: EA and RA significantly reduced cell adhesion of human endometrial stromal cell line (T-HESC) and endometrial epithelial carcinoma cell line (ECC-1).

What is known already: EA is a natural poliphenol, which has been widely demonstrated to have properties as antioxidant and anti-tumoral in-vitro and in-vivo. RA is the active metabolite of vitamin A (retinol). It is known that retinoids have many biological effects, including a variety of immunomodulatory and anti-inflammatory activities, and have already shown satisfactory results in cancer research. In our previous work was demonstrated that 10 μM RA significantly inhibited T-HESC and ECC-1 proliferation, while 100 μM EA inhibited ECC-1 proliferation and no interfere with T-HESC viability. In the same way, we observed a significant tendency towards T-HESC cycle arrest after RA and EA treatment.

Study design, size, duration: Both cell lines, T-HESCs and ECC-1, were incubated with EA or RA for 24 h, and subsequent evaluated for plastic adhesion.

Participants/materials, setting, methods: Cell adhesion was evaluated by plastic adhesion assay in ECC-1 and T-HESC cultures. Briefly, cells were treated with 50-100 μM EA and 5-10 μM RA. After 24 h cells were trypsinized and allowed to recover for 40 min in fresh serum-free medium. Then were sowed and 60 min later adhered cells were washed, trypsinized and counted.

Main results and the role of chance: We observed that 24 h treatment reduced plastic adhesion of both cell lines under study. ECC-1 treated with 50 and 100 μM EA significantly reduced their plastic adhesion ability ($p < 0.01$ and $p<0.001$ respectively). In the same way, ECC-1 treated with 5 and 10 μM RA, showed a significant reduced adhesion ($p<0.01$ and $p<0.001$ respectively). As well as ECC-1, T-HESC treated with both assayed doses of EA and RA showed reduced plastic adhesion ($p<0.05$ and $p<0.01$ respectively).

Limitations, reasons for caution: Further studies should be aimed at fully understanding of the mechanism behind the obtained results, as well as in vivo trials to subsequently assess these compounds safety for patients with endometriosis.

Wider implications of the findings: Current treatments for endometriosis consist on laparoscopic-surgery and continuous hormonal treatments. As their efficiency is under discussion, non-adverse effect long-term therapies remain a field of strong research. From these results, and taken together with the background, we highlight the idea to investigate natural compounds as novel and promising therapeutic treatment.

Trial registration number: not applicable.

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P-283 Active biomolecules present in Scutellaria baicalensis and Rosmarinus officinalis induce cell cycle arrest in in-vitro model of endometriosis

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Study question: The objective was to evaluate the effect of Wogonin (WG), Carnosic Acid (CA) and Rosmarinic Acid (RA) on human endometrial stromal cell line (t-HESC) growth.

Summary answer: Results suggest that inhibition of t-HESC proliferation by CA and WG might be associated to cell cycle arrest induction in G2/M phase.

What is known already: Current medical therapies available for endometriosis result inefficient due to their long term side effects and high levels of recurrence. Natural compounds are being investigated because of their beneficial properties. WG, a flavonoid isolated from *Scutellaria baicalensis* root, is the most active constituent of Chinese Herbal Medicine. CA and RA are two of the main antioxidant compounds found in *Rosmarinus officinallis* leaves. Anti-tumoral effects of these polyphenols have already been reported in several types of tumor cell lines. Previous studies developed in our laboratory demonstrated that these compounds inhibit endometrial stromal cell proliferation in both *in-vitro* and *in-vivo* endometriosis model.

Study design, size, duration: T-HESC cell line was incubated with WG, CA or RA for 24 hours or 48 hours for apoptosis assay or cell cycle analysis respectively. After incubation, cells were harvested and fixed, and TUNEL technique or propidium iodine staining was performed. Apoptosis and cell cycle analysis were measured by flow cytometry.

Participants/materials, setting, methods: After 24-h incubation with CA (5 and 7.5 μg/ml), RA (50 and 100 μg/ml) and WG (40 and 80 μM), cells were fixed to assess the percentage of apoptosis by the TUNEL technique. After 48-h incubation with CA (2.5 and 5 μg/ml), RA (50 and 100 μg/ml) and WG (40 and 80 μM), cells were fixed and DNA was stained with propidium iodine. Subsequently, apoptosis and cell cycle were measured in t-HESC by flow cytometry.

Main results and the role of chance: Pro-apoptotic effect of WG, CA and RA has not been evidenced by TUNEL technique in t-HESC cell line after 24-h incubation with each compound. WG 40 and 80 μM significantly decreased the percentage of cells in the G0/G1 phase and complementarily increased the percentage of cells in G2/M phase ($p<0.001$ vs. basal). In the same way, CA 5 μg/ml decreased G0/G1 percentage with an increase of G2/M phase percent ($p<0.01$ vs. basal). Cell cycle deregulation has not been observed after RA incubation. These results suggest that inhibition of endometrial stromal cell proliferation induced by WG and CA might be associated to cell cycle arrest induction at G2/M stage.

Limitations, reasons for caution: More studies should be addressed to fully understand the mechanism behind the results obtained.

Wider implications of the findings: Previous results demonstrated that WG, RA and CA exerted inhibitory effect on *in-vitro* cell proliferation and *in-vivo* endometriosis-like lesions development. New results indicate that this antiproliferative effect could be associated to cell cycle arrest. Our studies support further investigation of novel, safe and well-tolerated botanical products as future endometriosis treatments.

Trial registration number: not applicable.

P-284 Searching for endometriosis biomarkers with proteomics

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Study question: We aimed to identify novel biomarkers of endometriosis in eutopic and ectopic endometrium and verify these biomarkers in serum.

Summary answer: We did not identify any biomarkers that performed better than CA - 125 alone.

What is known already: Endometriosis is a common gynaecological disorder affecting 5-10% of women of reproductive age and occurs when endometrial cells implant on surfaces in the abdomen and pelvis. There they form lesions that respond to hormones of the cycle and stimulate inflammation. Women with endometriosis experience painful periods, pain on intercourse and defecation and may have difficulties conceiving. Definitive diagnosis is surgical, requiring laparoscopy under general anaesthetic, exposing patients to potentially serious complications.

Study design, size, duration: Prospective observational cross sectional study of women presenting to a tertiary referral centre with a history of pelvic pain or for prophylactic surgery (control) recruited between March 2013 and March 2016.

Participants/materials, setting, methods: We collected eutopic and ectopic endometrial tissue from 28 patients with endometriosis and 18 controls. We used quantitative 2D difference gel electrophoresis and multiplex mass tagging of peptides linked to 3D liquid chromatography-based separation and tandem mass spectrometry to select candidates (LUM, CPM, TNC, TPM2, PAEP/glycodelin). We verified these by ELISA in 109 serum samples from the same and additional women. Reported biomarkers (CA125, sICAM1, FST, VEGF, MCP1, MIF and IL1R2) were also tested.

Main results and the role of chance: Cycle phase and endometriosis-associated changes were identified in the eutopic tissue expression profiles from over 1,400 identified gene products. Bioinformatics analysis revealed enrichment of adhesion/extracellular matrix proteins and progesterone signalling. The best single marker for discriminating endometriosis from controls remained CA125 (AUC = 0.72). The best cross-validated multi-marker model (CA125, sICAM1, FST) gave an AUC of 0.75 for discriminating endometriosis from both control groups.

Limitations, reasons for caution: We have performed a comprehensive proteomic analysis of eutopic tissue samples from patients with endometriosis, relevant pain controls and healthy women identifying candidate biomarkers. These and previously reported markers were tested in a larger set of serum samples taking cycle stage into account.

Wider implications of the findings: The best models gave only marginal improvement on using CA125 alone, and would thus be unsuitable as standalone diagnostic tests or for use in triaging for surgery.

Trial registration number: N/A.

P-285 Protein expression and cellular localization of Estrogen Receptors alpha and beta in endometriosis-associated epithelial ovarian cancer cell lines

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Study question: To evaluate the protein expression and the cellular localization of Estrogen Receptor alpha (ERα) and beta (ERβ) in cell lines derived from human endometrioid and clear-cell Epithelial Ovarian Cancer (EOC).

Summary answer: Both endometriosis-associated cell lines expressed these steroid receptor proteins. ERα was mainly localized into the cellular nucleus, whereas ERβ was mostly localized into cytoplasm.

What is known already: The pathophysiological mechanisms involved in the etiology of the endometriosis-associated EOC, such as endometrioid and clear-cell ovarian tumors, are not entirely known yet. However it is well-known that the local hormonal environment that promotes the development of these ovarian carcinomas is similar to the peritoneal hormonal environment found in patients with endometriosis, where the steroid hormones levels, mainly

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estradiol, are particularly high. Therefore, it is crucial the development of suitable experimental models in order to allow an accurate characterization of the potential role exerted by the endometriotic lesions-released estrogens on the occurrence and progression of endometriosis-associated EOC.

Study design, size, duration: Human epithelial ovarian cancer cell lines were cultured under standard conditions until confluence to further analysis of ERα and ERβ protein expression and cellular localization.

Participants/materials, setting, methods: TOV-112D cell line (ATCC CRL-11731) derived from a human endometrioid-type ovarian carcinoma and TOV-21 G cell line (ATCC CRL-11730) derived from a human clear-cell ovarian carcinoma were purchased from the American Type Culture Collection (ATCC). The protein expression and cellular localization of ERα and ERβ in each cell line was analyzed by Western blot and indirect immunofluorescence (IFI) respectively, in 4 independent experiments.

Main results and the role of chance: We found that both EOC cell lines expressed ERα and ERβ proteins, being the ERα expression qualitatively stronger than ERβ in both cell lines. These results suggest that these endometriosis-associated EOC cell lines have the necessary molecular machinery to respond to steroid hormones stimuli. In addition, we found that in both cell lines ERα was mostly located into the nucleus, indicating that this receptor had been activated by its canonical ligand, dimerized and translocated to the cellular nucleus, where it regulates the transcription of its target genes. ERβ was mainly found in the cytoplasm, where it is mostly inactive.

Limitations, reasons for caution: This is an initial descriptive study, so it is very important to make it clear that functional studies will be addressed to fully understand the role of the estradiol secreted by endometriotic lesion on the pathophysiological mechanisms underlying the etiology of endometriosis-associated EOC.

Wider implications of the findings: These cell lines are endowed with the necessary molecular machinery (i.e. ERα and ERβ) to respond to estradiol released by endometriotic lesions, thus becoming a suitable *in vitro* model to study the potential role of steroid hormones on the onset and development of endometriosis-associated EOC.

Trial registration number: not applicable.

P-286 A comparison of IVF cycles in women with endometriomas pre-treated with either depolurpolide acetate or depoluprolide acetate and letrozole

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Study question: Does the addition of an aromataze inhibitor improve IVF outcomes when pre-treating women with gonadotropin releasing hormone agonists with endometriomas?

Summary answer: The combination of depoluprolide-acetate 3.75 mg monthly for 60 days combined with letrozole 5 mg daily orally, has better clinical outcomes at IVF than depoluprolide-acetate alone.

What is known already: At meta-analysis and in five randomized studies, three to six months of depolurprolide pre-IVF improves clinical outcomes, as compared to placebo. Most clinicians give depoluprolide for two months to prevent extended delays in care. Depoluprolide causes a pseudo-menopausal state decreasing estradiol, through which it diminishes endometriosis. Aromatase inhibitors affect the endogenous aromatase in the endometriosis lesions. Combining both can results in decreasing diameter or resolutions of the cysts. Comparison of both protocols have not been previously tried in IVF patients with endometriosis.

Study design, size, duration: A prospective cohort study was performed from 2011 until 2016. 62 women with endometriomas were offered depolupron and 52 completed care. 64 women with endometriomas were prescribed depoluprolide and letrozole and 50 completed care.

Participants/materials, setting, methods: Patients with a failed previous IVF cycle and all subsequent frozen embryo transfers, ground glass appearance persistent cysts consistent with endometriomas on ultrasound, treated at a University IVF center were non-randomly assigned to one or the other treatment pre-fresh IVF. Statistics were evaluated with t tests and chi-square tests. Data is presented as Mean ± SD.

Main results and the role of chance: Among letrozole (negative vs. positive) treated subjects respectively, demographics did not differ for: female age (34.2 ± 3.2 vs. 34.6 ± 3.4 years, p = 0.54), antral follicle count pre-luprolide (6.7 ± 2.4 vs. 6.2 ± 1.9, p = 0.25), serum basal FSH level pre-luprolide (10.6 ± 2.6 vs. 11.3 ± 2.3 IU/L, p = 0.15), mean largest endometrioma diameter pre-luprolide (3.4 ± 0.7 vs. 3.5 ± 0.7 cm, p = 0.47) or number of failed embryo transfers (1.4 ± 0.2 vs. 1.4 ± 0.2, p = 1.0). Post luprolide the AFC differed between the letrozole (negative vs. positive) treated groups (6.4 ± 2.5 vs. 10.3 ± 2.0, p = 0.0001), as did the mean endometrioma maximum diameter (3.2 ± 0.8 vs. 1.8 ± 0.4 cm, p = 0.0001). At IVF, the gonadotropin dose used was less in the letrozole treated subjects (3716 ± 1314 vs. 2079 ± 1119 IU, p = 0.0001), the number of mature oocytes collected was greater (4.0 ± 1.7vs. 9.1 ± 2.4, p = 0.0001), the number of 2 PN embryos was greater (1.3 ± 1.0 vs. 7.3 ± 2.9, p = 0.0001), the number of blastocysts was greater (0.6 ± 0.2 vs. 3.1 ± 1.1, p = 0.0001). The number of transferred embryos were similar. The clinical pregnancy rates was higher in the letrozole treated group (12/52 vs. 25/50, p = 0.0047) as was the ongoing pregnancy rate (9/52 vs. 20/50. p = 0.01).Note non-of the subjects had complete resolution of the endometriomas with letrezole as seen in non-fertility studies.

Limitations, reasons for caution: This preliminary study was performed to gather evidence for the development of a prospective randomized trial. The non-randomized allocation of subjects may mask a hidden bias.

Wider implications of the findings: Data suggests that among women with endometriomas ovarian reserve tests and clinical outcomes can be improved with pretreatment of several months of gonadotropin releasing hormone agonist combined with an aromatase inhibitor. Data from the prospective trial will be needed to confirm these results.

Trial registration number: Not aplicable.

P-287 Pharmacokinetics, pharmacodynamics, and safety of relugolix, a potent oral once-daily gonadotropin-releasing hormone (GnRH) receptor antagonist, as monotherapy and in combination with estradiol/norethindrone acetate add-back therapy

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Study question: To evaluate pharmacokinetics/pharmacodynamics (PK/PD) and safety/tolerability during 6 weeks of treatment with relugolix or relugolix plus low-dose estradiol/norethindrone acetate add-back therapy in healthy premenopausal women.

Summary answer: Relugolix plus estradiol/norethindrone acetate hormonal add-back therapy resulted in estradiol plasma concentrations that mitigated bone turnover and vasomotor symptoms associated with administration of relugolix alone.

What is known already: Relugolix, an oral GnRH receptor antagonist, has demonstrated clinical benefit in the symptomatic treatment of women with uterine fibroids or endometriosis in phase 2 clinical studies when used at doses resulting in a high degree of estradiol suppression. However, these doses are associated with time-dependent loss of bone mineral density and burdensome hot flashes. Low-dose hormonal add-back therapy, comprised of an estrogen and progestogen, has been shown in prior studies with other agents to mitigate these adverse effects with minimal impact on symptom control. This is the first study evaluating the PK/PD and safety of relugolix combined with add-back therapy.

Study design, size, duration: This was a phase 1, open-label, parallel-group, PK/PD study. Forty-eight women were randomized to receive relugolix 40 mg

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or relugolix 40 mg plus estradiol/norethindrone acetate (1 mg/0.5 mg) once daily for 6 weeks. The first day of dosing occurred on Day 1 to 6 of the menstrual cycle. PK/PD samples were collected throughout the study, and 24-hour PK profiles were obtained at Week 3 and 6. Subjects returned for a follow-up visit at Week 8.

Participants/materials, setting, methods: Healthy premenopausal women were enrolled at 4 clinical sites in the United States. In addition to laboratory safety assessments, serum was sampled for follicle-stimulating hormone, luteinizing hormone, estradiol, and progesterone concentrations. Relugolix, estradiol, estrone, norethindrone, and ethinyl estradiol PK concentrations and PK parameters were determined. Bone resorption markers N- and C-telopeptide were also determined at baseline, Week 3, and Week 6. Vasomotor symptoms and menstrual bleeding were captured using a daily diary.

Main results and the role of chance: Relugolix PK were not impacted by estradiol/norethindrone acetate.

After treatment with relugolix alone, Week 6 median C_{trough} and C_{max} estradiol concentrations were 5.77 and 7.22 pg/mL, respectively. With estradiol/norethindrone, the median values increased to 21.4 and 49.2 pg/mL, respectively.

After 6 weeks, the % change from baseline in N-telopeptide and C-telopeptide were significantly elevated after treatment with relugolix, but not after treatment with relugolix plus estradiol/norethindrone acetate, indicating reduced bone resorption.

The proportions of women reporting no menstrual bleeding other than minor spotting over the last 28 days of treatment were 88.0% and 47.8% after treatment with relugolix alone or relugolix plus estradiol/norethindrone acetate, respectively. The proportion of women reporting hot flashes (any grade) was significantly mitigated by the addition of estradiol/norethindrone acetate (60.0% for relugolix alone vs 17.4% with estradiol/norethindrone during Week 6).

The most commonly (≥10%) reported adverse events in the study were hot flash, headache, nausea, and events of uterine bleeding, consistent with GnRH antagonist pharmacology. With the exception of severe hot flash, adverse events were mild or moderate in intensity. One subject experienced 2 serious adverse events (syncope and chest pain) unrelated to study drug. There were no deaths, withdrawals due to AE, or reported pregnancies.

Limitations, reasons for caution: In this study, treatment with relugolix plus estradiol/norethindrone acetate was limited to 6 weeks in healthy premenopausal women. The impact of relugolix co-administered with add-back therapy in patients with endometriosis or uterine fibroids requires further evaluation in longer duration phase 3 studies.

Wider implications of the findings: This study characterized the effects of relugolix co-administered with hormonal add-back therapy and provides a rationale to evaluate this combination in women with symptoms associated with uterine fibroids or endometriosis. The data suggest add-back therapy may mitigate bone mineral density loss and vasomotor symptoms associated with estradiol-suppressive doses of relugolix.

Trial registration number: NA.

P-288 Anatomical visualization of inferior hypogastric nerves during uterosacral ligamentectomy for deep infiltrating endometriosis contributes to the prevention of postoperative dysuria

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Study question: This study was performed to establish the optimal surgical strategy for deep infiltrating endometriosis to prevent postoperative dysuria.

Summary answer: Anatomical visualization of inferior hypogastric nerves during uterosacral ligamentectomy for deep infiltrating endometriosis is suggested to contribute to the prevention of postoperative dysuria.

What is known already: Uterosacral ligamentectomy is an established surgical method to improve pain related to deeply infiltrating endometriosis, including menstrual, sexual, bowel, and chronic pelvic pain. Owing to the closed anatomical location of the ureter, hypogastric nerve, and pelvic plexus to the uterosacral ligament, dysuria can be caused by the damage to these tissues. Approximately 30% of patients who underwent extensive ligamentectomy without attention to tissue injury were reported to have urinary disorders that required transient or permanent self-catheter urination. However, the surgical strategy to prevent these postoperative complications has not been elucidated.

Study design, size, duration: In this study, 48 patients who underwent surgery for endometriosis in our institute between August 2013 and December 2015 without uterosacral ligamentectomy (group A, n = 14), with bilateral uterosacral ligamentectomy (group B, n = 24), and with unilateral uterosacral ligamentectomy (group C, n = 10) were enrolled. Postoperative dysuria in each group was retrospectively assessed based on the Core Lower Urinary Tract Symptom Score (CLSS) and statistically evaluated.

Participants/materials, setting, methods: In this study, we introduced a procedure to prevent tissue injury by anatomically visualizing the hypogastric nerve during uterosacral ligamentectomy. From among the CLSS evaluation items, 'Slow urinary stream," 'Need to strain when urinating," and 'Feeling of incomplete emptying of the bladder after urination" were evaluated and rated in four stages as follows: no, 0; rare, 1; sometimes, 2; and often, 3. The total scores were compared between the groups and statistically analyzed.

Main results and the role of chance: In contrast to the previous report, none of the patients in either group showed severe postoperative dysuria that required catheter urination. The scores of the urinary disorders were not significantly different among the groups (A: 1.14 ± 0.48, B: 0.92 ± 0.31, C: 0.60 ± 0.40), indicating that postoperative dysuria after deep endometriosis can be prevented by visualizing the hypogastric nerve anatomically and selectively resecting only the sacral uterine ligament itself without damaging surrounding tissues.

Limitations, reasons for caution: Owing to the small sample size of this study, further investigation with more patients is necessary to confirm our preliminary findings.

Wider implications of the findings: Not only pursuing the improvement of the symptoms of the original disease but also developing a surgical strategy by paying attention to the prevention of complication will contribute to the improvement of the true postoperative quality of life of patients with deep endometriosis.

Trial registration number: not applicable.

P-289 Is pregnancy the panacea for pain symptoms associated with endometriosis?

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Study question: What is the long- term impact of pregnancy on endometriosis-related pain?

Summary answer: Women with endometriosis experience symptoms relief during and immediately after pregnancy. However, as for hormonal therapy, symptoms rapidly recur in the vast majority of cases.

What is known already: Albeit based on scanty evidence, pregnancy is commonly believed to confer symptomatic benefit to women with endometriosis. In fact, the supposed beneficial effects of pregnancy have inspired modern therapy of endometriosis. The idea of curing the disease with progestins, thus mimicking pregnancy, has guided investigators for decades. However, while pregnancy is claimed to determine long-term benefits that can definitely cure endometriosis or at least determine a long-term protection from recurrences, the benefits of progestins are well-known to rapidly vanish once treatment is

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discontinued. This inconsistency is intriguing and merits more in-depth investigation.

Study design, size, duration: Retrospective cohort study performed at a tertiary care center for the treatment of endometriosis. We included women who had a live birth after at least one surgery for endometriosis and who reported pre-pregnancy moderate to severe pelvic pain symptoms. The study period lasted from 2008 to 2013.

Participants/materials, setting, methods: 144 women were identified, aged between 18 and 45 years. Data were primarily extracted from patients' charts. They referred to the last pre-pregnancy clinical evaluation and to the assessment made at two years after delivery. Comparisons were made for pain symptoms, quality of life (using the SF-12 questionnaire), psychological status (using the Hospital Anxiety and Depression scale) and sexual functioning (using the Female Sexual Function Index).

Main results and the role of chance: Pre-pregnancy moderate to severe dysmenorrhea, deep dyspareunia, non-menstrual pelvic pain and dyschezia were reported in 137 (95%), 91 (63%), 71 (49%) and 60 (42%) cases, respectively. Sixty women (42%) initiated medical therapy during the follow-up period. Specifically, 29 (20%) started because of pain complaints, 10 (7%) for prevention purposes, 3 (2%) for contraception and the remaining 18 (12%) because of both pain recurrence and need for contraception. Four women (5%) required a surgical intervention for endometriosis. Overall, 49 women had a clinically relevant recurrence requiring treatment, corresponding to 34% (95%CI: 27-42%). This proportion rose to 54% (95% CI 39-68%) when restricting the analysis to women who were already assuming medical therapy because of pain before pregnancy seeking. Two years after delivery, 84% of women (95% CI 77-90%) reported at least one moderate-severe pain symptom. A statistically significant improvement was observed for SF-12 and HADS scores but not for FSFI scores. For SF-12, improvements were observed for both physical and mental health scales. Similarly, the HADS scores showed improvements for both anxiety and depression scales.

Limitations, reasons for caution: The retrospective design potentially implicates inaccuracies even if in our center information is systematically collected at every visit. Moreover, pre-post pregnancy comparisons were complicated by the heterogeneous therapeutic trajectories of the population, and a selection bias cannot be ruled out, as women who did not refer after pregnancy were excluded.

Wider implications of the findings: The pregnancy-related hormonal milieu and the psychological benefits of parenthood may benefit women. However, as for hormonal therapy, symptoms rapidly relapse in the vast majority of cases. Pregnancy represents a period of substantial but only transient symptoms relief, and cannot be viewed as a definitive cure of the disease.

Trial registration number: Not applicable.

P-290 Epigenetic alteration of Thymic Stromal Lymphopoietin, TSLP, in endometriosis

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Study question: Is there any changes in expression and epigenetic profiles of Thymic Stromal Lymphopoietin and its receptor in endometrium of women with endometriosis?

Summary answer: TSLP is up-regulated in ectopic endometrial tissues of women with endometriosis through hyper-acetylation and hypo-methylation of histone H_3 region in its promoter.

What is known already: Endometriosis which is defined as the presence of endometrium like tissue outside of uterine is an estrogen dependent and inflammatory disease. It has been suggested that immunological factors play important roles in the pathogenesis of endometriosis. TSLP is an interleukin 7–like cytokine that triggers dendritic cell–mediated T helper2 inflammatory responses. TSLP receptor, also known as cytokine receptor- like factor 2 (CRLF2), forms a functional heterodimeric complex with IL-7 R to bind with TSLP. On the other hand, epigenetic aberrations such as DNA methylation and histone modifications appear to be involved in various diseases such as endometriosis.

Study design, size, duration: In this case-control study, ectopic and eutopic endometrial samples were obtained through laparoscopic procedure from 15 women with endometriosis. As control group, endometrial tissue biopsies were collected by pipelle from 16 healthy fertile women.Women with any other uterine abnormalities were excluded. Informed written consent was obtained from all women according to local ethical approval.

Participants/materials, setting, methods: The relative mRNA expressions of TSLP and TSLPR were studied by the use of quantitative-PCR technique. Chromatin Immunoprecipitation (ChIP) coupled with real-time PCR was used to monitor histone modifications (methylation/acetylation) on lysine 9 of histone 3 (H3K9me/ ac) in the regulatory region of TSLP gene.

Main results and the role of chance: Expression profile of TSLP and TSLPR genes revealed significant increase ($p < 0.05$) in ectopic lesion of patients with endometriosis in comparison to endometrium of eutopic samples and control group.

In ectopic tissues of endometriosis patients, a significant H_3K_9 hyper acetylation parallel to hypo methylation was detected in regulatory region of TSLP gene compared to eutopic and control groups ($p < 0.05$). These epigenetic changes were aligned with its expression changes.

Limitations, reasons for caution: For getting more information, we need to investigate TSLP genes in a large number of endometriosis patients and control group and also to evaluate expression of other related genes. Other Epigenetic studies such as DNA methylation of TSLP promoter region are recommended.

Wider implications of the findings: These data collectively identify TSLP and TSLPR as a candidate gene critically involved in development of endometriosis beyond its role in promoting Th_2 immune responses.

Trial registration number: -.

P-291 Targets for improvement in endometriosis care in a secondary and tertiary clinic

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Study question: What are, according to patients, differences in strengths and targets of improvement regarding endometriosis care in a tertiary and a secondary clinic in the Netherlands?

Summary answer: We found 3 corresponding strengths and 9 targets for improvements. Patients experienced 9 aspects variously as strength or as targets for improvement between the clinics.

What is known already: Endometriosis is a chronic disease with high impact. Therefore, it is important to optimize the quality of endometriosis care. One of the cornerstones for high quality of care is patient-centeredness, which can be assessed with the Endocare questionnaire (ECQ). Patients' quality of life (QoL) differ between a secondary and tertiary clinic, which might mean that the patient-centeredness of care also differs between tertiary and secondary clinics. To date, the ECQ has solely been used in two tertiary care facilities.

Study design, size, duration: A cross sectional study was conducted in surgically diagnosed patients with endometriosis. A total of 401 of them received the ECQ between 2015 and 2016. Two reminders were sent.

Participants/materials, setting, methods: All patients were selected from a Dutch tertiary and a Dutch secondary clinic. Patients underwent endometriosis surgery in 2013 or 2014.

The ECQ asks patients to score performance in different care aspects and asks patients how important those aspects are, combining these results in the patient-centeredness scores (PCS). Patient-centeredness was deducted from

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the ECQ's using the previous published scoring system. Strengths and targets for improvement were identified using an importance-performance matrix.

Main results and the role of chance: The overall response rate was 56.9%. In total, data from 209 patients could be analyzed (133 and 76 respectively for the tertiary and secondary clinic).

In both clinics 'shared decision making', 'managing expectations' and 'kindness of caregivers' were experienced as strengths. Targets for improvement were 'advise on self-care and recovery after surgery', 'receiving consistent information from different caregivers', 'ability to discuss daily complaints', 'having one regular caregiver', 'accessibility of caregiver in urgencies', 'waiting periods', 'indicated limits of competences' and 'pro-activity of doctors'.

Care aspects that were strengths in the tertiary clinic, but were targets for improvement in the secondary clinic were 'being taken seriously', 'information on pain medication' and 'short diagnostic delay'. Conversely, strengths in the secondary clinic were 'treated as a person instead of a number', 'clarifying information', 'understanding and caring caregivers', 'being reassured', 'known head practitioner', and 'trust in the experience of caregivers', whereas in the tertiary clinic these items were targets for improvement.

Limitations, reasons for caution: The differences in strengths and targets for improvement between secondary and tertiary clinics might be due to a difference in what patients value or what they experience between type of clinics.

Wider implications of the findings: The two investigated clinics could learn from another how to improve the patient-centeredness, and thereby quality of care, since the strengths from one clinic are the targets for improvement in the other. Improvement projects need to be set-up and evaluated.

Trial registration number: not applicable.

P-292 Effect of cabergoline and letrozole on subcutaneous experimental model of endometriosis

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Study question: What is the effects of cabergoline and letrozole on induced subcutaneous endometrial lesion in experimental endometriosis model of rat?

Summary answer: cabergoline and letrozole administration resulted in decreased size of the induced subcutaneous endometrial lesions in experimental endometriosis model of rat.

What is known already: A great number of medications were examined in preclinical models of endometriosis and suggest that letrozole (non-steroid third generation aromatase enzyme inhibitor) is effective in treatment of patients with endometriosis (reducing size and symptoms), But there is less information regarding the effects of cabergoline (dopamine agonist) on endometriosis.

Study design, size, duration: Twenty four female Sprague-Dawley mature rats were used for an experimental study in the Avicenna Research Institute.

Participants/materials, setting, methods: Endometriosis was surgically induced in 24 rats by transplanting of autografted endometrial tissue subcutaneously. After 3 weeks (for establishment of implants), animals were randomized into three groups and treated with cabergoline (0.5 mg/kg/day) (group I),letrozole (0.18 mg/kg/day) (Group II) and vehicle solution (Group III,control), subcutaneously. Three weeks later, the rats were euthanized and the implants were assessed for volume and histopathological grade of implants.

Main results and the role of chance: All the animals had comparable baseline characteristics. We found that the volume of endometrial lesions decreased significantly in cabergolin and letrozole groups ($p < 0.001$) compared to the control group. The histopathology grade of implants had significant difference between groups with $\alpha = 0.1$ (p:0.078).

Limitations, reasons for caution: The study was done on animal subjects. Assessment of effect of drugs in human is difficult because it needs surgery.

Wider implications of the findings: cabergoline resulted in decreased size of the induced endometrial lesions in rat.cabergoline appear to be a virtual therapeutic agent for treatment of endometriosis probably due to its potent antiangiogenic properties.Also The model of subcutaneous endometriosis is a considerable,low-cost,easy to perform, and proper for the study of the effects of drugs.

Trial registration number: Not applicable.

P-293 Evaluation of coagulation variables in endometriosis

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Study question: Is there a strong relation between endometriosis and clotting abnormalities and inflammation blood parameters?

Summary answer: We confirm the presence of a shorter, but still in the normal range, APTT in women with ovarian endometriomas compared to a control population.

What is known already: Subtle alterations of coagulation and fibrinolysis parameters have been recently reported in endometriosis patients supporting a potential hypercoagulable status associated with this disease.

Study design, size, duration: This cross-sectional study was carried out retrospectively querying our prospective surgical database at San Raffaele Scientific Institute in Mlilan. A total of 314 Patients who underwent surgery between January 2013 and December 2015 were considered. The case group (n = 169) included

The case group (n = 169) included patients with a surgical diagnosis of endometriosis, at any stage of disease.The control group (n = 145) included women with a surgical diagnosis of benign gynecologic pathology.

Participants/materials, setting, methods: Both the surgical and the histopathological examinations confirmed that no stage of endometriosis was present in the control group.

The routine preoperative included complete blood count parameters, NLR, platelet-lymphocyte ratio (PLR), TT ratio, APTT ratio and INR.

A univariate linear regression analysis was conducted in order evaluate the association of each variable with different phenotypes of endometriosis, that were included in the regression analysis as n-1 dummy variables.

Main results and the role of chance: No difference were found for thrombin time, INR, platelet count, and platelet-to-lymphocyte ratio between women with endometriosis and controls. Conversely, patients with endometriosis had significantly shortened activated partial thromboplastin time (APTT) as compared with controls (1.08 + 0.06 and 1.12 + 0.19, respectively; $P < 0.01$). In the subgroup analysis, women with ovarian endometriosis had significantly shortened APTT values in comparison with women without this form (controls and women with peritoneal and deep endometriosis). In multivariate logistic regression analysis, after controlling for potential confounders, a shortened APTT remained associated to the disease.

Limitations, reasons for caution: Limits included the retrospective querying and the presence of only one type of controls, the surgical population. All the subjects considered had a laparoscopic diagnosis, were not on oral contraceptives and we could include a large sample size with a great variety of forms and manifestations of the disease.

Wider implications of the findings: The evidence is insufficient to claim a crucial role of a systemic hypercoagulable state in the origin of the disease. Our evidence does not exclude a role of the local coagulation system in the pathogenesis of endometriosis.

Trial registration number: not applicable.

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P-294 Reduced ovarian sensitivity but similar chance of cumulative live birth after IVF/ICSI and high frequency SET in women with endometriosis-associated infertility

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Study question: Does endometriosis associate with a reduced ovarian sensitivity and worse IVF/ICSI outcome estimated as cumulative live-birth rates per ovum pickup (OPU) than other infertility causes?

Summary answer: Endometriosis patients had significantly reduced ovarian sensitivity compared to infertile women without endometriosis; however, IVF/ICSI with predominantly SET resulted in similar cumulative live-birth per OPU.

What is known already: Single-embryo transfer (SET) with subsequent frozen-embryo transfer (FET) was shown to result in similar cumulative live-birth rates compared to double-embryo transfer in infertile patients < 36 years. Several studies reported a negative effect of endometriosis on the ovarian reserve and on IVF/ICSI outcome, suggesting negative effects in both oocyte yields and the endometrial environment resulting in implantation failure. If endometriosis would predominately affect endometrial factors, fresh and FETs should be affected in such patients while ovarian factors might negatively affect cumulative live-birth rates.

Study design, size, duration: Retrospective cohort study of 2757 consecutive patients undergoing IVF/ICSI at a single center from January 2009 until December 2013 with reported data on live birth. A total of 2757 patients underwent 8236 treatment cycles (4598 fresh and 3638 frozen). Infertile women with symptomatic and demonstrated endometriosis during their infertility work-up undergoing IVF/ICSI (cases N = 172) were compared to infertile women without endometriosis (controls N = 2585).

Participants/materials, setting, methods: Approximately 2/3 of the women with endometriosis had previous adnexal surgery and nearly 40% had endometriomas. Patients underwent IVF/ICSI followed by SET in most cases and single FET in consecutive cycles. Statistical analysis was done using a generalized estimated equation (GEE) model accounting for dependences between consecutive treatments. Primary outcome measures: Ovarian sensitivity index and cumulative live-birth per OPU.

Main results and the role of chance: Women with endometriosis were similar to controls as regards to age (33.56 vs. 33.59 years, p = 0.82) or BMI (p = 0.60). Patients with endometriosis required significantly longer stimulation (p = 0.0008) and higher FSH doses (p = 0.0001). There was no difference in cycle cancellations (p = 0.76) or miscarriages (p = 0.83) between the two groups. In both groups, over 70% of the patients received SET and about 80% received frozen-thawed SET in consecutive cycles (p = 0.49). In fresh cycles, live-birth rates per OPU were similar between endometriosis vs. controls, (26.41% vs. 25.52%, respectively, p = 0.71) and per ET (29.18% vs. 28.39%, respectively, p = 0.78). Live birth rate was also similar after FET in both groups (22.22% vs. 26.16%, respectively, p = 0.43) and the cumulative live-birth per OPU did not differ between the groups (35.56% vs. 34.67%, respectively, p = 0.73). The results did not change after adjusting for age, and they were also similar irrespective of presence of ovarian endometriomas.

Limitations, reasons for caution: Retrospective character of the study that allows to examine associations but not causality. A minor proportion of the study population received DET (< 20%).

Wider implications of the findings: SET and consecutive FET conclusively can be encouraged in patients with endometriosis. Endometriosis patients had a reduced ovarian sensitivity with lower oocyte yields but similar live-birth rates per ET compared to controls, suggesting that the effect of endometriosis is more pronounced on ovarian factors rather than on the endometrial environment.

Trial registration number: not applicable.

P-295 Cell migration, apoptosis and myofibroblastic metaplasia are differentially regulated by seminal plasma in regard to endometriosis pathogenesis

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Study question: Can seminal plasma (SP) induce metaplasia-associated pathogenic properties in endometriosis
(EM)?

Summary answer: While SP induces changes in metaplasia marker expression, it inhibits endometriotic cell migration and apoptosis, suggesting a possible effect on endometriotic cell behavior.

What is known already: EM pathogenesis involves different biological steps; detachment of the endometrium, retrograde transit, prolonged cell survival, implantation on the peritoneum, metaplasia and angiogenesis. SP comes in contact with the endometrium following intercourse, delivering many growth factors (especially TGFβ1) to endometrial cells as a possible mechanism to contribute to EM. In our previous study (presented on ESHRE annual meeting 2016, O-024), SP (1:10) induced an early myofibroblastic metaplasia observed 6 h after incubation with endometrial cells. In this study we investigated a longer incubation time using a more diluted SP to study cell migration, apoptosis and metaplasia on endometrial cells.

Study design, size, duration: SP collected from semen samples of normozoospermic men (n = 23) were centrifuged after liquefaction, pooled and used for in-vitro experiments in 1:10 and 1:100 dilutions. Peritoneal fluid (PF) (n = 14) was collected during laparoscopy from EM patients. Premenopausal women (n = 4) underwent transcervical endometrial biopsy. In-vitro studies were carried out in 5 groups; controls, SP1%, SP1%+TGFβ1 Activin receptor-Like Kinase Inhibitor (ALK-I) to block TGFβ1 receptor signaling pathway, ALK-I and TGFβ1.

Participants/materials, setting, methods: TGFβ1 level in SP and PF was assessed using ELISA. 12Z (endometriotic epithelial), St-T1b (endometrial stromal) cell lines as well as endometrial biopsies were used for the in-vitro studies. Flow cytometry analysis to assess **apoptosis** using the Annexin V/PI method. To assess cell **migration** a scratch wound assay was done. A quantitative real-time PCR assessed the mRNA expression of a **metaplasia** marker (alpha smooth muscle actin 'ASMA", a marker of myofibroblasts).

Main results and the role of chance: TGFß1 concentrations. TGFß1 in SP (average= 92.88 ng/ml) as well as in SP-pool (88.17 ng/ml) was significantly higher than in PF (average= 6.79 pg/ml) (p < 0.001).

Cell Migration. SP 1% inhibited cell migration of endometrial epithelial cells which were not reversed by blocking TGFβ1 in SP. Nevertheless, TGFβ1 alone stimulated cell migration, which could be partially blocked by adding TGFβ1-inhibitor.

Apoptosis. SP 1% forced endometriotic cells into apoptosis; the latter was partially reversed by blocking TGFβ1 in SP. Notably, cell cycle analysis revealed that more cells shifted into the G1 phase in 12Z, whereas a shift to the G2 phase occurred in St T1b. SP10 % massively induced apoptosis and reduced cell viability after prolonged incubation times (24 h), resulting in cell detachment from culture plates.

Myofibroblastic metaplasia. While SP 10% robustly up-regulated the ASMA-expression in both endometrial tissue as well as cell lines, the effect of SP 1% was yet more variable.

Limitations, reasons for caution: All experiments are in-vitro and they need to be reconfirmed in in-vivo studies, before translation into clinical practice can be accomplished.

Wider implications of the findings: These findings together with our previous results propose a possible role of SP on the different biological steps of EM

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pathogenesis. They necessitate yet a study on a possible role of SP on immunomodulation and angiogenesis as further crucial steps of the disease.

Trial registration number: Irrelevant.

P-296 Regression rates of symptomatic and asymptomatic endometrial polyps after 3-month of subcutaneous progesterone administration in premenopausal woman: a preliminary retrospective analysis

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Study question: Can medical therapy through the administration of 3 cycles of luteal progesterone improve the regression rate of symptomatic and asymptomatic endometrial polyps in premenopausal woman?

Summary answer: Three cycles of luteal phase subcutaneous progesterone administration were associated with a regression rate four times greater than that seen with watch-and-wait approach.

What is known already: In premenopause, 25% of endometrial polyps regresses spontaneously in 1 year. According to guidelines, given that most premenopausal polyps are not malignant, there is an option for expectant approach with no surgical intervention. Studies on the efficacy of medical treatments for endometrial polyps are also recommended by gynaecologic societies, with the aim of finding cost-saving not invasive strategies to manage this common pathology. Up to now nobody has investigated the effect of progestin administration on polyps, but molecular and clinical data suggest that the antiestrogenic effect of this hormone can be exploited to increase and speed-up their regression rate.

Study design, size, duration: A retrospective analysis of the data of all the premenopausal women with endometrial polyps managed in our unit from January to December 2016 was conducted. The regression rates of women managed with watch-and-wait approach and of those treated with 3 cycles of luteal progesterone were compared. Ninety-three out of 127 women completed the follow-up and were included in this study. Sixty-one accepted the therapy while 32 chose to wait for the spontaneous regression of polyp.

Participants/materials, setting, methods: Polyps were diagnosed through vaginal ultrasonography performed in follicular (94%) or luteal (6%) phase. Doppler examination was performed to confirm the diagnosis by visualization of the polyp feeding vessel. Treatment consisted of 7 days of 25 mg subcutaneous progesterone administered from 18° to 25° day of the menstrual cycle and repeated for 3 cycles. Patients refusing therapy were re-evaluated after 3 menstrual cycles, as per our standard protocol. Persistent polyps were treated by hysteroscopic resection.

Main results and the role of chance: Mean age of the study population was 37.2+3.7 years. Among 93 women included, 32% consulted our Unit for infertility, 58% had abnormal uterine bleeding (AUB), 10% were diagnosed with asymptomatic polyps during a routine check-up. Asymptomatic women were less inclined to undergo therapy for their polyps, whereas infertility and AUB were factors positively related with the choice of the patients to undergo to progesterone. Thirty-two out of 61 and 28 out of 32 polyps persisted in the progesterone treatment and watch-and-wait groups, respectively, with a regression rate of 47.5% and 12.5% in patients managed by medical therapy and in those who refused progesterone, respectively. Persistent endometrial polyps had a longer diameter significantly greater at inclusion (13.2+3.89 mm) compared with polyps that regressed spontaneously or after therapy (8.27 +1,85 mm) ($p < 0,001$). Although not disappeared, polyps persisting after progesterone administration significantly reduced in size after therapy (14.93 +2.98 mm vs. 10.87 + 3.32 mm $p<0,001$), with a mean reduction of 4.06 ± 2.81 mm. In the watch-and-wait group, persistent endometrial polyps had a longer diameter at inclusion greater than those spontaneously regressed (11.46+3.54 mm vs 7.5+0.57 mm in progesterone and watch-and-wait groups, respectively; $p<0,001$). The histological diagnosis of all polyps persisting at 3-months re-evaluation was "endometrial polyp".

Limitations, reasons for caution: The retrospective design of the study currently limits the exploitation of our results in the general population but it gave us useful data to calculate the sample size required for an adequate prospective randomized trial on the topic.

Wider implications of the findings: Although hysteroscopy is recognized as a safe and effective treatment for endometrial polyps, medical management could become a fascinating alternative because of its lower cost and invasiveness. Among infertile population, progesterone administration can increase the regression rate of polyps, allowing faster conception through natural and Assisted Reproductive Technology attempts.

Trial registration number: Not applicable.

P-297 Sensibility and specificity of the molecular vs classical diagnosis of chronic endometritis

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Study question: Is the molecular diagnosis of chronic endometritis (CE) comparable to classical hysteroscopic, histological and/ or microbial diagnosis?

Summary answer: Compared to the classical diagnosis, the molecular detection showed a sensitivity and specificity of 0.73 and 1, respectively, and allowed the identification of non-cultivable bacteria.

What is known already: CE is a persistent inflammation of the endometrial lining with 40% prevalence in infertile patients, causing recurrent implantation failure and clinical miscarriage. The most common bacteria causing CE are: Enterobacteria, Gardnerella vaginalis, Enterococcus faecalis, Streptococcus, Staphylococcus, Mycoplasma, and Ureaplasma spp.

Current diagnosis of CE is based on hysteroscopy of the uterine cavity with endometrial biopsy in which plasmatic cells are identified, while specific treatment can be only set out based on microbial culture to identify pathogens causing CE. However, bacterial culture is not routinely performed because is expensive, with turnaround time of one week and not all microorganisms are culturable.

Study design, size, duration: Endometrial biopsies from 28 patients, previously diagnosed for CE using at least one (hysteroscopy) or several classical methods (histology and/or microbial culture) in an independent center were blindly processed and analyzed by quantitative PCR (qPCR) using specific primers for the variable regions of the 16 S rRNA gene of the most common bacteria causing CE. The results of our molecular analysis were compared with their prior diagnosis by the indicated classic methods in an independent center.

Participants/materials, setting, methods: Twenty-eight endometrial biopsies from clinically diagnosed patients for CE were used in this study. Total DNA was isolated upon enzymatic digestion for difficult-to-lyse bacteria and subjected to qPCR to detect the presence of the most prevalent infectious agents of CE. The organisms tested were: Enterobacteria, Enterococcus faecalis, Streptococcus and Staphylococcus spp., Gardnerella vaginalis and Mycoplasma spp. Known amounts of total DNA from these bacteria were used as positive controls.

Main results and the role of chance: In 14 patients diagnosed of CE by microbial culture, hysteroscopy and histology, we identified bacterial pathogens in 12 out of 14 cases.

In 12 patients diagnosed of CE by hysteroscopy or histology without, or with negative, culture, molecular detection of pathogens was confirmed in 7 out of 12 cases. From the 5 remaining in which no bacterial DNA was detected by PCR, 4 samples were diagnosed only based on hysteroscopy, but were negative for histology.

In the 2 women diagnosed as negative for CE by all classical methods, our molecular test confirmed the negative results in both cases.

Overall, the sensitivity and specificity of the molecular diagnosis versus at least one classical method was 0.73 and 1.00, respectively. The positive

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predictive value and the negative predictive value of the molecular method were 100% and 22%, respectively. The false positive rate and false negative rates of PCR compared to the current diagnosis with at least one of the classical methods were 26% and 0%, respectively.

Limitations, reasons for caution: The main limitation of the method is the establishment of the minimum amount of bacterial DNA that causes the disease, as the copy numbers for the interrogated gene could be variable between different bacterial species and this effect could cause a slight deviation on the number of bacteria estimated.

Wider implications of the findings: The proposed molecular method represents an objective, fast and cheap diagnostic tool that allows us to detect culturable and non-culturable bacteria improving the diagnostic and treatment of this ghost endometrial pathology.

Trial registration number: This study has been approved by the local Ethical Committee at the University of Bary, where it has been registered with the number 4880.

P-298 Toxicogenomic profiling and chemical-gene interaction reveal the potentiation of environmental toxins risk on endometriosis

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Study question: How can we study and evaluate the synergistic risks or mechanisms of multiple environmental chemicals on pathogenesis of endometriosis?

Summary answer: By integrating the endometriosis-related genes and chemical-gene interactome, the combinatory and potentiated risks of environmental chemicals co-exposure in endometriosis was investigated.

What is known already: Several transcriptomic analysis of endometriosis-related genes have been profiled; whereas, we have already identified 52 endometriosis-related genes (52 Endo-genes). Although, the genes related chemical exposures are also discovered as well; the synergistic effects of the environmental toxins, such as dioxins, polychlorinated biphenyls (PCB), benzo (a)pyrene, alfatoxins, arsenic, and phthalates, on endometriosis via the integrated databases of toxicogenomcis and protein-protein interaction/conectome has not been described.

Study design, size, duration: According to our database and literature survey, the gene expression profile of endometriosis has been established. The toxicogenomic database and putative signaling analysis were performed and analyzed via the comparative toxicogenomic database (CTD), TOXNET, AltTox, and STRING to study the chemical-gene interaction and protein-protein intercatome.

Participants/materials, setting, methods: For the intersection of the environmental chemicals and the 52 Endo-genes, ten common environmental toxins were selected for the analysis, including tetrachlorodibenzodioxin, benzo (a)pyrene, alfatoxin A1, arsenic, and dibutyl phthalate. For the individual of the intersect genes, the combinatory analysis of the synergistic effects of the target Endo-genes of the environmental chemicals were evaluated and confirmed via Q-PCR.

Main results and the role of chance: Here, we have identified 52 endometriosis-related genes (52 Endo-genes), including FRZB, MGP, PDLIM5, TNS1, SPEG, and MEF2C signaling which may controlling the cell migration, immune regulation and cell survival on endometriosis; also, we try to identify the intersection of the endometriosis-related and the environmental chemical-induced genes or hubs. Interestingly, several important genes in endometriosis could be synergistically regulated via different environmental chemicals, such as dioxins and polychlorinated biphenyls (PCBs), which have been suggested to play a significant role in the development of endometriosis. We found that tetrachlorodibenzodioxin could induce 42 of the 52 Endo-genes; whereas, benzo (a)pyrene could up-regulate 38 Endo-genes. Most importantly, there are 34 Endo-genes could be regulated via both tetrachlorodibenzodioxin and benzo(a) pyrene. Furthermore, we can found that TNS1, the regulator to promote cell migration, could be regulated via not only dioxins and benzo(a)pyrene, but also dibutyl phthalate, bisphenol A and alfatoxin B1. Whereas, clustering (CLU) and FGL2 could be regulated via estrogen, dioxins, bisphenol A, indicating the transcriptional signaling of MEF2C, CTGF, ER and AHR may co-contribute on these pathological pathways. These results indicate the combinatory risks as co-exposure to the environmental chemicals; also discover the potential signaling pathway for the prevention or therapeutic interventions for endometriosis.

Limitations, reasons for caution: Thousands of environmental chemicals might need to be analyzed and should be further validated in vitro or even in vivo study to exam the potency and power of the analysis of the chemical-gene interactome approach. Furthermore, the methodology also needs optimization in different or certain types of the response cells.

Wider implications of the findings: Such approach could be helpful for evaluate the potential interactions or potentiation of multiple environmental chemicals on diseases, such as endometriosis. Under the risk of the complicated environmental hazards, we should learn how to make the risk assessment via the integration of the genomic databases and the big data analysis.

Trial registration number: Not applicable.

P-299 How do endometriosis and adenomyosis and their subtypes affect women's reproductive life-course from the quality of oocyte to birth?

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Study question: How do endometriosis and adenomyosis affect reproduction from oocyte development to neonatal outcomes and how do these outcomes relate to the various disease subtypes individually?

Summary answer: Endometriosis and adenomyosis negatively impact on fertility. Subtypes of the disease present specific complications relating to either the oocyte quality and/or the endometrium.

What is known already: Endometriosis is known to cause hormonal, cellular and immunological alterations within the endometrium as well as DNA damage to oocytes, resulting in subfertility. Severe endometriosis is associated with a worse reproductive outcome in those undergoing assisted reproduction. Aberrant decidualisation and placentation within a disturbed uterine environment is known to influence the developmental programming of health and disease (Barker's hypothesis). It is unclear if and how such early aberrant reproductive developments relate to later pregnancy outcomes in endometriosis and adenomyosis and if these are subtype or severity specific.

Study design, size, duration: A systematic literature review of various databases was performed and screened by two reviewers independently from which a meta-analysis was conducted.

Participants/materials, setting, methods: A systematic literature review identified all comparative and observational studies published between 1980 and 2016 in any language on endometriosis and adenomyosis (all severity, stages and subtypes) and fertility, obstetric and neonatal outcomes (23 search terms). A proforma was used to extract data for meta-analysis. 1493 papers were reviewed by title and abstract. 118 full texts were reviewed, 60 papers were finally selected for data extraction. A standardised checklist was used to evaluate study quality.

Main results and the role of chance: The majority of studies have examined reproductive outcomes of in-vitro fertilisation and intra-cytoplasmic sperm injection treatment. Some population based studies included spontaneous conception. Stage I-II endometriosis specifically was associated with reduced fertilisation rate per oocyte (stage I-II: OR 0.82; 0.75-0.90 95% CI) whereas stage III-IV endometriosis was associated with reduced implantation rate per embryo transfer (OR 0.72; 0.58-0.90 95% CI) and reduced clinical pregnancy rate per cycle (OR 0.71; 0.53-0.95 95% CI). The live birth rate, however was unaffected by endometriosis regardless of severity. Patients with adenomyosis were found to have lower implantation rate (OR 0.51; 0.49-0.88 95%CI), pregnancy rate (OR 0.51; 0.38-0.69 95% CI) and live birth rate (OR 0.54; 0.36-0.80 95% CI) with an increased risk of miscarriage (OR 3.62; 2.01-6.51 95% CI). The

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presence of endometriosis and/or adenomyosis is associated with increased obstetrics complications including preterm birth and placenta praevia but more studies are required to draw meaningful statistical conclusions.

Limitations, reasons for caution: The Downs and Black checklist revealed weaknesses mainly in internal validity and confounding and selection bias in many of the included studies. Meta-analysis demonstrated that quantitative and qualitative heterogeneity between studies varied considerably but I^2 is <50% in the majority of studies.

Wider implications of the findings: Endometriosis may have subtype-specific impacts across the entire reproductive process. In seeking diagnostic and therapeutic strategies, a reproductive life course systems approach is necessary in order to achieve useful long-term reproductive health impact.

Trial registration number: Not applicable.

P-300 Downregulation tensin 1 following GnRHa treatment in tissues and serum women with endometriosis

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Study question: Is tensin 1 associated with gonadotropin-releasing hormone agonist (GnRHa) treatment in the patient with endometriosis?

Summary answer: Treatment with GnRHa on the women with endometriosis down regulates the expression of tensin 1 in mRNA and protein level both in tissues and serum.

What is known already: The underlying pathogenesis of endometriosis is still poorly understood, many cell migration and invasion-related molecules have been reported to be associated with endometriosis disease progression. Tensin 1 is focal adhesion phospohoprotein molecule localized in cytoplasm, which play a role in cel migration as the transmembrane junctions between the extracellular matrix, and the cytoskeleton. Currently, one of the most widely used agents for the medical treatment of endometriosis is gonadotropin-releasing-hormone agonist (GnRHa). To date, there is no report related with expression of tensin 1 in women with endometriosis following GnRHa treatment.

Study design, size, duration: Tissues and serum were collected from the endometriosis patients with (n = 48) and without (n = 41) GnRHa treatment. Serum from patients with non-endometriosis (adenomyosis and myoma, n = 16) were used as comparasion for investigating serum level of tensin 1 in women with endometriosis

Participants/materials, setting, methods: Messenger RNA and protein level of tensin 1 from patients with and without GnRHa treatment were examined by qPCR and western blot respectively. Immunolocalization was examined by IHC. Serum level of tensin 1 was examined by ELISA in the patients before and after GnRHa treatment and also compared with non-endometriosis patients. We also investigated expression of tensin 1 in tissues and serum based on the menstrual cycle and also duration of GnRHa treatment.

Main results and the role of chance: Tensin 1 mRNA in endometriotic tissues showed significantly lower expression in the GnRHa treatment group than in controls (P = 0.0064). The level of tensin 1 protein was consistent with the level of mRNA (P = 0.0024). From IHC experiment we observed cytoplasmic tensin 1 protein expression in both epithelial and stromal cells and tensin 1 expression in endometriosis tissue from controls was higher than in the treated group. Follow up study showed after GnRHa treatment, the average concentration of tensin 1 was significantly decreased by 27.27% from pretreatment levels (from 2732 ± 710.3 to 1987 ± 620.9 pg/ml). There was significant difference serum level of tensin 1 between women with adenomyoma and women with moderate-severe endometriosis (676.7 ± 27754.99 VS 1016 ± 45.55 pg/ml, P = 0.04) but no difference between women with myoma and endometriosis at any stage. Based on the different stage of endometriosis there was no significant difference in serum levels of tensin 1 between patients with minimal-mild and moderate-severe (993.4 ± 142 vs. 1016 ± 45.55 pg/ml). There was no difference in tensin 1 expression in the proliferative and secretory phases of the menstrual cycle in both tissues and serum.

Limitations, reasons for caution: Our results should be further confirmed in a larger cohort of patients and functional assay is needed for further investigation related to involvement of tensin 1 in the pathogenesis of endometriosis.

Wider implications of the findings: Down regulated expression of tensin 1 by GnRHa may contribute to the inhibition of cell motility of endometriotic cells. The results emphasise the importance of migration-related factor of tensin 1 in the pathogenesis of endometriosis. Tensin 1 may be target for therapeutic intervention by GnRHa treatment in women endometriosis

Trial registration number: not applicable.

P-301 Uterine Natural Killer Cell Phenotype: Predicting ART (Assisted Reproductive Technology) success in endometriosis-associated infertility

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Study question: Do women with endometriosis-related infertility display differences in blood or uterine NK cell profiles that may be predictive of ART outcomes?

Summary answer: Uterine, but not blood, NK cell phenotype was found to differ between endometriosis patients with successful and unsuccessful implantation following ART.

What is known already: Uterine natural killer (uNK) cells are the dominant mucosal immune cell in the uterus, are abundant early in gestation and contribute to placentation. Their role in implantation is currently unclear. We have previously shown maturation of lymphoid progenitors in human endometrium and increased uterine haematopoetic precursors in women with infertility. We also found increased proportions of uNK progenitor cells in women with endometriosis and infertility compared to fertile controls. The aim of this study was to examine mature NK cells and their progenitors in the endometrium and blood of women with endometriosis who underwent ART.

Study design, size, duration: Patients were prospectively recruited to a cohort study between March 2013 and June 2015. Endometrial tissue from proliferative or secretory phase endometrium was obtained by aspiration biopsy from thirty-one women with a diagnosis of endometriosis-associated infertility (21 stage 1-2, 10 stage 3-4). Matched peripheral blood samples were also collected. Ethical approval was granted by the National Maternity Hospital. Patients underwent ART treatment (IVF, ICSI or IUI) within a mean interval of 9.5 months of surgery.

Participants/materials, setting, methods: Study participants were similar with regard to age, BMI, parity, AMH levels and duration of infertility at endometrial biopsy. Endometrial tissue was processed and peripheral blood mononuclear cells were isolated using established protocols. NK cells were analyzed by flow cytometry using antibodies to CD45, CD56, CD1, CD34, CD117 and CD94. Successful implantation was defined as evidence of a clinical pregnancy. Data were analyzed using Mann-Whitney or Kruskal–Wallis test, with Dunn's multiple comparison test.

Main results and the role of chance: Of thirty-one patients with a confirmed surgical diagnosis of endometriosis who underwent ART in a nonconsecutive cycle, twenty-one women (68%) had successful implantation and ten (32%) were unsuccessful. No difference in age, number of treatment cycles or stage of endometriosis was noted between patients with a positive outcome and those without. CD34+ haematopoietic progenitor cells were increased in the endometrium of women with successful implantation (4.9% vs 1.2% P < 0.05). This difference was more pronounced in proliferative endometrium. While there was considerable heterogeneity in NK phenotypes and numbers, women with successful implantation had fewer mature $CD94^+$ uNK cells (22.6% vs 38.2% P = 0.07) and higher levels of immature $CD10^+CD117^+$ uNK

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cells (5.2% vs 1.0% P = 0.02) than women with failed implantation. Furthermore, uNK expression of the NK cell receptor NKp46 was markedly lower in women who progressed to clinical pregnancy. In contrast, levels of mature peripheral blood NK cells were higher in women with successful implantation while levels of blood NK progenitors were similar in both groups.

Limitations, reasons for caution: The study cohort was limited in size and interval between endometrial biopsy and ART intervention ranged between four and twenty-one months (mean of 9.5 months, standard deviation 4.5).

Wider implications of the findings: This work demonstrates the marked differences in NK repertoires and developmental pathways in blood and uterine compartments. Moreover, these findings emphasise the limitations of focusing on blood NK cells for diagnostic or therapeutic interventions in female infertility.

Trial registration number: not applicable.

P-302 Differential transcript profile of cumulus cells of infertile women with and without advanced endometriosis who underwent ovarian stimulation

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Study question: Are the cumulus cells (CC) of infertile women with advanced endometriosis molecularly different from the CC of infertile women without endometriosis?

Summary answer: CC of infertile women with advanced endometriosis seems to be molecularly different from those of infertile women without endometriosis.

What is known already: Several researches report a deleterious effect of endometriosis on oocyte quality. Since CC play a crucial role in oocyte competence acquisition, studies suggest that gene expression analysis of these cells can be used as indirect biomarkers of oocyte quality. Our group was pioneer in this theme demonstrating altered genes potentially related to compromised oocyte competence. However, the study of individual genes in CC restricts the exploration of deregulated metabolic pathways, whose investigation is essential to understand the mechanisms of infertility related to endometriosis. Until now no research evaluate the complete transcriptome of CC from infertile women with endometriosis.

Study design, size, duration: This prospective case-control study, was conducted between August of 2014 and February of 2016 at the Human Reproduction Division of the University Hospital, FMRP-USP. The study size was established based on previous researches that used the global transcripts profile screening methodology. Therefore, we used 9 patients per group, grouped in 3 pools/samples of 3 women each.

Participants/materials, setting, methods: CC were collected after controlled ovarian stimulation (COS) for intracytoplasmic sperm injection (ICSI). RNA next generation sequencing (RNA-Seq) was performed in 3 infertile women groups (controls without endometriosis, with endometriosis III/IV without endometrioma, and with endometriosis III/IV with endometrioma) by the Illumina platform HiSeq2500, High Output, pair-end. Data normalization and differential expression analyses were performed using the R statistical environment and STAR package.

Main results and the role of chance: The comparison between endometriosis III/IV with and without endometrioma to control group revealed 461 and 66 altered genes, respectively. No difference was achieved between endometriosis groups. Further, analysis of the top deregulated genes among groups of advanced endometriosis and control patients evidenced genes associated with oxidative phosphorylation pathway, acetylation, ubiquitination processes, and genes related to cholesterol and estradiol regulation. These data suggest that CC of infertile woman with advanced endometriosis carry essential molecular alterations linked with follicular and gametic development. Through enrichment of the top deregulated genes, we can point out the potential pathways altered in this process toward oocyte impairment and infertility.

Limitations, reasons for caution: The main limitation was the small sample size evaluated owing to restrictive eligibility criteria adopted and the difficulty in obtaining adequate samples for RNA-Seq, which compromises the generalizability of the study. On the other hand, the strict eligibility criteria increase the internal validity of this study.

Wider implications of the findings: The evidenced differentially expressed transcripts suggest molecular alterations in CC of infertile women with advanced endometriosis with and without endometrioma undergoing COS. As the identified top deregulated genes are related to pathways potentially involved with oocyte quality, these findings open new perspectives on the mechanisms involved in advanced endometriosis-related infertility.

Trial registration number: Not applicable.

P-303 Specific biopsychosocial variables affect sexual function in endometriosis patients

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Study question: Is sexual function in endometriosis affected only by disease severity or additional biological and psychosocial variables are also associated with sexual dysfunction?

Summary answer: Dyspareunia, length of pain and specific psychobiological variables affect sexuality. Hormonal treatment and previous surgeries were also associated with increased risk of sexual dysfunction.

What is known already: Sexual function is an important aspect of health and quality of life and is influenced by both medical conditions and health-care interventions, especially when gynecologic disorders are involved. Coital pain is among the main factors that affect sexual functioning, and this symptom is reported by almost half of women suffering from endometriosis. However, sexuality is a complex phenomenon driven by social, psychological and biological/hormonal factors and the presence of endometriosis might further affect domains of sexual function and the quality of a sexual relationship.

Study design, size, duration: Cross-sectional design. All participants were recruited among consecutive inpatients scheduled for laparoscopic surgery at the Geneva University Hospital based on signs and symptoms suggestive of endometriosis (ie, dysmenorrhea, nonmenstrual pelvic pain, ovarian cysts, and infertility) between January 2014 and September 2015. The study was reviewed and approved by the institution's Ethics Committee.

Participants/materials, setting, methods: A total of 198 women with histologically confirmed endometriosis were enrolled in this study. Before laparoscopy, a semi-structured questionnaire was used to collect demographic data, medical history (including infertility status, hormonal treatment and pain) and disease characteristics. Female Sexual Function Index was used to assess sexual function and Endometriosis Health Profile 30 (EHP30) questionnaire was utilized to investigate measures of quality of life. All sub-scores of FSFI and EHP30 (core questionnaire 5 scales) were analyzed.

Main results and the role of chance: 171 with histological proven endometriosis completed questionnaires. Crude prevalence of sexual dysfunction according to FSFI score was 71%. Low FSFI score was correlated with higher EHP30 values. Dyspareunia and dyschesia scoring, the presence of deep lesions in the pouch of Douglas and advance staged were associated to sexual dysfunction. Length of pain symptoms, intensity of pain, current use of hormonal treatment and previous surgery were likely associated sexual dysfunction. After multiple backward logistic regression analysis including also demographic variables, only the length of pain symptoms (any type of pelvic pain), dyspareunia scoring, current use of hormonal treatment and previous surgery were significantly associated with female sexual dysfunction. Compared to women with no history of surgery for endometriosis, those who received one or more surgeries are at higher risk of having sexual dysfunction after adjustment for confounding factors.

Limitations, reasons for caution: This study analyzed patients with a surgical indication for endometriosis treatment (pain or infertility) and it couldn't be representative of the entire population of women affected by endometriosis.

Wider implications of the findings: Endometriosis negatively affects different domains of sexual function. Specific quality of life measures of endometriosis patients, disease charactestics and medical interventions may further affect quality of sexual function in endometriosis

Trial registration number: 09-193 R.

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P-304 Efficacy of preimplantation genetic screening (PGS) for successful pregnancy in infertile, endometrioma patients

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Study question: Wound PGS be effective on infertile women having endometrioma?

Summary answer: Endometrioma may result the poorer quality of embryos, but PGS may not be recommended routinely for women having endometrioma

What is known already: Although there have been improvements in process of in vitro fertilization-embryo transfer (IVF-ET), implantation rates are reported to be approximately 30% in women less than 35 years old and less than 10% in women more than 40 years old. Approximately 50-75% of spontaneous miscarriages are due to numeric chromosomal abnormalities of embryos. Endometriosis is known to have detrimental effects on oocyte and embryo qualities. Recently, preimplantation genetic screening (PGS) seems to be one of the methods in screening chromosomally abnormal embryos. Therefore, in this study, we are to study on effectiveness of PGS on quality of embryos in endometrioma patients.

Study design, size, duration: Retrospective cross sectional study

Total 100 cases of infertile women having endometrioma

From August 2014 to January 2016

Participants/materials, setting, methods: Fifty cases of infertile women who had endometrioma and 50 who did not have endometrioma underwent IVF and agreed to do PGS at Gil hospital were enrolled. Data were collected retrospectively. From a good quality embryo (grade I, II), embryo biopsy was done on day 3 and WGA with CMA were done on the same day. Percentages of euploidy status were calculated and analyzed according to the presence of endometrioma.

Main results and the role of chance: Total 234 embryos were sent for PGS. One hundred thirty embryos were from women with endometrioma, and 104 embryos were from women without endometrioma. The total retrieved numbers of embryos were similar, but the proportion of good quality embryos (Grade I, II) was statistically higher in non-endometrioma patients (49 % vs. 67 %, p-value = 0.02). However, the the results of euploidy proportions from PGS were statistically similar (57 % vs. 54 %, p-value = 0.79).

Limitations, reasons for caution: Single centered study

Infertile reasons were not all excluded

Wider implications of the findings: Although PGS has becoming the new issue for higher pregnancy rate, it should not be routinely recommended to endometrioma infertile couples.

Trial registration number: not applicable.

P-305 The cytokines analysis suggest the immune system activity in endometriosis

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Study question: To compare cytokines profile between endometriosis grade I-II patients and recurrent implantation failure (RIF) patients

Summary answer: Despite of fact that endometriosis is considered an inflammatory disease, RIF patients are comparable in this immunological response.

What is known already: Cytokines can help in the diagnosis of endometriosis through its inflammation response. Their paper in Recurrent Implantation Failure is still unknown, althouth the association with different cytokines together with T Helper Lymphocytes families are being investigated.

Study design, size, duration: Peripheral blood samples were obtained from sixty five women who attended Instituto Bernabeu between February and September 2016. Fifty five of them with a previous history of recurrent embryos implantation failure (RIF) and ten with unilateral mild endometriosis. We considered recurrent failed implantation when at least four good quality embryos were transferred in three transfers. Blood was centrifugated and serum frozen at -20 °C until its determination.

Participants/materials, setting, methods: A prospective study was carried out to achieve our objective. After serum thawing, we determined immune profile that included biomarkers as IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-δ, TNF-α, IL-1-α, IL-1β, MCP-1, EGF. For the analysis, we used "Cytokine and Growth Factors High Sensitivity" Randox Biochip. This technique consists of a sandwich chemiluminescent immunoassay. For the statistical analysis we used T Student analysis

Main results and the role of chance: All of the results of the levels of cytokines between groups (Endometriosis vs RIF) were comparable with the exception found in IL-1-α levels in which differences statistically significant were found (Endometriosis group: 0,14 vs RIF group: 0,39; p = 0.0069)

Limitations, reasons for caution: The main limitation is the sample size.In order to evaluate the effect of cytokines in implantation clinical data would be added. Patients with different indications

Wider implications of the findings: RIF patients could have associated an special immunological response. The fact that IL-1-α levels are lower in women with unilateral mild endometriosis could be interpreted as these patients have a different immunologic profile. So, IL-1-α levels could represent an early biomarker before endometriosis diagnosis.

Trial registration number: No trial

POSTER VIEWING SESSION
ETHICS AND LAW

P-306 Indications of the global prevalence of inheritable mitochondrial modifications

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Study question: At present, how widely are inheritable mitochondrial modifications (IMMs) permitted and provided in countries with different reproductive policies?

Summary answer: IMMs are currently permitted and provided at fertility clinics in at least 12 countries.

What is known already: Various types of IMMs have been developed to treat unexplained female infertility or prevent the inheritance of mitochondrial disease by offspring. Most IMMs require oocyte donation, but some do not. Clinical uses of ooplasmic (cytoplasmic) transfer and pronuclear transfer for infertility treatment led to regulatory responses in the US and China, respectively. Recently, MII spindle transfer for preventing mitochondrial disease has been implemented in Mexico. Furthermore, pronuclear transfer in Ukraine and AUGMENT in Canada, Japan and Spain are ongoing as an infertility treatment. However, the current state of the global clinical provisions and the regulations of IMMs remain unclear.

Study design, size, duration: First, in addition to relevant medical reports and clinical trial data, the uses indicated in the IFFS 2016 report as well as relevant information in the websites of reproductive tourism and clinics were investigated. Those results were then compared, and several countries were selected for further legal analyses regarding oocyte donation and germline genetic modifications for reproduction. Finally, the wider implications of the findings were discussed from regulatory and ethical standpoints.

Participants/materials, setting, methods: The surveyed reports include works published until December 2016 in English, Chinese or Japanese that were available through PubMed. Relevant trials were surveyed using ClinicalTrials.gov. The internet survey was based on the information available at the end of December 2016.

Main results and the role of chance: An analysis of document records and clinical trial data identified eight countries where IMMs had been clinically practiced or were being practiced. Further investigations of IFFS reports and tourism websites and clinics added 28 countries. Among the total 36 countries, 16 countries satisfying least 1 of our 4 criteria were selected for a legal analysis. Oocyte donation was allowed or permissible with some limitations in all 16 countries. In conclusion, cytoplasmic transfer or other IMMs are permitted and provided in at least 12 countries (Canada, Czech Rep., India, Israel, Japan, Mexico, Northern Cyprus, Panama, Russian Fed., Spain, UK and Ukraine). However, the regulatory positions differ among such countries; some simply do not regulate IMMs, other implicitly or explicitly regulate IMMs. Pronuclear transfer is or may be prohibited in Canada,

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Czech Rep. and Japan. Currently, there is no evidence that IMMs are provided in China, Italy, Taiwan and the US. China largely prohibits IMMs except for AUGMENT and granular cell mitochondrial transfer. Italy implicitly prohibits IMMs. Taiwan and the US regulate IMMs by reviewing relevant clinical trials.

Limitations, reasons for caution: This study does not intend to encourage reproductive tourism. The results in part depend on the accuracy of information available on the internet. Furthermore, the accessibility and interpretation of legal documents might influence the regulatory analysis. We ask physicians to consult regulators in their own countries.

Wider implications of the findings: IMMs may prove attractive to female patients who value genetic relatedness. However, their unregulated use might globally cause social problems such as health problems in offspring and increasing oocyte transactions. The technical definitions and medical purposes should be contemplated t in their regulations, or the trials should be carefully reviewed.

Trial registration number: Not applicable.

P-307 Zika Virus Outbreak - Assisted reproduction patients should avoid pregnancy?

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Study question: Women should avoid pregnancy, because of the current Zika virus (ZIKV) outbreak, as recommended by public health authorities and government leaders of some countries?

Summary answer: ZIKV infection risk deserves special attention; however infection with ZIKV during pregnancy does not lead to increased risks than any other previously known pathogens.

What is known already: Zika virus (ZIKV) is predominantly spread via the *Aedes* species of mosquitos. An outbreak is currently spreading throughout South America, resulting in a large number of cases of microcephaly, Guillain–Barre syndrome, and meningoencephalitis. The World Health Organization (WHO) declared the ZIKV epidemic a Public Health Emergency of International Concern, and the CDC Emergency Operations Center elevated their response to ZIKV to level 1, the highest level of activation, for only the fourth time in the history of the organization. Moreover, public health authorities and government leaders in multiple countries have issued first-ever recommendations for women to simply avoid pregnancy.

Study design, size, duration: This study was performed in a university-affiliated *In vitro* fertilization center in Brazil, in which the issue concerning the ZIKV infection in pregnant women is an extremely real problem, with drastic consequences for the reproductive population and assisted reproduction centers. Considering the fast spread of ZIKV worldwide, a critical discussion on the risk of microcephaly due to ZIKV infection, and incidence rate (IR) of others harmful pathogens to vulnerable pregnant women and infants was conducted.

Participants/materials, setting, methods: Brazil has experienced over 1.5 million cases of ZIKV, and WHO estimates that potentially 4 million people in the Americas may become infected in 2016. The Brazilian health officials warned women to avoid pregnancy until the ZIKV outbreak passes. Governments in Colombia and Ecuador made similar recommendations. Recommendations in El Salvador are even more extreme, and women there have been advised to avoid pregnancy until 2018. This study discusses whether such recommendations are justified.

Main results and the role of chance: Pregnant women are exposed to many infectious agents that are potentially harmful. Intrauterine bacterial infection, such as: *Chlamydia trachomatis* (IR=3.8%); *Neisseria gonorrhea* (IR=1.9%); and *Treponema pallidum* (Syphilis, IR=0.15-1.5%), viruses infection, such as: Parvovirus B19 (IR= 1-5%); Cytomegalovirus (IR=1-3%); Varicella Zoster (chickenpox) (IR=0.02%); Herpes Simplex Virus (IR=2%);; and parasites infection, such as: *Trypanosoma cruzi* (Chagas disease, IR= 0.5-10%); *Toxoplasma gondii* (IR=0.4%); *and Plasmodium sp* (Malaria, IR= 2.9% in endemic regions), may represent major risks for pregnant women and fetus. Investigators studying the 2013–2014 ZIKV outbreak in French Polynesia estimated that the risk of microcephaly due to ZIKV infection in the first trimester of pregnancy was 0.95% and recently, the magnitude of the risk of microcephaly in Brazil (IR) is estimated to be approximately 0.88%. However, patients who face infertility and the fact that their ovarian reserve is declining, specially older patients, when receiving adequate orientation, did not postpone their IVF cycles, in Brazil. In fact, in the present assisted reproduction center, despite the recommendation of health authorities and government leaders in 2015, 844 cycles were performed, in which 34.0% of the patients were >38 y-old, while in 2016, 769 cycles were performed and 36.8% of the patients were >38 y-old.

Limitations, reasons for caution: This is a literature review and authors opinion.

Wider implications of the findings: The ZIKV infection risk is extremely high, especially in endemic regions. However the microcephaly risk due to ZIKV is not higher than the risk of miscarriage and birth defects due to other recognized pathogens. Therefore, it is prudent to take precautions to avoid ZIKV as any other infection during pregnancy

Trial registration number: None.

P-308 What legal aspects worry ART professionals?

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Study question: Which aspects generate most legal doubts among ART professionals in order to establish adequate mechanisms to solve these concerns?

Summary answer: During 2016, questions addressed to the Scientific Society's Legal Consultancy were mainly focused on aspects related to Donation (35.4%) and gametes and embryos Cryopreservation (21.2%).

What is known already: European Tissues and Cells Directive (EUTCD) applies to all member states. Moreover, each country has its own legislation according to its idiosyncrasy and specific context. Despite this, many legal questions arise in ART practice that are difficult for users to understand or that are not specified in the legal texts.

Scientific societies play an important role publishing position papers and articles about those aspects of ART that need to be treated more extensively. Having a Legal Consultancy service available to all members of the society is another way to contribute to it.

Study design, size, duration: Longitudinal descriptive study.

The use of the Society's Legal Consultancy has been quantified from the beginning of the service in 2006 to 2016. A total of 1290 legal questions was received. Those received during the last year (n = 226) have been analysed and classified according to their subjects.

Participants/materials, setting, methods: Legal questions from members were addressed to the Legal Consultancy on line through the society's Website. All questions were recorded and transferred to the lawyer specialist in ART legislation who advises the society and handles members' queries. The reply to each question according to Spanish legislation and the EUTCD was sent to the member in a maximum of 2 days. Questions were classified in 10 different groups according to their topic.

Main results and the role of chance: The implementation of the Legal Consultancy in our society was in 2006 coinciding with publication of the new law on ART in our country and two EU Commission Directives implementing the EUTCD. Since its incorporation as a free service for all the membership of the society, the number of questions received has shown a great increase not only due to the higher number of members but also to the growing interest in legal questions related to ART. While in the first 5 years (2006-2010) the annual number of questions was <100 (2-54) with a mean rate of <0.1 questions/member, in the next 2 years (2011-2012) it exceeded 100 questions (0-1-0.2 questions/member) and in the last 4 years (2013-2016) it was >200 queries/year (>0.2 questions/member). A peak was observed in 2014 coinciding with the publication of new national rules. The most frequent questions received during the last year were related to gamete and embryo donation (35.4%; 80/226), cryopreservation (21.2%; 48/226), shipping and distribution of reproductive samples (5.7%; 13/226), preimplantation genetic diagnosis (4.9%; 11/226), posthumous reproduction (3.5%; 8/226); surrogacy (3.1%; 7/226), consent forms (8%; 18/226), status of the embryologists (4%; 9/226), ART centres (4.9%; 11/226) and others (9.3%; 21/226).

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Limitations, reasons for caution: Descriptive analysis that includes only questions addressed to one specific scientific society that mainly consists of embryologists. Questions like medical concerns might not be sufficiently reflected in the sample analysed.

Wider implications of the findings: Our scientific society will offer different specific actions and events focused on the topics that have been detected as the most conflictive in order to give its members necessary tools for better knowledge and understanding of the current legislation on ART.

Trial registration number: None.

P-309 What raises ethical concerns on oocyte cryopreservation for social indications in lay people? Results from a factorial survey in Germany

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Study question: Which factors of oocyte cryopreservation for non-medical reasons raise ethical concerns in the general population?

Summary answer: They evaluate the specific social implications and consequences of cryopreservation in order to avoid harm and stick to social norms of a mother's age.

What is known already: Very few studies on lay peoples' attitudes towards cryopreservation have been published. They report high levels of support, also for social indications. However, participants for these studies where recruited at reproductive care centres or on dedicated websites. Results on factors that raise ethical concerns in lay people have not been published yet. We assume that support for cryopreservation is based on its benefit to avoid suffering from involuntary childlessness. Likely this benefit is balanced by potential harm for the (unborn) children resulting from cryopreservation. There is no study on situational and individual factors influencing acceptance of cryopreservation for non-medical reason.

Study design, size, duration: We collected data of 749 German speaking people from an online access panel. Every participant rated 12 short descriptions (vignettes) of different women using cryopreservation for non-medical reasons. The vignettes contain controlled variation with respect to the factors expected to influence acceptance. The different categories of the factors represent the treatments and controls as in experimental designs. Therefore, effects are unbiased and causal interpretation is valid (Auspug/Hinz 2015:9; Dülmer 2014a: 721 f.).

Participants/materials, setting, methods: We identified 5 factors from ethical discussions about cryopreservation expected to influence lay peoples' acceptance: age at cryopreservation (28 or 33 years), maximum age for using preserved oocytes (40 or 55 years), strength of wish for a child (weak or strong), number of oocytes for preservation (4, 12 or 30) and motives (financial security, career or self- realization). We performed a multilevel analysis that simultaneously accounts for the variation between respondents and within each respondent.

Main results and the role of chance: Cryopreservation for social indications is highly controversial in the sample: The mean rating is 6.14 (sd=3.40) on the 11 point scale (0 "not justifiable at all" 10 "completely justifiable"). The woman's age at the time of oocyte preservation does not influence acceptance ($p>0.1$). All other factors significantly influence acceptance of cryopreservation ($p<0.01$): The strongest effect yields the maximum age until which the women plan to use the preserved oocytes for IVF (-2.094). Compared to a woman that plans to become pregnant up to age 40, acceptance is two scale points lower for woman that plan IVF up to age 55. As expected, acceptance is higher for women who have a strong wish for a child (0.124). Supporting arguments from pro-life critiques of cryopreservation, a greater number of preserved oocytes decreases acceptance. The difference is 0.211 scale points for 12 oocytes compared to 30 and 0.247 scale point for 4 oocytes compared to 30. Egoistic motivations (career, self-realization) also decrease the acceptance. The effect size for the pursuing a career before parenthood (0.218) is smaller than for self-realization (0.251). This difference is not significant, indicating that respondents do not differentiate between economic and other egoistic motives.

Limitations, reasons for caution: The online access panel we drew the sample from is not representative for the German population. Although factorial surveys are quasi-experimental designs allowing for causal interpretation of the results, we have to be careful with generalizing the results to the general population.

Wider implications of the findings: To address ethical concerns regarding cryopreservation for social indications more information on potential harm and benefit of late parenthood for child development should be provided to potential users and the general public.

Trial registration number: This study is not a clinical trial.

P-310 Ethical issues raised by offering ovarian tissue cryopreservation to girls with Turner syndrome

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Study question: What are the ethical issues raised by offering ovarian tissue cryopreservation (OTC) to girls with Turner syndrome (TS)?

Summary answer: An overview of ethical issues raised by offering OTC to girls with TS with focus on four basic ethical principles: autonomy, beneficence, non-maleficence and justice.

What is known already: OTC has been well described in girls undergoing gonadotoxic cancer treatments. Auto transplantation of cryopreserved-thawed ovarian tissue in cancer survivors has resulted in restoration of ovarian function and pregnancy, with a life birth rate of 25% per transplantation. This has led to a new debate to expand OTC as a fertility preservation option to girls with TS. Due to an accelerated loss of oocytes, most girls with TS undergo ovarian failure before reaching adulthood. Although OTC has been experimentally performed in these patients, the promise of fertility preservation is at present hypothetical according to what we know.

Study design, size, duration: A two-round ethical Delphi method including a questionnaire survey and a consensus meeting.

Participants/materials, setting, methods: A literature search was undertaken to identify the ethical issues associated with OTC in girls with TS. All arguments identified were divided into four subcategories based on basic ethical principles (autonomy, beneficence, non-maleficence, and justice). A multidisciplinary expert panel (including patients and parents) rated and prioritized all arguments extracted from the literature during the questionnaire round. Key arguments were selected by using a strict consensus methodology. Group consensus was reached during a consensus meeting.

Main results and the role of chance: OTC in girls with TS raises a number of ethical considerations. The primary objective of OTC remains to promote the autonomy of patients by giving them the hope of having genetic concordant children in the future, and thus improve their psychosocial well-being. However, patients younger than 16 do not have the legal capacity to make their own decision, requiring an approved consent from both parents. Furthermore, besides the potential risks associated with the surgical retrieval it is important to be aware that, at present, the promise of fertility preservation in girls with TS is still hypothetical and may produce false hope. Still, we think that it is defensible to offer OTC to patients with TS who are demanding this fertility preservation option, provided that these girls (and their parents) are well-informed about all aspects of the experimental procedure.

Limitations, reasons for caution: Since all arguments were retrieved from published data only, potential publication bias with an underrepresentation of negative study results may have occurred.

Wider implications of the findings: This ethical analysis forms a basis for further debates and may be helpful for the development of research protocols to collect more data on the possible benefits and harms of OTC in girls with TS.

Trial registration number: not applicable.

P-311 Banned for 10 years, ART gametes donation cycles now allowed in Italy: first national data collection

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Study question: Analyze the number and type of gametes donation cycles collected by the Italian Assisted Reproductive Technology Register (IARTR). After the change of the law that banned these procedures for 10 years

Summary answer: IARTR collected aggregate data on 3,046 gametes donation cycles performed from May 2014 till December 2015 by 76 clinics

What is known already: The postponement of childbearing age in Italy determine a large number of older women wanting a baby. In these cases and in many others gametes donation could represent the only option in infertility treatments. Recent change of the Law in Italy, allows gamete donations, but not any form of compensation for donors. In the absence of Italian donors especially for oocytes, almost all the gametes utilized in Italy comes from abroad (80.9%)

Study design, size, duration: In this study IARTR analyzed retrospectively data on 3,046 gametes donation cycles performed on 2,699 patients. 205 ART clinics and 172 IUI clinics sent data to the National Italian Register and participating in the study with 73 out of 205 (35.6%) and 3 out of 172 (1.7%) performed donation cycles

Participants/materials, setting, methods: All ART centres which has performed at least one cycle with gametes donation, that have sent data during the study period. Parameters regarding number of patients, number of cycles, treatment indications, age classes, pregnancies and live births rates were analysed. Data were statistically analysed using SPSS statistic 22.0

Main results and the role of chance: IARTR collected 2,496 gametes donation from ART cycles and 550 from IUI, by 76 clinics. Among the clinics only 8 were public. Most of cycles were oocyte donation cycles (47.3%), while semen donation were 1,151 Cycles (37.8%) and 454 cycles were done with cryopreserved embryos obtained from gametes donation (14.9%). The principal cause for oocyte donation was the advanced maternal age in 41.6% of cases, while for semen donation 95.2% of cases were pathologies affecting sperm viability. Almost all the gametes utilized for treatments (85.7 % of oocytes and 72.9 % of semen) comes from abroad. Pregnancy rate per transfer was 41.7% for fresh donor eggs, 32.1% for frozen donor eggs cycles and 33.9% for cryopreserved embryos. Pregnancy rate per cycle in sperm donations was 24.8%. Multiple delivery rates were 20.7%, 39.3%, 18% and 17.1% respectively. During the study period 663 infants were born

Limitations, reasons for caution: With the limitation of donors due to the impossibility to give any form of compensation or benefit to these persons, the picture of the situation in Italy on gametes donation is far from exhaustive. Few public clinics have had the possibility to apply those procedures, due to lack of resources

Wider implications of the findings: the Italian situation regarding gametes donation policy, arise the question of equity in access to these procedures. The impossibility to compensate the donor determine the difficulties of public services to perform these procedures due to the lack of resources to import gametes

Trial registration number: not applicable.

P-312 Legally Enforceable IVF Surrogacy in the U.K as an Exclusive Treatment Option for Recurrent Miscarriage of Unknown Aetiology- Time for Debate

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Study question: Can a legally enforceable and regulated system of IVF surrogacy be a treatment option offered by the UK National Health Service to couples facing recurrent miscarriage of unknown aetiology?

Summary answer: It is ethically unacceptable to allow gestational surrogacy without legal enforcement while permitting medical intervention and therapies of unproven efficacy in managing recurrent miscarriage

What is known already: Traditional and gestational forms of surrogacy are either commercialised or altruistic and the U.K law permits the latter arrangement. Furthermore, in the U.K although IVF surrogacy is fully regulated in HFEA centres, it remains unenforceable.

Optimal management of recurrent miscarriages of unknown aetiology remains elusive and current therapies offered to emotive couples often lack therapeutic efficacy.

Study design, size, duration: Literature Review

Participants/materials, setting, methods: A literature search of surrogacy and management of recurrent miscarriages of unknown aetiology was made in Pub Med & COCHRANE databases using search terms [recurrent pregnancy loss and IVF surrogacy] or [recurrent miscarriage and IVF surrogacy] and relevant studies reviewed

Main results and the role of chance: The current legal position in the UK of fully regulating gestational surrogacy in licensed HFEA centres still does not provide legal enforcement for the arrangement. The ethics of failing to offer a couple an intervention with proven efficacy as is that of carefully selected and screened altruistic surrogates has to be debated

IVF performance and conception rates in gestational surrogacy are comparable to standard IVF including the perinatal outcomes of the children that result. Current therapies of recurrent miscarriage of unknown aetiology include hCG, Progesterone, anticoagulation, immunotherapy and all but psychological care do not have the evidence to support their efficacy.

Awareness and campaign for legislation to enforce IVF surrogacy in a contained pathway as a treatment option exclusively for couples with recurrent miscarriages is long overdue but lacking. For this purpose we have coined the term 'therapeutic gestational surrogacy' to encapsulate the exclusive nature of this proposed arrangement.

Limitations, reasons for caution: Literature concerning IVF surrogacy in recurrent pregnancy loss is sparse and the topic poorly researched. The conclusions drawn here are therefore limited and further research in this area is needed.

Wider implications of the findings: The success of UK HFEA centres can be extended to include legally enforceable gestational surrogacy as an exclusive evidence based treatment option. The quest for a 'miracle cure' to recurrent pregnancy loss has denied serious debate and campaign for enforcement of IVF surrogacy which would be welcomed by affected couples.

Trial registration number: N/A.

P-313 Will it be possible once and for all to establish the single Embryo Transfer (sET) as gold (and unique) standard in IVF procedures?

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Study question: Despite the general agreement that multiple pregnancy represents a serious (and avoidable) complication of assisted reproduction, in most procedures, 2 (even 3) embryos are transferred.

Summary answer: The implementation of a strict sET policy does not represent a limitation in IVF success and multiple pregnancy rates (and their complications) are reduced dramatically.

What is known already: Spanish legislation allows the transfer of up 3 embryos in each IVF procedure. Although some limitations are advised and there is a general agreement that among good prognosis patients a sET should be the preferred choice, in more than 70% of treatments 2 or even 3 embryos are transferred in Spain. The complications of multiple pregnancies (MP) are (should be) well known by assisted reproduction specialists and include both maternal and perinatal events. Unfortunately, the lack of legal limitation constitutes a "pathway" to keep on with this inappropriate and sometimes hazardous policy.

Study design, size, duration: A retrospective cohort analysis with a study group of IVF cycles undertaken between June, 2015 and May, 2016 at an University associated ART center were assessed and the results were compared to those published by Spanish Fertility Society (SEF) (www.registrosef.com) corresponding to the activity of Spanish centers in 2014 (last reported year). Participation in such registry is mandatory in Spain. A total of 225 centers (91,80% of the officially accredited) reported their results in 2014.

Participants/materials, setting, methods: A total of 397 IVF cycles (203 fresh and 194 vitrified-embryos cycles) (study group) were compared to the

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72598 IVF cycles reported in the SEF 2014-Report (51591 fresh and 21007 frozen-embryos cycles). Cycles in which donor eggs were used were not included.

The frequencies of single, double and triple embryo transfer were compared. Pregnancy rates per transfer and differences in multiple pregnancy rates were also assessed. Pearson's Chi-squared test was used to analyze the differences.

Main results and the role of chance: A sET was performed in 184 out of 203 fresh embryo transfers (ET) (90,64%). Regarding vitrified-thawed ET, in 184 out of 194 cases, a decision was taken to transfer one single embryo (94,85%). Overall in 368 out of 397 (92,70%) a sET was performed. No triple embryo transfer was undertaken. The clinical and ongoing pregnancy rates were 37,19% and 29,65% per transfer respectively.

Among the 51591 ET performed in the control group, in a 25,43%, 68,20% and 6,37%, one, two and three embryos were transferred respectively. The clinical and ongoing pregnancy rates per transfer were 37,16% and 29,31% respectively.

The transfer of more than one embryo was not associated to a higher chance of pregnancy. Furthermore, the multiple pregnancy rate in our study group was 3,69% whereas a 20,87% and 0,43% of the pregnancies achieved in the control group were twins and triplets respectively (p = 0,000). The delivery of the double pregnancies of the control group was premature in 50,47% of gestations (9,81% <32 weeks of amenorrhea) and in 100% of triplet gestations (58,52% <32 weeks of amenorrhea).

Limitations, reasons for caution: The retrospective nature of the study constitutes a limitation. Furthermore, we have to be cautious because 2 different groups are being compared in terms of size. Moreover, two different periods of time are being compared (2014 vs. 2015). Nevertheless, the evolution in embryo transfer policy has not changed so dramatically.

Wider implications of the findings: The availability of both, embryos and gamete effective preservation procedures, such as vitrification and the huge improvement in laboratory conditions including video-time-lapse systems and genetic screening procedures should become mandatory a change in some countries' legislation, by banning the achievement of high risk pregnancies (including MP, specially among aged women).

Trial registration number: Not applicable

POSTER VIEWING SESSION
FEMALE (IN)FERTILITY

P-314 Impact of vitamin D deficiency on ICSI outcomes

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Study question: Is there an association between the vitamin D levels and ICSI (Intracytoplasmic Sperm Injection) outcomes in a large population of patients in a sun-rich environment?

Summary answer: Women with sufficient levels of vitamin D are significantly more likely than those with insufficient levels to achieve a pregnancy following ICSI

What is known already: Vitamin D deficiency is highly prevalent in the general population, and especially in women in reproductive age. The role of this vitamin in ovarian steroidogenesis is well established, and recent evidence has shown its effect on uterine receptivity. In addition, vitamin D has also been shown to be involved in the pathophysiology of some disorders among women of childbearing age that are most commonly encountered among infertile women undergoing IVF procedures.

Study design, size, duration: It is a prospective observational study including 92 women who underwent ICSI cycles between July 2013 and July 2014. Patients were divided into two groups according to their vitamin D plasma levels: Group A (n = 61) < 30 ng/ml, and Group B (n = 31) ≥30 ng/ml. The vitamin D level in follicular fluid was also assayed.

Participants/materials, setting, methods: Stimulation by long agonist protocol, FSH ≤10 (IU/L), age ≤38 years and first or second ART cycle were the inclusion criteria. Severe male factor, endometriosis, white puncture and cycles without transfer were the exclusion criteria. Vitamin D was assayed by *ELISA 25-OH Vitamin D total, DRG Diagnostics*. The mains parameters compared between the two groups were clinical parameters and ICSI outcomes (pregnancy and implantation rates).

Main results and the role of chance: The mean age of patients was 31,8 years, the mean level of serum vitamin D was 26,01 ng/ml and the mean level of follicular fluid vitamin D was 18,87 ng/ml with a deficiency vitamin D prevalence of 66.3%. The clinic and paraclinic characteristics, the stimulation parameters, the ovarian response, the embryo development, and the number of embryos transferred were similar in both groups. The body mass index was significantly higher in women with insufficient vitamin D levels than in those with sufficient levels (p = 0.03). The thickness of the endometrium, was significantly higher in group B in comparison to group A (p = 0.002).A Better embryo quality was found when vitamin D levels were higher (p = 0.035). In addition, the study of ICSI outcomes based on serum vitamin D showed significantly a better pregnancy rate in group B compared to group A (74.19 % vs. 14.75 %, p = 0.000) and a higher implantation rate in group B (36.3% vs 4.9 %). Finally, a positive correlation between vitamin D levels in follicular fluid and serum levels (r=0.7) has also been established.

Limitations, reasons for caution: The observational character of the study, and the reduced size of the samples studied constitute major limitations. In addition, there are several possible confounding factors such as age and BMI which are both risk factors for infertility and vitamin D deficiency.

Wider implications of the findings: We confirm prior results about the association between vitamin D levels and ICSI outcomes by improving endometrial thickness.

Assessment of vitamin D status might be used in routine before artificial reproductive treatment, especially in women with high BMI, as vitamin D supplementation may be a way to improve pregnancy rates.

Trial registration number: It is not a clinical trial.

P-315 In vitro culture of vitrified and stimulated human ovarian tissue

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Study question: What is the effect of vitrification procedure on the integrity, morphology, follicular development and gene expression of stimulated human ovarian tissue after warming and two weeks in vitro culture?

Summary answer: The vitrification had no adverse effects on the morphology, development of follicles and gene expression of stimulated human ovarian tissue

What is known already: It was shown that vitrification and warming can guarantee the good preservation of follicular and stromal tissue structure in non-stimulated ovarian tissue.

Study design, size, duration: The biopsies of ovarian cortexes were collected from ovarian stimulated women that are candidate for transsexual surgery during laparoscopic surgery and cut into small pieces and were divided into non-vitrified and vitrified groups. Some of the tissues fragments in both groups randomly were culture for two weeks.

Participants/materials, setting, methods: The morphology of tissues was evaluated using hematoxylin and eosin and masson's trichrome staining. The expression of genes related to folliculogenesis was evaluated using real-time RT- PCR. The 17-β estradiol (E2) and anti mullerian hormones (AMH) analysis were done in collected culture media in the beginning and end of culture period.

Main results and the role of chance: The structure of human stimulated ovarian tissue after vitrification and culture was normal. In spite of high rate of normal follicles in both non-cultured tissues, these rates were significantly

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decreased after in vitro culture ($P < 0.05$). There was no significant difference in the percentage of normal follicles at different developmental stages between vitrified and non-vitrified groups before and after of culture period. The percent of growing follicles (secondary follicles) was increased in both cultured tissues than their non-cultured groups ($P < 0.05$). The expression of folliculogenesis related genes in vitrified-warmed tissues were not changed compared to non-vitrified ones. During in vitro culture the expression of GDF-9 and FSHR genes were increased and the expression of FIGLA and KL genes were decreased ($P < 0.05$). An increase in E2 level was observed after 14 days of culture in both vitrified and non-vitrified groups. The AMH concentration was not statistically increased during culture period.

Limitations, reasons for caution: Human sampling

Wider implications of the findings: The heterogeneity of the human ovarian sample. The distribution of follicles within the human ovarian tissue is not uniform.

Trial registration number: 52 / 883.

P-316 Is more better? Inseminated volume 0.2 ml vs. 0.5 ml in donor intrauterine insemination (dIUI) cycles: a prospective randomized controlled trial

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Study question: What is the impact on live birth rates when dIUI is performed with a volume of 0.5 mL versus 0.2 mL?

Summary answer: Live birth (LB) rates (LBRs) after dIUI cycles are not different when performed with 0.5 mL versus 0.2 mL.

What is known already: dIUI has an important role in the treatment of severe male infertility, and is often used in same-sex female couples and single parents. Different variables have been studied as factors correlated with clinical outcomes (IUI scheduling, ovarian stimulation, sperm parameters) but little is known about the inseminated volume. The use of conical bottom test tubes could contribute substantially to the loss of inseminated spermatozoa because it precludes the total recovery of the sample. Additionally, the insemination catheter could uphold this reduction causing sperm adhesion on the inner walls of the insemination catheter, decreasing even more the total inseminated volume.

Study design, size, duration: A prospective RCT, including patients undergoing natural or stimulated dIUI was performed between March 2013-April 2015. dIUI cycles (n = 293) were randomized to undergo insemination with 0.2 mL (control) or 0.5 mL (study group), of which 24 were excluded (protocol deviation) and 269 received the allocated intervention. Study was designed with 80% power to detect a 20% difference in LBR with a reference of 20% and a two-tailed 5% significance level. The required sample size was 125/group.

Participants/materials, setting, methods: There were 143 cycles (0.2 mL group) and 126 cycles (0.5 mL group). A multivariate logistic regression model was constructed to adjust for potential predictor variables regardless of association within a univariate model. Additionally, cycles that resulted in LB were compared to those that did not (total and by group) by Mann-Whitney test. Lastly, a ROC curve was developed to determine whether LB was associated with total motile sperm (TMS, in millions) as total and by group.

Main results and the role of chance: Groups (0.2 mL group vs. 0.5 mL group, respectively) were similar in age (35.8 ± 3.9 vs. 35.4 ± 4.0), AMH (2.2 ± 1.8 vs. 2.0 ± 1.5), basal antral follicle count (13.2 ± 6.4 vs. 13.6 ± 6.0), BMI (23.5 ± 3.9 vs. 23.7 ± 4.1), follicles >17 mm (1.1 ± 0.5 vs. 1.1 ± 0.5), total GND dose (553.1 ± 366.3 vs. 494.6 ± 237.1), and TMS count (8.22 ± 7.1 vs. 7.7 ± 5.7). Similar clinical pregnancy rate (18.9% (27/143) vs. 19.8% (25/126), NS), live birth rate (15.4% (22/143) vs. 19.0% (24/126), NS) and miscarriage rate (18.5% (5/27) vs. 4.0% (1/25), NS) were observed between groups. After adjusting in a logistic regression model for variables mentioned before, the inseminated volume was not shown to be associated with LB (OR 1.1 (95% CI 0.6 – 1.9). Comparison of all patients who achieved a LB versus those that did not, no demographic differences were observed in the included variables, both as a total (LB vs. no LB) or by subgroup (LB vs. no LB in the 0.2 mL group, and LB vs. no LB in the 0.5 mL group). Lastly, an ROC curve did not demonstrated correlation of the TMS inseminated with the probability of obtaining a LB.

Limitations, reasons for caution: Study was not powered to detect a statistical difference in miscarriage rate. Furthermore, a similar study in a larger number of women is required to confirm these results.

Wider implications of the findings: This is the first RCT showing that the inseminated volume is not correlated with LB. We speculate the lower miscarriage rate observed in the 0.5 mL group could be related to the presence of contractions similar of those generated during sexual intercourse, implicated in the inception of early biochemical embryo-endometrium communication.

Trial registration number: The trial was registered at clinicaltrials.gov (Identifier: NCT03006523).

P-317 Results of ovulation induction with pulsatile GnRH using a novel portable device (LutrePulse®)

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Study question: We investigated the practical applicability and success rates of a new one-way portable pump in the pulsatile delivery of GnRH for ovulation induction.

Summary answer: LutrePulse® proved to be an easy-to-handle and reliable system. Ovulation could dependably be induced, and live birth was achieved in 2/3 of the patients.

What is known already: Pulsatile GnRH stimulation has long been established for ovulation induction of patients with hypothalamic amenorrhea. Previously used automatized systems suffered from patients' discomfort in daily use and were cumbersome to work with for medical staff. In spite of therapeutic efficacy many physicians were reluctant to use this particular form of stimulation. In 2012 with the introduction of a new comfortable insulin pump system to pulsatile GnRH therapy handling was vastly improved.

Study design, size, duration: We performed a retrospective analysis of pulsatile GnRH therapy using LutrePulse® in all patients with hypothalamic ovarian failure from six German fertility centers. 62 patients were treated from 2013 thru 2016. After diagnosis was established, stimulation was individually designed according to the physician's judgment.189 treatment cycles could be evaluated. Duration of GnRH application as well as pregnancy rate and live-birth rate were analyzed.

Participants/materials, setting, methods: Infertility patients were selected by clinical and laboratory features qualifying them for the diagnosis of hypothalamic ovarian failure. Male factor infertility was excluded by repeated sperm analysis. Patients with suspected tubal factor infertility were also not included. Duration and dose of GnRH therapy until the day of ovulation induction were evaluated. The number of treatment cycles needed to achieve a clinical pregnancy, live-birth rates as well as drop-out rates were determined as primary outcome parameters.

Main results and the role of chance: The mean age of patients was 30.3 years. Patients were treated from 1 up to 10 cycles. Mean BMI was 21 kg/m^2. Doses depending on clinical features ranged from 5–40ug with a pulse interval of 90 min. The dose in 1/3 of all treatment cycles was 15ug every 90 min.

Duration of treatment until ovulation induction ranged from 8 to 53 days. In 22% of all cycles, treatment was prolonged for more than 20 days, while mean duration of therapy was 17 days.

Luteal support differed between treatment centers. In 35 patients, pulsatile GnRH was continued after ovulation induction for luteal support. During the luteal phase application interval was prolonged to 120 min in luteal phase without changes in GnRH dose. The remaining patients were treated with

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progesterone and hCG for luteal support. No differences in pregnancy rate could be seen with respect to luteal phase management.

52 pregnancies and 39 live-births could be achieved. 5 abortions and 1 termination due to malformation in ultrasonographic screening were reported. Pregnancy rate was 84% and live birth resulted in 63% of treatment cycles.

As of today, 7 pregnancies are still ongoing and were obviously not included in live-birth rate.

Limitations, reasons for caution: Data were collected in 6 fertility centers in Germany. Most centers collected patients according to clinical features, while one center additionally performed a GnRH-test to evaluate patients for treatment. Although no differences in outcome could be seen, luteal support differed in treatment centers and might influence pregnancy outcome.

Wider implications of the findings: Pulsatile GnRH therapy is well established in fertility treatment of hypothalamic amenorrhea. While excellent pregnancy rates have been presented in literature, the focus of the pharmaceutical industry has shifted more towards patients' and physicians' satisfaction with practical treatment modalities. LutrePulse® combines high treatment comfort and safety with excellent pregnancy rates.

Trial registration number: none.

P-318 IUI in the stimulated cycle versus IUI in the natural cycle: a cohort study from China

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Study question: What is the effectiveness of different ovarian stimulation protocols as compared to natural cycle treatment in an intrauterine insemination (IUI) program?

Summary answer: In an IUI program, ovarian stimulation significantly increased the clinical pregnancy rate with 3% as compared to natural cycle IUI.

What is known already: In many countries, IUI is the treatment of first choice for couples with unexplained and mild male factor infertility. While IUI can be applied in the natural cycle, in many settings ovarian stimulation aiming to establish multiple follicle growth is applied. It is however controversial whether ovarian stimulation improves fertility outcomes in an IUI program. Here, we compare the success- and twin rates of natural and stimulated IUI cycles in a large centre in Beijing, China.

Study design, size, duration: We performed a retrospective cohort study in the Reproductive Medicine Centre of Peking University Third Hospitalin. Couples with unexplained and mild male subfertility were treated with IUI with or without ovarian stimulation. Women with polycystic ovary syndrome (PCOS) were excluded. Here, we compare IUI cycles stimulated with clomiphenes citrate (CC), letrozole, and gonadotropins to IUI cycles without ovarian stimulation.

Participants/materials, setting, methods: We compared the baseline characteristics among the groups. We then compared the number of dominant follicles, clinical pregnancy, miscarriage and live birth between groups with CC, letrozole, gonadotropins stimulation and natural cycle IUI. We also report on multiple pregnancies. We calculated relative risks (RR) and 95% confidence intervals (CI) for stimulated versus natural cycles, as well as Numbers Needed to Treat (NNT). ANOVA, t-test and Chi square analysis were conducted in SPSS 19.0.

Main results and the role of chance: Between January 2015 and September 2016, we performed 5, 177 IUI cycles, including 1,003 CC, 743 letrozole, 583 gonadotropins and 2,848 natural cycles. Baseline characteristics did not differ between the groups. The mean number of follicles was higher in stimulated IUI cycles with CC (1.64 ± 0.85), letrozole (1.37 ± 0.66), and gonadotropin (1.39 ± 0.79), as compared to natural cycle IUI (1.01 ± 0.16) (all P-values < 0.001).

After stimulation with CC, letrozole, or gonadotropin, clinical pregnancy rates were 11.1%, 11.6%, and 11.5% respectively. In natural cycle group, the clinical pregnancy rate was 8.7%. Compared to natural cycle, the pregnancy rate in stimulated IUI cycles with CC (RR 1.28, 95% CI1.03 to 1.58), letrozole (RR 1.33, CI1.06 to 1.68) and gonadotropins (RR1.33, CI1.03 to 1.71) were significantly higher compared to unstimulated IUI cycles. The NNTs to establish one additional pregnancy with IUI with ovarian stimulation as compared to natural cycle IUI were 42, 34 and 35 for CC, letrozole and and gonadotropins, respectively. Live birth rates were 10.9%, 10.6% and 6.5% in the CC, letrozole, and gonadotropins group versus 6.37% in the natural cycle group. Multiple pregnancy occurred in 3/111, 0/86, 2/67, 1/247 in the CC, letrozole, gonadotropins and natural cycle groups.

Limitations, reasons for caution: This is a retrospective study that could be biased by indication. Since we did not include cancelled cycles, results might be biased, probably in favour of ovarian stimulation. Also, we reported results per cycle and not per couple. Our data should inform a randomised controlled trial on the subject.

Wider implications of the findings: This study showed that ovarian stimulation in an IUI program improves pregnancy rate. However, as the numbers to treat for ovarian stimulation versus natural cycle were between 34 and 42, natural cycle IUI can be considered as the treatment of first choice.

Trial registration number: N/A.

P-319 Is intrauterine insemination more effective than expectant management in couples with unexplained subfertility? A secondary analysis of a prospective cohort

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Study question: What is the benefit of intrauterine insemination (IUI) compared to a wait-and-see policy i.e. expectant management in couples diagnosed with unexplained subfertility?

Summary answer: Intrauterine insemination seems to increase the chances of ongoing pregnancy compared to expectant management in couples with unexplained subfertility.

What is known already: Intrauterine insemination with or without ovarian stimulation is often the first-line treatment for couples with unexplained subfertility although the effectiveness of IUI over expectant management has never been demonstrated.

Study design, size, duration: A prospective cohort studying the long term follow up of 1946 couples diagnosed with unexplained subfertility after expectant management and after IUI, mostly with ovarian stimulation, included in 7 centres in the Netherlands between January 1999 and October 2005. Couples with bilateral tubal occlusion, anovulation, or a total motile sperm count <1 x 10^6 were excluded.

Participants/materials, setting, methods: The primary endpoint was time until conception leading to an ongoing pregnancy. Follow up time was censored at the start of IVF treatment or at the last date of contact. We used the sequential Cox approach where we compared in each month couples who started IUI with couples on expectant management for their six month outcome. We used inverse probability weights to balance the known predictors for conception between groups.

Main results and the role of chance: Data of 1946 couples were available. Follow up was truncated at one and a half years after the fertility workup due to insufficient sample sizes for our approach thereafter. The median time of expectant management follow up was 7-8 months after the workup, median time until initiation of IUI was 6-7 months and median time spent in an IUI regimen was 4-5 months. 391 (20%) couples conceived naturally within one and half years after the fertility workup (rate: 0.30 per couple per year). 803 couples had at least one IUI cycle within one and a half years after the workup of which 140 (17%) couples conceived (rate: 0.41 per couple per year). Most treated couples (82%) used ovarian stimulation in at least one IUI cycle.

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The sequential Cox approach estimated a fecundability ratio of 1.56 (95%CI: 1.20-2.03) for conceiving after starting IUI compared to expectant management. The predicted chance for a couple to conceive within one year of expectant management after the fertility workup was 27.3%. If this couple would instead opt for six months of IUI after six unsuccessful months of expectant management, their predicted chance to conceive within this year increased to 32.2%.

Limitations, reasons for caution: The effect estimates are based on a prospective cohort. Although we balanced the known predictors of pregnancy between treated and untreated couples using inverse probability weighting, these estimates do not allow for causal interpretation. The results need to be confirmed by large randomised controlled trials.

Wider implications of the findings: IUI may benefit unexplained subfertile couples as first-line treatment although the absolute difference in cumulative pregnancy chances is modest.

Trial registration number: Not applicable.

P-320 Low dosing of gonadotropins in IVF cycles for women with poor ovarian reserve: Systematic review and meta-analysis

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Study question: How does low dosing of gonadotropins and high doses of gonadotropins in ovarian stimulation regimens compare in terms of ongoing pregnancy per fresh IVF attempt in women with poor ovarian reserve?

Summary answer: We found no evidence of a difference in pregnancy outcomes between low dosing of gonadotropins and high doses of gonadotropins in ovarian stimulation.

What is known already: Ovarian stimulation regimens for women with poor ovarian reserve include high doses of gonadotropins combined with various protocols of GnRH analogues. These regimens are of long duration, expensive and burdensome. Low dosing of gonadotropins has been suggested as an alternative, either by reducing the dose of gonadotropins itself or by a combination of gonadotropins and oral compounds such as antiestrogens or aromatase inhibitors which shorten the duration of stimulation and thereby lower the dose.

Study design, size, duration: A systematic review and meta-analysis of randomized controlled studies that evaluate the effectiveness of low dosing of gonadotropins compared to high doses of gonadotropins in women with poor ovarian reserve undergoing IVF/ICSI treatment.

Participants/materials, setting, methods: We searched the PubMed, EMBASE, Web of Science, the Cochrane Library and the Clinical Trials Registry using Medical Subject Headings and free text terms up to June 2016, without language or year restrictions. We included truly randomized controlled studies (RCTs) enrolling subfertile women with poor ovarian reserve undergoing IVF/ICSI treatment and comparing low doses of gonadotropins versus high doses of gonadotropins. The primary outcome was ongoing pregnancy rate per woman randomized.

Main results and the role of chance: We retrieved 788 records. Fifteen RCTs (N = 2183 women) were included in the analysis. Six studies (N = 796 women) compared low doses of gonadotropins versus high doses of gonadotropins. There was no evidence of a difference in ongoing pregnancy rate ((3 RCTs: RR 1.05, 95% CI 0.66 to 1.65, $I^2 = 0\%$)). This suggests that for a woman with a 13% chance of achieving an ongoing pregnancy with the use of high doses of gonadotropins, the chance of an ongoing pregnancy with the use of low doses of gonadotropins would be between 7% and 17%.

Nine studies (N = 1387 women) compared ovarian stimulation using gonadotropins combined with the oral compounds letrozole (n = 6), or clomiphene citrate (CC) (n = 3) versus the high doses of gonadotropins only. There was no evidence of a difference in ongoing pregnancy rate (3 RCTs: RR 0.90, 95% CI: 0.63 to 1.27; $I^2 = 0\%$) This suggests that for a woman with a 13% chance of achieving ongoing pregnancy with the use of high doses of gonadotropins, the chance of an ongoing pregnancy with the use of a gonadotropins combined with oral compounds would be between 8% and 16%.

Limitations, reasons for caution: Although we used strict inclusion criteria in the conduct of the systematic review, the included studies had several methodological considerations and clinical heterogeneity that have to be taken into account when interpreting the results.

Wider implications of the findings: We found no evidence of a difference in pregnancy outcomes between low doses of gonadotropins and gonadotropins combined with oral compounds compared to high doses of gonadotropins in ovarian stimulation regimens. Whether the low doses of gonadotropins or the gonadotropins combined with oral compounds is to be preferred is unknown

Trial registration number: CRD42016041301.

P-321 A genetic test to predict recurrent implantation failure based on the association of SNPs

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Study question: Can single-nucleotide polymorphisms (SNPs) in genes involved in the implantation process be used in a test to predict recurrent implantation failure (RIF)?

Summary answer: A genetic test (GT) to predict RIF was created from the association of several SNPs in genes involved in the implantation process.

What is known already: It is known that single genes can be associated with the regulation of invasion, angiogenesis and endometrium-embryo communication during the implantation process and that SNPs in these genes can increase susceptibility to RIF. Thus, there is a gene pattern signature of endometrial receptivity or embryo communication. The development of next-generation sequencing (NGS) technologies has allowed the identification of large sets of SNPs in several genes that can potentially be related to that phenomenon.

Study design, size, duration: A cross-sectional study was conducted with the blood samples from 49 women (35.2 ± 2.7years) representing the RIF group (RIF-G) that underwent IVF/ICSI, and from 47 fertile women constituted the control group (C-G). The RIF-G inclusion criteria: ≥2 failed IVF/ICSI attempts/≥5embryos transferred, age≤39years and normal karyotype. The exclusion criteria: uterine defects, hydrosalpinx, infections, endocrine problems, coagulation defects or thrombophilia and autoimmune defects. The C-G: volunteers with at least 2 live births without treatment and history of miscarriage.

Participants/materials, setting, methods: Genomic DNA was extracted from the peripheral blood of the RIF-G and C-G. The coding regions (exons), untranslated regions in both 3'/5' ends (UTRs) and some intronic regions of 36 selected genes were analysed by NGS using TruSeq Custom Amplicon/MiSeq-Systems. GATK's Unified Genotyper was used as a variant caller to detect SNPs on the full alignments, and logistic regression (LR) analysis was used to compare the SNP frequencies in the RIF-G and in C-G.

Main results and the role of chance: Sequencing showed 12 prevalent SNPs in the RIF-G and 10 prevalent SNPs in the C-G. LR multivariate analysis (considering RIF-G=1 and C-G=0) identified a significant association of 7 SNPs: 3 are prevalent in RIF-G(B1,B2,B3), and 4 in C-G(G1,G2,G3,G4). That association is represented by the equation $Logit(y)=1/(1+e^{-y})$, y=0.377+1.638xB1 +1.408xB2+1.348xB3-1.194xG1-1.232xG2-0.974xG3–1.548xG4 (Table 1). Considering the result of this equation/Logit(y) between 0.99 and 0.01, the GT classified the patient as RIF if the value was closer to 0.99 and as a control if the value was closer to 0.01. This GT applied on the RIF-G/C-G patients exhibited 81.6%/sensitivity, 76.6%/specificity, and 79.1%/efficiency, and the false-positive and false-negative rates were 23.4% and 18.4%, respectively. These data support the use of this GT to predict RIF.

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Table 1. Variables in the Equation.

	B	S.E.	df	Sig.	Exp(B)	95% C.I. for EXP (B)Lower	95% C.I. for EXP (B)Upper
B1(VEGFA)	1.638	.536	1	.002	5.142	1.798	14.706
B2(PGR)	1.408	.611	1	.021	4.087	1.235	13.529
B3(LIF)	1.348	.552	1	.015	3.850	1.306	11.351
G1(LEPR)	-1.194	.546	1	.029	.303	.104	.884
G2(TP63)	-1.232	.541	1	.023	.292	.101	.843
G3(ESR1)	-.974	.527	1	.050	.378	.135	1.060
G4(IGF1R)	-1.548	.598	1	.010	.213	.066	.687
Constant	.377	.331	1	.254	1.458		

Limitations, reasons for caution: Additional validation of the analysed SNPs (i.e., increasing the number of cases and/or expanding to different ethnic groups) is needed to provide more information regarding the potential use of this GT for RIF prediction.

Wider implications of the findings: The ability to predict RIF with a reliable GT can give the physician tools to analyse the risks for each patient.

Trial registration number: Not applicable. All patients provided written consent, and the local Research Ethics Committee approved the study.

P-322 The association between preconception maternal caffeinated and non-caffeinated beverage intake on IVF outcomes

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Study question: Does maternal intake of caffeinated or non-caffeinated beverages before IVF affect intermediate and clinical outcomes of IVF?

Summary answer: While total caffeine intake was unrelated to IVF outcomes, higher sugar sweetened beverages consumption may decrease the chances of pregnancy and live birth following IVF.

What is known already: Caffeine is a stimulant of the central nervous system. Increased caffeine consumption is associated with lower estrogen and progesterone levels in the luteal phase, shorter menstrual cycles (<25 days), and increased rates of spontaneous abortion. The effect of caffeinated beverages on fecundity is still controversial. Sugar sweetened beverages, both with and without caffeine, are associated with increased insulin resistance and weight gain which might lead to reduced fecundity. Moreover, soda drinkers are potentially exposed to higher levels of chemical contaminants such as bisphenol A, which is embedded in soda cans.

Study design, size, duration: A prospective study of 340 women undergoing IVF at a Tertiary, University Affiliated Hospital. Participants were enrolled from January 2014 through August 2016 during ovarian stimulation and completed a questionnaire on the first day of stimulation and/or on the day of egg retrieval. Intermediate (i.e., number of oocytes retrieved, mature oocytes, fertilization and day 3 top embryos) and clinical (i.e., positive beta-hCG, clinical pregnancy and spontaneous abortion) IVF outcomes were abstracted from medical records.

Participants/materials, setting, methods: Women reported on their usual consumption of 14 different beverages including coffee and espresso drinks, tea, hot chocolate, soda, and energy drinks. Total caffeine intake was estimated by summing the caffeine content for specific beverages multiplied by their frequency of intake. Associations between specific types of beverages and IVF outcomes were analyzed using Poisson regression models adjusting for age, BMI, smoking status and nationality, as well as other beverage intake.

Main results and the role of chance: Neither overall caffeine (range: 0-816 mg/day) nor coffee (range: 0-10 cups/day) intake was associated with intermediate or clinical IVF endpoints. A significant inverse association was observed with higher intake of caffeinated tea, sugar sweetened beverages, and energy drinks and lower total, mature, and fertilized oocyte counts following ovarian stimulation. Specifically, women who consumed energy drinks had, on average, 4.4 fewer oocytes retrieved, 2.9 fewer mature oocytes retrieved, and 1.6 fewer fertilized oocytes compared to women who did not consume energy drinks. Of these beverages, only intake of sugar sweetened beverages was significantly associated with clinical IVF outcomes. Compared to women in the lowest category of sugar sweetened beverages intake (0 cups/day), the adjusted difference in percent of cycles resulting in live birth for women consuming 0.1-1 cups/day and >1 cup/day were -9% and -13%, respectively (p-trend=0.04). A similar inverse association was observed for clinical pregnancy (p-trend=0.04). No associations were found between diet soda consumption and intermediate or clinical IVF outcomes.

Limitations, reasons for caution: Due to the observational nature of this study, we cannot rule out residual or unmeasured confounding due to other diet or lifestyle characteristics. We also had limited power to detect small differences in clinical outcomes given our sample size.

Wider implications of the findings: Among women undergoing IVF, tea, sugar sweetened beverages and energy drinks, may have adverse effects on oocyte and embryo quality. Higher intake of sugar sweetened beverages may decrease a women's chance of clinical pregnancy and live birth following IVF. These associations do not appear to be driven by caffeine intake.

Trial registration number: n/a.

P-323 Progesterone to Oocyte Index – a novel predictor for fresh IVF cycle outcome

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Study question: What is the relative predictive value of Progesterone to Oocyte Index (POI) for fresh IVF-ET cycle outcome?

Summary answer: POI is inversely related to clinical pregnancy (CP) and live-birth (LB), similar to Progesterone to Follicle Index (PFI) and superior to serum Progesterone (SP).

What is known already: The possible deleterious effect of elevated serum progesterone on the day of hCG administration on implantation is controversial in the relevant literature and no consensus exists about the detrimental threshold level. PFI has been suggested as a superior parameter to predict clinical pregnancy rate, in a single study. The number of oocytes aspirated may serve as a more objective parameter than the number of follicles measured by ultrasound.

Study design, size, duration: This is a retrospective analysis of a cohort of fresh IVF/ICSI-ET cycles (n = 2493) performed in a single IVF center during the period of 2010–2015.

Participants/materials, setting, methods: Patients who underwent COH for IVF/ICSI using GnRH-antagonist protocol. PFI and POI were calculated by dividing SP level (ng/mL) by the number of follicles (≥ 15 mm) on the day of hCG or the number of oocytes retrieved, respectively. A multivariate logistic regression analysis, after adjustment for patient's age and BMI, cycle number, E2 level, and endometrial thickness was performed to evaluate the prediction value of P level, PFI, and POI for CP and LB.

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Main results and the role of chance: Although the mean values for many parameters differ significantly between conception and non-conception cycles, multivariate analysis indicate that whereas P had no significant association with CP and LB, with odds ratio of 0.85 (95% CI 0.61–1.2, P = 0.37) and 0.74 (95% CI 0.49-1.10, P = 0.14) respectively, PFI and POI were inversely associated with clinical pregnancy adjusted OR 0.177 (95% CI 0.052-0.60, P = 0.006) and 0.063 (95% CI 0.016-0.249, P < 0.001) respectively and with live birth adjusted OR 0.054 (95% CI 0.011-0.272, P < 0.001) and OR 0.036 (95% CI 0.007-0.199, P < 0.001) respectively. For prediction of LB, the area under the curve (AUC) was 0.66 (95% CI 0.62-0.69, p<0.001) for the PFI model, and 0.68 (95% CI 0.64-0.71, p<0.001) for the POI model. Dividing POI to deciles, after the 9th decile - (POI of 0.360), CPR and LB decreased to 8.0% and 5.9% respectively.

Limitations, reasons for caution: This is a retrospective analysis of data collected during a 3-year period, and although the effect of the most important confounders was controlled for in the multivariable analysis, the presence of residual bias cannot be excluded.

Wider implications of the findings: This study offers a new and simple index for prediction of fresh ET outcome, emphasizing the need for a multivariable approach.

Trial registration number: 88-15-BRZ (this is aregistration number in our hospital)

P-324 Centromere protein C(CENP-C) antibody causes spindle defects and chromosome misalignment during meiosis of mouse oocyte

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Study question: Whether centromere protein C(CENP-C) antibody developed by active immunized method could impact oocyte meiosis.

Summary answer: oocyte meiosis was severely impaired by CENP-C antibody.

What is known already: Anticentromere antibodies were associated with lower oocyte maturation rate, fertilization rate, cleavage rate, pregnancy rate and implantation rate and higher abortion rate. CENP-C linked the centromeric chromatin with the outer kinetochore and was more closer to cell division than other centromere proteins.

Study design, size, duration: Mice were divided into two groups, one was experimental group immunized with human centromere protein C in Freund's adjuvant (CFA), the other was control group injected with CFA only.

Participants/materials, setting, methods: Serum and oocytes of BALB/c mice immunized with human centromere protein C(CENP-C) in complete Freund's adjuvant (CFA) or injected with CFA only were studied for the development of CENP-C antibody. Rates of germinal vesicle breakdown (GVBD), first polar body (Pb1) extrusion, abnormal spindle morphology, chromosome misalignment and aneuploidy were compared between experimental group and control mice.

Main results and the role of chance: Following immunization, CENP-C antibody was observed in serum and oocytes of mice immunized with centreomere protein C antigen, compared with mice injected with CFA only. Decreased first polar body (Pb1) extrusion rate was found among the centromere-immunized mice compared with the CFA-injected mice (P < 0.01). Higher percentage of spindle defects, chromosome congression failure and aneuploidy were detected in immunized mice oocytes, (spindle defects:64.67 ± 1.16% vs 9.27 ± 2.28% control; chromosome misalignment:50.80 ± 2.40% vs 8.30 ± 1.16% control; aneuploidy:55.83 ± 3.63% vs 9.60 ± 1.86% control; p < 0.01 for both).

Limitations, reasons for caution: The impacts of CENP-C antibody on oocyte meiosis were only examined in mice.

Wider implications of the findings: This study revealed immunologic factors could affect oocyte meiosis and cause oocyte aneuploidy, which could provide some new thoughts and clues for improving reproductive outcomes of anticentromere antibody positive women.

Trial registration number: not applied.

P-325 Obese women have a different polunsaturated fatty acid pattern in their oocytes

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Study question: Is the fatty acid oocyte composition related with maternal weight?

Summary answer: Obese women and also overweight women had a different fatty acid profile when compared with normal weight women.

What is known already: Obsesity complications are well known in medicine, gynecology and reproductive medicine. The siblings of obese women have higher weights when newborn infants and have an increased rate of infant obesity. Polyunsaturated FA are of paramount importance in a healthy diet. Current western diets have an excess of n6 FA and a relative lack of n3 FA. This disbalance has been associated with obesity. We have previously shown that a number of FA can be detected in human oocytes (Matorras et al, 1998), and that their n6/n3 ratio was inside the ranges of the recommended dietary allowances.

Study design, size, duration: During a 18 month period, 922 oocytes (381 germinal vesicle, 208 metaphase I, 333 unfertilized metaphase II), corresponding to 237 women performing IVF/ICSI in two different centers were recruited. Three groups were established: normal weight (BMI 20-25), overweight (BMI 25-30), obesity (BMI > 30).

Participants/materials, setting, methods: The obtained oocytes were grouped according BMI and maturation status (germinal vesicle, metaphase I or II). They were thawed and later were subjected to chromatography analysis in groups of 15-20 oocytes (since a lower number precluded the FA analysis). Analysis was performed using capillary gas chromatography. Results were expressed in percentages of total FA amount.

Statistical analysis was performed by means of non-parametric analysis (Willcoxon, Mann Withney)

Main results and the role of chance: A different FA profile was observed in the majority of FA in the three body groups considered.

The "classical" n3 FA, Docosahexaenoic acid (DHA) was significantly lower in normal weight (0.58) than in overweight (0.81) than in obesity (1.01). On the other hand eicosapentaenoic acid (also a n3 FA) was much higher in normal weight (0.74) than in obesity (0.07). Thus, when analysed toghether, total n3 FA were significantly different in women with normal weight (1.82) than in overweight women (1.64) than in obese women (1.55). n6 fatt acids were significantly increased in obese women (11.59) vs 12.31 in normal weight (12.31) and overweight (12.54).

A number of quotients were significantly different among the three aforementioned populations: the arachidonic acid/DHA was higher in normal weight and lower in overweight whereas the opposite occurred with arachidonic/linoleic acid and the Docosahexaenoic acid (DHA)/α linolenic acid were lower in normal weight and higher in obese.

When the analysis was restricted to the same oocyte maturation status, the aforementioned differences persisted.

Limitations, reasons for caution: Our MII population was restricted to unfertilized MII oocytes, thus the results may not be extrapolated to MII oocytes which do fertilize.

Wider implications of the findings:

(1) The well-known poor outcome in obese women may be also related to an unfavourable FA pattern

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(2) It could be speculated that an unfavorable oocyte FA pattern could be an early determinant of obesity in the infant and the adult

Trial registration number: None.

P-326 Impact of zinc, selenium and malondialdehyde acid levels in follicular fluid on the IVF outcome

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Study question: Have zinc, selenium and malondialdehyde acid (MDA) follicular concentrations any impact on the early embryonic development?

Summary answer: Zinc concentration in the follicular fluid (FF) is positively correlated to the oocyte maturity, fertilization, cleavage and Top quality embryos rates.

What is known already: It has been shown that there are detectable levels of zinc and selenium in human follicular fluid and it is already known that these trace elements exert an important antioxidant activity.

Study design, size, duration: Prospective study, including 190 women of infertile couples undergoing IVF cycles from January to June 2016.

Participants/materials, setting, methods: FF samples were obtained form 190 female of infertile couples undergoing IVF cycles. Patients were divided into four groups based on IVF indications: Group I (n = 35) idiopathic infertility, group II (n = 50) tubal diseases, group III (n = 50) endometriosis and group IV (n = 55) male infertility. After oocyte retrieval, FF were collected and centrifuged (400 g, 15 minutes). The supernatants were frozen at -80°c until analysis. Flame and furnace atomic absorption spectrophotometry were respectively adopted for zinc and selenium dosage.

Main results and the role of chance: In comparison with group I, zinc follicular concentration in groups III and IV showed significant decrease (respectively 17,5 and 39,79 mg/l vs 67,64 mg/l; p<0.001). Selenium concentrations were significantly higher (P < 0.001) in group I compared to patients with tubal disease, endometriosis and male factor of infertility. Concerning MDA, we noted elevated rates in all patient groups (GII= 56,20 µmol/l, GIII=17,5 µmol/l and G IV=39,79 µmol/l) compared to the control group (GI=0,006 µmol/l). Regarding the early embryonic development, all patient groups demonstrated a significantly decrease in comparison with control group. Follicular zinc concentration was highly correlated to the oocyte maturity (r= 0.384**, P < 0.01), the fertilization rate (r=0.338**, P < 0.001), the cleavage rate (r=0.347**, P < 0.001) and Top quality embryos rate (r=0.269**, P < 0.001). No correlation was found between selenium and MDA concentrations and IVF outcomes.

Limitations, reasons for caution: Much larger sample size is requested, all data are limited to the power provided by the number of test subjects included.

Wider implications of the findings: It is proved that the non-enzymatic antioxidant statue and the lipid peroxidation in the follicular microenvironment may play a role in the gamatogenesis and fertilization process and they would be the best predictors of IVF outcome.

Trial registration number: It is not a clinical trial.

P-327 endometrial thickness: impact on live birth outcomes of FET from freeze-all cycles

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Study question: The objective of this study was to examine the independent effect of endometrial thickness on FET outcomes of blastocyst freeze-all cycles.

Summary answer: Endometrial thickness as an independent variable in art-FET does not significantly affect reproductive outcomes, even for endometrial thickness of ≤6 mm.

What is known already: Sonographic assessment of endometrial thickness as a measure of endometrial receptivity remains part of the diagnostic work-up and follow-up of IVF patients, because of it being a relatively accurate non-invasive assessment. Its value in the prediction of IVF success, however, has been controversial. In general, a thin endometrium, defined as a thickness of ≤7 mm, has been regarded to be associated with lower implantation. Investigating the value of endometrial thickness in patients undergoing frozen embryo transfer (FET), because of the absence of adverse effects of controlled ovarian stimulation (COS), may be more revealing in terms of pregnancy prediction.

Study design, size, duration: This is a retrospective of analysis of the first artificial frozen embryo transfer (art-FET), subsequent to 1338 freeze-all cycles in a segmented-IVF program performed between February 2015 and January 2016.

Participants/materials, setting, methods: The impact of endometrial thickness on pregnancy, live birth, and pregnancy loss was assessed. Of the 1338 freeze-all cycles 1182 first FET outcomes were analysed, excluding cycles with no FET (i.e., due to patient choice, pre-implantation genetic diagnosis, blastocyst degeneration) and cycles with incomplete data. The same artificial endometrial preparation was used for all FET, with sonographic endometrial measurements performed on day 14. All blastocysts were vitrified and were transferred on the day of warming.

Main results and the role of chance: The incidence of thin endometrium (≤7 mm) in this population of infertile patients was 8.8%. The non-significantly different pregnancy, live birth (LB), and pregnancy loss rates were 63.5%, 47.1%, and 25.8%, and 74.5%, 58.0%, 22.2% for cycles with endometrial thickness of ≤7 mm and >7 mm cycles, respectively. Although numerically reduced, the pregnancy and LB rates for the group with ≤6 mm endometrial thickness were also non-significantly different at 53.9% and 46.2%, respectively. In a subgroup analysis, with endometrial thickness sub-groups increasing in increments of 1 mm, ≤7, 7.1-8, 8.1-9, 9.1-10, 10.1-11, 11.1-12, and >12 mm, and female age, number of oocytes retrieved, and number of blastocysts transferred non-significantly different the pregnancy and LB rates were non-significantly different across the sub-groups. The sub-group 9.1-10 mm, however, had the lowest numerical pregnancy loss rate compared to the other groups (16.3 vs ≥21.5%).

Limitations, reasons for caution: This was a retrospective study; only analyzing patients 1st FET cycles, restricted to patients under 43 years of age and including pre-implantation genetic diagnosis cycles.

Wider implications of the findings: Endometrial thickness may not be an independent variable of significance in the prediction of live birth in FET, as the live birth was non-significantly different across all categories of endometrial thickness.

Trial registration number: N/A.

P-328 The lncRNA landscape of human oocytes changes with age and ovarian reserve

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Study question: Do long non-coding RNA (lncRNA) expression levels change with age and ovarian reserve in human MII oocytes?

Summary answer: The expression levels of several lncRNA changes according to a woman's age and ovarian reserve.

What is known already: Age and ovarian reserve both affect oocyte developmental competence, i.e. its ability to sustain early embryonic development. Ovarian reserve decreases with age, and oocyte competence decreases with it. The molecular mechanisms underlying a diminished ovarian reserve are poorly characterized. Long non-coding RNA (lncRNA) are involved in controlling gene expression, and have been associated with important cellular processes such as proliferation, lineage commitment and development, suggesting a role of lncRNA in early human embryonic development. The present study on lncRNA differential expression aims to dissect the effect of age and ovarian reserve on the lncRNA oocyte landscape.

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Study design, size, duration: 27 in vivo matured MII oocytes from 22 fertile women were divided into 3 experimental groups n = 9 each) according to age and ovarian reserve measured by antral follicular count (AFC): Young with Low AFC (YL; 24 ± 2 y.o. and 8 ± 1 AFC); Young with High AFC (YH; 21 ± 1 y.o. and 24 ± 3 AFC) and Old with Low AFC (OL; 34 ± 2 y.o. and 7 ± 1 AFC). Oocytes were individually processed and analyzed for expression of 31 lncRNA.

Participants/materials, setting, methods: Thirty-one lncRNA were selected as candidates from a preliminary internal study. MII oocytes were dezoned and individually lysed. Total RNA extraction, single-cell whole transcriptome amplification, and quantitative PCRs (qPCR) were performed. Student's t and two-way between-subject ANOVA ($p<0.1$) tests were performed to analyze qPCR results.

Main results and the role of chance: There was high variability in the expression of the 31 lncRNA candidates analyzed within and among groups. Overall, we did not find any lncRNA more expressed in the YL compared to the other groups and the OL group tends to express higher amounts of lncRNA transcripts than YL and YH. Nevertheless, 8 out of 31 lncRNA showed differential expression ($p<0.1$) among specific two-way group comparisons. When comparing YL with OL, 5 lncRNA showed higher expression in the OL group, 3 of them (lincCCDC140, lincSLC5A12 and lincCOL1A2-2) at intergenic locations and 2 overlapping to coding genes (lincADCYAP1R1 and lincCC2D1B). The comparison between the YL and YH groups showed 2 intergenic lncRNA (lincARRDC4 and C9Orf3) with higher expression in the YH group. Finally, lincCOL1A2-1 was expressed at higher levels in OL and YH groups compared to the YL group. Among the 8 lncRNA candidates, ENCODE analysis revealed DNAse I hypersensitive genomic regions at lincCOL1A2-1, indicating the presence of a distal enhancer for the COL1A2 gene, and suggesting a regulatory role for this gene in human oocytes. Moreover, lincADCYAP1R1 could regulate the expression of an oocyte-specific receptor for Pituitary adenylate cyclase-activating polypeptide (PACAP), which controls final oocyte maturation and oocyte developmental competence.

Limitations, reasons for caution: The women included in the study were fertile and up to 35 years old, thus our results cannot readily be extrapolated to older or infertile women.

Wider implications of the findings: The relationship between diminished ovarian reserve and age on oocyte developmental competence is still poorly understood. The present study of lncRNA in the oocyte indicates that lncRNAs are differentially expressed in relation to both factors, and might serve as biomarkers of oocyte fitness.

Trial registration number: NA.

P-329 Does pituitary suppression lead to low antral follicle count during IVF Cycles in north Indian females?

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Study question: To study the effect of short term pituitary suppression on antral follicle count (AFC).

Summary answer: Short-term pituitary suppression has a negative impact on AFC, suggesting the suppressive impact of exogenous hormones on the ovary during stimulation.

What is known already: To control the hypothalamic–pituitary–ovarian axis through the induction of pituitary 'down-regulation' using a continuous and supraphysiological dose of gonadotrophin-releasing hormone (GnRH) agonist (Marcus and Ledger, 2001), prevents a premature surge in luteinizing hormone (LH), which occurs in up to 23% of cycles when gonadotrophins alone are used and facilitates the timing of oocyte retrieval therefore (Janssens et al., 2000). The use of GnRH agonists in assisted reproduction treatment (ART) has been shown to increase the number of oocytes retrieved, to improve pregnancy rates and to significantly reduce the chance of cancellation (Hughes et al., 1992).

Study design, size, duration: Retrospective study.

Study was done on 407 infertile females attending the Sparsh IVF clinic, Teerthankar Mahaveer Medical College & RC, Moradabad in north India (2013 to 2016).

Participants/materials, setting, methods: Study was done on 407 infertile females attending the Sparsh IVF clinic, Teerthankar Mahaveer Medical College & RC, Moradabad in north India (2013 to 2016). Base line AFC was noted, when no medications known to cause pituitary suppression were used.Follow up AFC (suppressed AFC) while on E2, GnRH agonist (GnRH-a) or oral contraceptive (OC) pills in preparation for ovarian stimulation, performed within 6 months of initial baseline AFC.

Main results and the role of chance: There was an average decline of 0.2, 1.1, 2.8, in AFC while patients were on E2, GnRH-a, OC pills, respectively. Decline in AFC is also influenced by increasing age.

Limitations, reasons for caution: Small size of population.

Wider implications of the findings: The study shows that exogenous e2 may cause least suression of antal follicle count as compared to other agents. Furtur studies with large no of subjects is needed for confirmation of findings.

Trial registration number: Trial is approved by instituitional ETICAL committee.

P-330 Differential patterns of miRNA expression in granulosa cells (GC) media and ovine follicular fluid (FF)

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Study question: Can miRNA detected in extracellular fluids such as FF or cell culture media be used as a growth progress marker for GC and follicles.

Summary answer: Specific miRNAs were differentially expressed in ovine FF relative to follicle size and GC and their media were relative to plating cell density.

What is known already: GC growth in culture from sparsely seeded to dense clustering has been shown to parallel changing patterns of gene and protein expression. This study aimed to determine the miRNA expression profiles and assess its value as a marker of GC growth in culture. It is also aimed to compare the determined profiles to that found in the FF of antral follicle. Research to date has shown that antral FF contained a wide range of miRNA but it is not known what contribution if any is made by GC. This present study aimed to provide some insight into this important question.

Study design, size, duration: Study was 2×4 factorial with hypoxic and normoxic culture conditions and the FF was collected from different size follicles. The expression of three miRNAs in the collected FF and in the GC lysate and their media at 16-96 hours of culture was the main endpoint. Outcomes were determined from at least three experiments done in duplicates.

Participants/materials, setting, methods: Sheep tissue was utilised as a physiological model species for the human. GC cultured under conditions to inducing either hypoxic or nomoxic growth and maintained for 16, 24, 48, and 96 hours, from which cells and media were analysed for three specific miRNAs. The same miRNAs were determined in FF from large (≥4 mm) and small (≤4 mm) size follicles.

Main results and the role of chance: A small sub-set of three miRNAs (miR-30c, miR-373p, miR-548p) were normalised against an endogenous control (miR-106b-3p). These were found to differentially express in the FF of different size follicles. Both miR-30c and miR-373p showed a statistically higher expression ($P < 0.05$) in the FF of larger size follicles compared to smaller ones. Variations in the levels of expression of these miRNAs were also found in GC cultured over time in hypoxia-inducing and normoxic conditions.

Limitations, reasons for caution: Part of the results have been derived from cell culture studies which, although designed to mimic the normal differentiative processes that occur in selected follicles in response to gonadotrophin-stimulation, may not fully reflect the complex physiological miRNA expression in a growing follicle.

Wider implications of the findings: This work suggests that miRNAs can be used as non-invasive markers of GC growth in cultures and in a clinical settings during follicular development.

Trial registration number: not applicable

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P-331 vascular endothelial growth factor A, VEGF receptors and interleukin 8 concentration in the blood serum after the final oocyte maturation with gonadotropin-releasing hormone agonist

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Study question: Does the administration of gonadotropin-releasing hormone agonist (aGnRH) reduce angiogenic cytokines concentration and prevent the ovarian hyperstimulation syndrome (OHSS)?

Summary answer: aGnRH triggering reduces VEGF concentration on the day of oocyte retrieval (OR) and VEGFR2 during OR and OR+5, which leads to the prevention of OHSS.

What is known already: The pathophysiologic mechanism that fully explains OHSS is still unknown. But it is mainly associated with the administration of human chorionic gonadotropin (hCG), because of an increase of angiogenic cytokines (VEGF, EGF-like growth factor, IL-8, bFGF, TNF-a etc.). Administration of aGnRH for oocyte maturation became a strategy for avoiding this complication of ovarian stimulation. However, the mechanism of aGnRH action in prevention of OHSS has not yet been identified.

Study design, size, duration: This prospective randomized study included 51 high-responder patients with tubal infertility. All patients underwent IVF program in GnRH antagonist cycles. Group 1 (n = 15) consisted of patients who received the aGnRH trigger plus 1500 IU hCG support on OR day. Group 2 (n = 18) included women who received the dual trigger (aGnRH+1500 UI hCG). Group 3 (n = 15) included patients who received the hCG trigger. Plasma samples were obtained on the OR and embryo transfer days (ET).

Participants/materials, setting, methods: Inclusion criteria: age <39 years; AMH > 2,5 ng/ml; AFC>14; 18 follicles measuring >11 mm on the day of the trigger. Exclusion criteria: uterine fibroids, endometriosis, embryo transfer cancellation. Concentrations of VEGFA, VEGFR1, VEGFR2, interleukin 8 (IL-8) in serum were evaluated with ELISA kit.

Main results and the role of chance: Only 2 cytokines showed significantly different expression levels in all groups (VEGFA, VEGFR2). Specifically, concentration VEGFA levels in Group 1 (M ± s: 432.39 ± 264.39) were almost 1.5-fold lower than in Group 3 (M±s: 664.36 ± 309.05) (p = 0.0029), VEGFR2 levels in Group 1 (M ± s: 17527.50 ± 6605.67) and in Group 2 (M±s: 15755.83 ± 6565.26) were significantly lower than in Group 3 (M±s: 23699.0 ± 9602.37) (p* = 0.0456; p**=0.0122) on the OR day. But only VEGFR2 concentration levels were significantly lower in Group 1 (M ± s: 4143.61 ± 4773.53) and in Group 2 (M±s: 16931.94 ± 5962.87) than in Group 3 (M±s: 24237.67 ± 814.48) (p* = 0.0004, p**=0.0082) on the OR+5 day. There were no significant differences in VEGFR1 and IL-8 levels on the OR and ET days between the groups. According to ROC-analysis, high level of VEGFA concentration on the OR day was associated with OHSS. For the baseline level of expression set at 736 pg/ml, the area under curve was estimated to be 0.768. The model sensitivity and specificity at the baseline level were 77.1% and 87.5%, respectively. No OHSS cases were seen in Group 1 and 2, whereas two cases of moderate late-onset OHSS occurred in Group 3 (13.3%).

Limitations, reasons for caution: Our study was carried out in a relatively small subset of high-risk patients for OHSS with tubal infertility. Therefore, the results obtained cannot be readily extrapolated on other groups of patients and need to be confirmed in larger trials.

Wider implications of the findings: This study opens new possibilities for understanding the mechanism of OHSS prevention after aGnRH triggering.

Trial registration number: N/A.

P-332 Cytokine profile in follicular fluid during ovarian ageing

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Study question: Does the follicular fluid of patients with diminished ovarian reserve express a specific cytokine profile, different from that of patients with a normal ovarian reserve?

Summary answer: The follicular fluid concentration of the platelet-derived growth factor BB (PDGF-BB) is significantly lower in patients with DOR as compared to the control group.

What is known already: Ovarian ageing is related to the gradual quantitative and qualitative decrease of the ovarian reserve (DOR). A large individual variability exists in the age at which this reproductive event occurs, and although early reproductive aging is relatively common, the pathogenesis of this disorder is still unclear. The follicular fluid (FF) constitutes an important microenvironment for oocyte development, and its composition is directly correlated with oocyte quality. Furthermore, both folliculogenesis and ovulation are regulated by intrafollicular cytokines, chemokines, and protein growth factors. To our knowledge, only a few cytokines have so far been evaluated in the context of ovarian ageing.

Study design, size, duration: This is a prospective, descriptive, cross-sectional, monocentric study, carried out at the Angers University Hospital between November 2015 and March 2016. Seventy-one women (aged 23-42 years), undergoing in vitro fertilization with or without intracytoplasmic sperm injection in our ART center, were included. Patients were classified according to the results of tests of their ovarian reserve and their response to ovarian stimulation.

Participants/materials, setting, methods: Oocytes were isolated for evaluation and culture from the FF of 34 patients with DOR and 37 patients with a normal reserve undergoing controlled ovarian stimulation. The remaining FF samples collected from the same patient were pooled and centrifuged to remove residual cells and the supernatants were stored until further analysis. The FF levels of 27 cytokines and growth factors were determined using the Bio-Plex Pro Human Cytokine 27-plex Assay panel.

Main results and the role of chance: Thirteen patients with uncertain ovarian profiles were excluded from the analysis. Univariate analysis and log transformation showed significant differences in the FF levels of 4 cytokines between the two groups using. The concentration of the platelet-derived growth factor BB (PDGF-BB) (p = 0.005), CCL5/regulated on activation, normal T-cell expressed and secreted (RANTES) (p = 0.023]), CCL2/monocyte chemoattractant protein-1 (MCP-1) (p = 0.030) and interleukin-1 receptor antagonist (IL-1RA) (p = 0.041) were significantly lower in the DOR group compared with normal reserve. After correction of the α-risk, using the Benjamini-Hochberg procedure, the PDGF-BB level was the only cytokine concentration significantly lower in the DOR group (7.34 ± 16.11 pg/mL) compared with controls (24.39 ± 41.38 pg/mL). No significant differences were found in the FF levels of the other 23 cytokines.

Limitations, reasons for caution: This observational study in a single ART center can only indicate possible associations between ovarian aging and the concentration of cytokines. Further studies will be needed to confirm the results reported and establish a link between ovarian ageing and PDGF-BB concentrations in the FF.

Wider implications of the findings: PDGF-BB promotes the activation of the primordial follicle *via* the KIT-KIT ligand system while increasing the growth of the secondary follicle and the proliferation of theca cells. The alteration of these processes in DOR patients may help to elucidate the mechanisms underlying ovarian ageing and ameliorate its management.

Trial registration number: Not applicable.

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P-333 Two LH β-subunit polymorphisms are equally correlated with ovarian response in oocyte donors

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Study question: To establish points of association between gene polymorphisms of gonadotropins and their receptors and ovarian response to stimulation protocols

Summary answer: A genetic variant of LH β-subunit determines a suboptimal ovarian response following a controlled ovarian stimulation protocol.

What is known already: The unpredictable variability in the response to gonadotropins represents one of the most intractable problems of an assisted reproductive treatment, with response ranging from poor to high, leading to cycle cancellation or undesired complications like ovarian hyperstimulation syndrome. Gonadotropin's and their receptors' gene carry several single-nucleotide polymorphisms (SNPs) resulting in endocrine genotypes modulating reproductive parameters leading to important implications for reproductive success. The study of SNPs is an interesting field of research that provide us with new information about the way each woman respond to exogenous gonadotropin administration during ovulation induction

Study design, size, duration: 158 oocyte donors were subjected to ovarian stimulation protocol. After oocyte recovery, they were classified into three groups depending on the number of oocytes obtained: suboptimal response (≤ 6 oocytes), normal-response (7-15 oocytes) and high response (> 15 oocytes). Then we identified genetic polymorphisms associated with FSH receptor gene, the β-LH subunit, estrogen receptor, GDF-9 and BMP-15 for describing a suitable genetic profile that helps predict most effective way ovarian response

Participants/materials, setting, methods: Subjects were assigned to receive daily doses of 150 UI rFSH; from day 6 of stimulation onwards, 0.25 mg GnRH antagonist were administered and a single dose of GnRH agonist (0.2 mg) was administered for triggering final oocyte maturation. Oocyte pick-up was programmed 36 hours after inducing ovulation. Oral epithelial cells were taken using FTA cards; the further analysis of genetic polymorphisms was performed by PCR with Taqman probes on a device StepOne Plus

Main results and the role of chance: After oocyte pick-up, 12.2% of oocyte donors were classified as suboptimal responders, 35.8% had a normal ovarian response, and 51.0% were considered to have a high-ovarian response. Data showed no differences among the three groups for the female ages; according estradiol levels, we found statistical differences between suboptimal (1122 ± 1450 pg/nl) normal (2021 ± 1214 pg/ml) and high-responders (2600 ± 1443 pg/ml), p<0.001.

Genotype frequencies were in the Hardy-Weinberg equilibrium. Univariate analysis revealed that any polymorphism but LH β-subunit was associated with ovarian response. Data were as follows; FSHR T307A (p = 0,191); FSHR N680S (p = 0.189); LHB W8R (p = 0.019); LHB 115T (p = 0,019); ESR1 Pvu T/C (p = 0.840); ESR1 Xba A/G (p = 0.870); GDF9 546 G/A (p = 0,375); BMP15 -9 C/G (p = 0,498).

Finally, we observed that LH β-subunit polymorphisms, LHB W8R and LHB 115T, were segregated together since the percentage of women carrying the wild genotype, the heterozygous genotype and the homozygous variant of the polymorphism were identical in each category of ovarian response for both genetic variants.

Limitations, reasons for caution: This study has been performed in oocyte donors, which form a homogeneous group in terms of age and ovarian response, so these results may not be extrapolated to other groups of women undergoing an assisted reproductive treatment

Wider implications of the findings: Donors with these LH variants seem to be less efficient in order to sustain multiple follicle growth and oocyte maturation. This genetic condition may be related with the endogenous LH-surge generated after GnRH agonist administrations and should be evaluated in further studies

Trial registration number: Does not apply.

P-334 Does retrieved oocytes quantity correlate with quality in advanced maternal age?

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Study question: Does high ovarian response (>10 retrieved oocytes) in advanced maternal age (AMA) correlate with better reproductive outcome?

Summary answer: In patients of AMA (age 40-43 years) high ovarian response does not predict higher clinical pregnancy and live birth rates

What is known already: The association between the number of retrieved oocytes and better reproductive outcome is well recognised, although most studies which demonstrated this association were performed on younger patients. It is yet to be established whether high response to ovarian stimulation and the retrieval of a high number of oocytes in patients of AMA is also associated with better reproductive outcome.

Study design, size, duration: A retrospective cohort study performed at a single academic reproductive center between January 2012 and December 2016. All fresh ovarian stimulation cycles in AMA patients were included (n = 1657). Oocyte donation and fertility preservation cycles were excluded (n = 110, 6.6%). Oocytes collection was cancelled or no oocytes were retrieved in 81 cycles (4.8%). No embryos were available for transfer in 184 cycles (11%).

Participants/materials, setting, methods: There were 1284 fresh IVF-ET cycles in patients of AMA available for analysis. Cycles were defined as high ovarian response (HOR) if more than 10 oocytes were retrieved. Normal ovarian response (NOR) was defined as cycles in which less than 10 oocytes were retrieved.

Main results and the role of chance: There were 227 (17.6%) fresh IVF-ET cycles in which more than 10 oocytes were retrieved, and 1057 (82.4%) cycles with less than 10 retrieved oocytes. Mean patients age was comparable in both groups) 41.5 vs. 41.6 years, p = 0.1). The mean number of retrieved oocytes was significantly higher in the HOR group (13.5 vs. 4.2, p<0.01), as was the number of transferred embryos (2.7 vs. 2.1, p<0.01). Clinical pregnancy rate (CPR) was comparable between study groups (17.6% in HOR vs. 13.5% in NOR, p = 0.07). Live birth rate (LBR) was also comparable (8.4% vs. 7.4%, p = 0.34). In order to adjust for repeated failures, sub group analysis of patient's first fresh IVF-ET cycle was performed. Outcome of these cycles also revealed comparable CPR and LBR in HOR group and NOR group (16.2% vs. 15.1%, p = 0.45, and 9.1% vs. 7.6%, p = 0.37; respectively).

When vitrified-warmed ET cycles were included in the analysis, the cumulative CPR was significantly higher in the HOR group (17.9% vs. 13.7%, p = 0.03). However, LBR were comparable (9.1% vs. 7.4%, p = 0.18).

Limitations, reasons for caution: The main limitation of our study is its retrospective nature.

Wider implications of the findings: In patients of AMA high oocytes yield is not associated with better oocytes quality and improved cycle outcome. These patients' age may be the predominant factor in success rates. However, high ovarian response enables vitrification of surplus embryos which may increase the cumulative pregnancy and live birth rates.

Trial registration number: N/A.

P-335 Oral dydrogesterone vs. micronized vaginal progesterone as luteal phase support in IVF: a systematic review and meta-analysis

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Study question: In IVF patients undergoing fresh embryo-transfer, is the probability of clinical pregnancy and live birth associated with the use of oral dydrogesterone vs. micronized vaginal progesterone (MVP)?

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Summary answer: Oral dydrogesterone is associated with an improvement in live birth rate as compared to micronized vaginal progesterone as luteal phase support in IVF.

What is known already: Dydrogesterone is an established oral retroprogesterone approved for the treatment of miscarriage and luteal phase insufficiency and has been used for luteal phase support (LPS) in IVF. A recent Cochrane analysis on LPS (van der Linden *et al.*, 2015) indicated no significant differences in live birth and ongoing pregnancy rates of oral dydrogesterone as compared to MVP. Recently, a large phase III trial (LOTUS I; NCT01850030) has been completed, proving non-inferiority of oral dydrogesterone 30 mg daily to micronized vaginal progesterone (MVP) 600 mg daily for LPS in IVF.

Study design, size, duration: Multiple databases (Allied & Complementary Medicine™, Analytical Abstracts, BIOSIS Previews®, Embase®, Embase® Alert, EMCare®, International Pharmaceutical Abstracts, MEDLINE®, ToxFile®, CENTRAL, ICTRP, ISRCTN, PQD, SCOPUS and ClinicalTrial.gov) were searched for randomized, fully published studies comparing dydrogesterone with MVP for LPS in patients undergoing controlled ovarian stimulation and IVF with fresh embryo transfer (up to 31-Dec-2016). Meta-analyses were performed using standard procedures in the software program RevMan v.5.3. All analyses were done per randomized patient.

Participants/materials, setting, methods: Six randomized controlled trials could be included. The comparisons were 600 mg MVP vs. 20 mg dydrogesterone (two studies), 600 mg MVP vs. 30 mg dydrogesterone (two studies), 400 mg MVP vs. 40 mg dydrogesterone and 800 mg MVP vs. 40 mg dydrogesterone (one study each). Primary outcome is the live birth rate. When live birth was not reported in a study, clinical pregnancy rate was converted to live birth using the abortion rate estimate from the LOTUS I trial.

Main results and the role of chance: Clinical pregnancy rate per randomized patient was significantly higher in patients receiving dydrogesterone (relative risk [RR] 1.16, 95% confidence interval [CI]: 1.05 to 1.29; $p = 0.005$; $I^2 = 39\%$; six RCTs, n = 3,211 subjects; fixed-effects model). In those studies reporting live birth, the RR for live birth was 1.17 (95% CI: 0.98 to 1.38; $p = 0.08$; $I^2 = 0\%$; two RCTs; n = 1,461; fixed-effects model). In those studies reporting clinical pregnancy rates and live birth rates, the abortion rate beyond achievement of clinical pregnancy was highly similar between treatments and between studies. After estimating live birth rate from clinical pregnancy rate in four of the six studies, the estimated RR for live birth per randomized patient with dydrogesterone as LPS was 1.19 (95% CI: 1.06 to 1.33; p = 0.004; $I^2 = 34\%$; six RCTs, n = 3,211 subjects; fixed-effects model). An analysis per-protocol yielded similar results. The number-needed-to-treat to achieve one additional live birth is estimated as 20 (95% CI: 13 to 50). No differences in side-effects or adverse events were found.

Limitations, reasons for caution: There was heterogeneity in dosing of drugs. 5/6 studies were of only moderate methodological rigor with only 1/6 studies being double-dummy and placebo controlled. 4/6 studies reported side effects or adverse events, but only one study reported comprehensively. Only one study reported child health indicating no differences between treatments.

Wider implications of the findings: There is evidence of a small improvement of the chance of success with the use of dydrogesterone. Since oral intake is preferred over vaginal administration by most IVF patients, and since dydrogesterone is a comparatively cheap drug, it may replace MVP as the standard of care for luteal phase support.

Trial registration number: NA.

P-336 PREPARE trial: A randomised double blinded controlled trial of a preconception Omega 3 and Vitamin D rich dietary supplement in couples undergoing assisted reproduction treatment

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Study question: Does dietary supplementation with omega 3 polyunsaturated fats (EPA and DHA) and Vitamin D for six weeks prior to IVF or IVF-ICSI improve embryo quality?

Summary answer: The dietary intervention increased blood levels of EPA, DHA and vitamin D in men and women and this correlated with markers of improved embryo quality.

What is known already: Observational cohort studies have suggested links between preconception diet and fertility treatment outcomes. A prospective observational study demonstrated an association between dietary intake of polyunsaturated fats and better embryo morphology. Data are also now emerging indicating that dietary vitamin D intake may be related to embryo and fetus development. Such observations from non-intervention studies have driven the uptake of Vitamin D and Omega 3 supplements. However, prospective randomized controlled trials demonstrating that supplementation improves outcome are lacking.

Study design, size, duration: 111 couples were recruited to this double-blinded randomised placebo controlled trial. The 6 week intervention prior to oocyte retrieval consisted of a daily drink, containing 2 g of DHA plus EPA and 10micrograms of vitamin D, and olive oil and olive oil spreads, all in unmarked containers. The control group received a placebo drink and sunflower oil and spreads, again in unmarked containers. 55 couples were randomised to the treatment group and 56 to placebo.

Participants/materials, setting, methods: Couples, in whom dietary questionnaires showed no consumption of oily fish or high dose omega 3 supplements, were eligible. Following IVF, embryos were cultured in an Embryoscope and validated morphokinetic markers of embryo quality were recorded; day 3 and 5 KIDScores (Known Implantation Data Score) were calculated for individual embryos. Mean embryo scores per couple were analysed. Blood EPA, DHA and vitamin D levels were measured before and after the intervention period.

Main results and the role of chance: 102 couples completed the study and 750 embryos were available for analysis of morphokinetic markers. There were no differences in age, BMI, background diet, caffeine and alcohol intake or AMH between the two groups. Men and women randomised to the intervention arm demonstrated statistically significant increases in the level of DHA ($p<0.001$) and EPA ($p<0.001$) in red blood cells (RBC) and in vitamin D in blood serum ($p<0.001$). There were no statistically significant differences between the two groups in the number of oocytes collected, the number of embryos formed or the proportion of embryos reaching the blastocyst stage. While overall, no statistically significant differences in mean embryo scores (KIDScore D3 and D5) between the two groups were observed ($p = 0.074$ and $p = 0.178$), those receiving the intervention and IVF without ICSI produced embryos with a significant increase in KIDScore on D5 ($p = 0.036$) and an improvement in CC4 ($p = 0.003$). Furthermore, embryo quality positively correlated with the women's RBC DHA and EPA: KIDScore D3 with EPA (coefficient=0.243, $p = 0.018$) and DHA (coefficient=0.201, $p = 0.050$) and KIDScore D5 with DHA (coefficient=0.258, $p = 0.012$). CC4 and S4 demonstrated a negative correlation with women's RBC DHA ($p = 0.031$ for CC4, $p = 0.014$ for S4) and EPA ($p = 0.024$ for CC4, $p = 0.022$ for S4).

Limitations, reasons for caution: While the intervention was shown to alter blood levels of the target nutrients, indicating compliance, the period of intervention may have been too short to impact significantly on gamete quality. The study was powered to investigate current morphokinetic markers and not pregnancy rates.

Wider implications of the findings: This study demonstrates that blood levels of omega 3 fats and vitamin D are increased by a six week dietary intervention and that this can have a significant impact on embryo quality, possibly by improving sperm quality and function. Further studies are required to clarify the mechanisms of action.

Trial registration number: ISRCTN50956936.

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P-337 Is selective reduction necessary for twin pregnancies in women with a unicornuate uterus after in vitro fertilization-embryo transfer

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Study question: Is selective reduction necessary for twin pregnancies in women with a unicornuate uterus after in vitro fertilization-embryo transfer (IVF-ET)

Summary answer: Twin pregnancies in women with a unicornuate uterus conferred worse perinatal outcomes and selective reduction to singleton could obtain similar pregnancy outcomes with singleton pregnancy.

What is known already: Unicornuate uteri account for 5.0-13.0% of all Müllerian anomalies and are caused by non-development of one Müllerian duct. This condition has been related to miscarriage, premature delivery, low birth weight, perinatal mortality and other complications. Little research has been conducted on the correlations between the unicornuate uterine anomaly and the perinatal outcomes as well as the outcomes of selective reduction of twins after IVF-ET till now. To the best of our knowledge, this study is the largest series to describe unicornuate uteri following IVF treatment at a single reproductive center to date.

Study design, size, duration: A retrospective, single-center cohort study was conducted with 206 women with a unicornuate uterus who delivered greater than or equal to 20 weeks' gestation from January 2012 to December 2015 after IVF-ET treatment. In order to avoid selection bias, only the first pregnancy of each patient were considered. Miscarriage, spontaneous reduction of twins to singleton and triple pregnancy were excluded from analysis.

Participants/materials, setting, methods: This study was approved by the ethics committee of the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). Written informed consent was obtained from all participants. The included 206 women were divided into singleton (n = 139), selective reduction of twins to singleton (n = 21) and twin (n = 46) groups. The perinatal outcomes were compared among these 3 groups and the main outcome measures included preterm delivery, perinatal mortality, live birth rate, gestational weeks at delivery and birth weight.

Main results and the role of chance: Compared with the singleton group, the twin group showed significantly higher rates of premature delivery (67.4% vs. 18.0%, OR 3.07; $P < 0.001$) and perinatal mortality (21.7% vs. 0.7%, OR 38.33; $p < 0.001$), significantly lower live birth rate (80.4% vs. 99.3%, OR 0.03; $p < 0.001$), gestational weeks at delivery (33.7 ± 4.7 vs. 38.2 ± 2.0, $p < 0.001$) and birth weight (2071.7 ± 766.9 vs. 3082.1 ± 556.0 kg, $p < 0.001$); however, selective reduction group had obtained similar rates of preterm delivery (9.5% vs. 18.0%), perinatal mortality (0 vs. 0.7%) and live birth (100% vs. 99.3%), similar gestational weeks at delivery (38.3 ± 2.3 vs. 38.2 ± 2.0) and birth weight (2989.1 ± 484.3 vs. 3082.1 ± 556.0) ($p > 0.05$). Additionally, compared with the twin group, the selective reduction group showed markedly lower rates of preterm delivery (9.5 % vs. 67.4%, OR 19.63; $p < 0.001$) and perinatal mortality (0 vs. 21.7%, $p = 0.022$), significantly higher live birth rate (100% vs. 80.4%, $p = 0.048$), gestational week at delivery (38.3 ± 2.3 vs. 33.7 ± 4.7, $p < 0.001$) and birth weight (2989.1 ± 484.3 vs. 2071.7 ± 766.9, $p < 0.001$).

Limitations, reasons for caution: All pregnancy outcomes were obtained via telephone calls or faxes, thus, some detailed obstetric complications, such as an abnormal fetal position or placenta previa, were not studied. Secondly, the results were confined to pregnancies greater than 20 gestational weeks and pregnancies with lower gestational weeks were not analyzed.

Wider implications of the findings: Our findings can be used to counsel women whose twin pregnancies are complicated by a unicornuate anomaly and help guide appropriate selective reduction, antenatal treatment and surveillance.

Trial registration number: None.

P-338 anti-adhesive effects of the newly developed two-layered gelatin sheet in dogs

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Study question: Does the two-layered gelatin sheet which we newly developed prevent the cauterized uterus adhesion more effectively than the conventional anti-adhesive materials?

Summary answer: The two-layered gelatin sheet had significantly superior anti-adhesive effects to the conventional anti-adhesive materials

What is known already: Hyaluronic acid carboxymethyl cellulose membrane (HA/CMC) is frequently used as a conventional anti-adhesive material clinically but the anti-adhesive effect of HA/CMC is controversial, concretely speaking it has reported to decrease severity of adhesion but not incidence in human and to have no effects to prevent pelvic adhesion in woman. Oxidized regenerated cellulose (TC7) is also a conventional material, however TC7 is only available to the non-bleeding site and provoke a large leucocyte response, which result in inducing adhesion.

Study design, size, duration: We have developed the two-layered gelatin sheet composed of gelatin film and gelatin sponge. Human fibroblasts and mesothelial cells were cultured on the materials for a week and the cell growth was evaluated. The dogs were randomly assigned to 4 groups: 1) control group, 2) two-layered gelatin sheet group, 3) HA/CMC group, and 4) TC7 group. The macroscopic and microscopic findings were evaluated on 3 and 6 weeks postoperatively.

Participants/materials, setting, methods: The viable cell number in each well was counted with the ATP assay. One side of the uterus horns was cauterized 40 mm long circumferentially with electric scalpel. Adhesions were scored with a grading scale macroscopically. The cauterized site of the uterus were stained with hematoxylin-eosin (HE) and investigated histologically.

Main results and the role of chance: Cell growth of human fibroblasts and mesothelial cells were proliferated significantly on the two-layered gelatin sheet compared with the HA/CMC and TC7. The score in the two-layered gelatin sheet group was significantly lower than those in the HA/CMC and TC7 groups macroscopically. The degree of lymphocyte infiltration in the two-layered gelatin sheet group was sparse compared with those in the HA/CMC and TC7 group within 6 weeks. A single-cell layer of matured mesothelium was formed in the two-layered gelatin sheet group, however peritoneal regeneration in the HA/CMC and TC7 groups were incomplete and delayed. Inflammation around the each anti-adhesive material was the weakest in the two-layered gelatin sheet group. The anti-adhesive effects of two-layered gelatin sheet is superior to the conventional materials in the canine cauterized uterus model. Early regeneration of the peritoneum, weak inflammation, and longtime remain of the material contributes these results. The two-layered gelatin sheet is considered to be a useful anti-adhesive material in deeply injured and hemorrhagic sites.

Limitations, reasons for caution: The results are not easy to apply to human, because the structure of canine uteri are different from those of human.

Wider implications of the findings: The two-layered gelatin sheet is easier to use than the HA/CMC and TC7 in deeply injured and hemorrhagic sites or organ, because the sheet is able to be bent but not brittle.

Trial registration number: N/A.

P-339 Short term intervention with liraglutide and metformin increased fertility potential in a subset of obese PCOS proceeding IVF

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Study question: Does short-term weight reduction more than 5% with metformin and liraglutide (COMBI) improve oocyte maturity and embryo quality in obese PCOS?

Summary answer: Weight loss with innovative anti-obesity therapy (COMBI) resulted in the highest percentage of mature oocytes and the highest blastocysts/patient rate.

What is known already: The maternal metabolic environment in obesity has a negative influence on oocyte development with more immature oocytes and lower fertilisation rate. Embryos reach the morula stage faster and the blastocysts contain fewer cells in the trophectoderm. A decreased blastocyst formation rate likely significantlly contributes to poorer reproductive outcome.

Few is known about the impact of weight reduction prior IVF and no information on impact of glucagon-like peptid (GLP-1) liraglutide in obese infertile PCOS. Research in rats suggested that GLP-1 plays a role in regulating the reproductive system by linking energy input to the short-term adjustment of the hypothalamic pituitary gonadal axis.

Study design, size, duration: A 12-week prospective randomized open-label study was conducted with 40 infertile obese PCOS patients. They were assigned to 3 groups: i) metformin (MET) (MET 1000 mg BID), ii) COMBI (MET 1000 mg BID and low dose liraglutide 1.2 mg s.c.) and iii) CON (control)group with no therapy. CON and patients from MET and COMBI who were good responders to treatment and lost at least 5% of the initial body weight proceeded with IVF.

Participants/materials, setting, methods: All the patients initially underwent the following evaluations: height, weight, whole-body composition with Hologic dual energy X-ray absorptiometry. At week 4, 8 and 12 measurements were repeated in MET and COMBI group. Short antagonist cetrorelix protocol (225 IU of follicle stimulating hormone QD s.c., 0.25 mg cetrorelix acetate QD s.c.≥ dominant follicle was 14 mm) was used for ovarian stimulation in all the patients.A buserelin acetate 0,6 mg s.c was used for oocyte maturation.

Main results and the role of chance: 11 women in MET, 13 in COMBI and 11 in CON group completed the study according to the protocol. Patients in MET lost on average 6,70 ± 6,70 kg (P<0.001) compared with 7,68 ± 3,74 kg loss in COMBI group (P<0.001), COMBI not being superior to MET (P = 0,103). COMBI group resulted in a significant reduction of visceral adipose tissue (VAT) area (-20,65 ± 7,40 cm2; P = 0,028). More than 5% of weight reduction was achieved in 76,9% of patients in COMBI group and 45,5% of patients in MET group. In high responders who lost more than 5% of initial body weight, numbers of blastocysts/patient were greater in both treatment arms than in controls (3,67 ± 4,82 in COMBI; 3,60 ± 6,95 in MET; vs 2,09 ± 2,07 in CON). High responders in COMBI also had the highest number of oocytes/patient 14,67 ± 9,59 and the highest percentage of mature oocytes 11,22 ± 9,27 among all three groups, although treatment differences were not statistically significant yet. Furthermore one patient in COMBI group become spontaneously pregnant before IVF and two become spontaneously pregnant after unsuccessful IVF. There were no spontaneous pregnancies in MET and CON groups

Limitations, reasons for caution: The most important limitation is in the size of the study with small number of participants.

Wider implications of the findings: This is the first study of anti-obesity treatment with GLP-1analog in specific infertile obese PCOS population. It shows promising results in weight loss and suggests the possible effect of weight loss on oocyte and embryo development. It should be tested also for improvement of ovulation frequencies and eventual spontaneous pregnancies.

Trial registration number: Clinical Trial: NCT03034941.

P-340 prognostic factors for the success of oocyte thawing

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Study question: What biological and biochemical factors can be considered predictive for oocyte survival, fertilization and for obtaining a pregnancy after oocyte thawing cycles?

Summary answer: None of the considered factors in fresh cycles is able to predict the trend of the oocyte thawing cycle.

What is known already: Currently the morphological selection of oocyte before cryopreservation is important to ensure a good survival rate, a development of good quality embryos and to achieve pregnancy in thawing cycles. There are several factors that affect oocyte quality, including the physiological and psychophysical state of the patient and the hormonal stimulation protocol to which she is subjected. Nowadays there are no data about what could predict whether a patient who undergoes In Vitro Fertilization (IVF) cycles can be considered a good candidate for oocyte freezing.

Study design, size, duration: This study was conducted on a total of 237 patients aged between 22 and 42 years scheduled for an IVF cycle at the Centro PMA of ASST Ospedale Papa Giovanni XXII between January 2012 and December 2015. Patients that cryopreserved supernumerary oocytes and subsequently were subjected to the oocyte thaw cycles were included in the study.

Participants/materials, setting, methods: Oocytes were cryopreserved using vitrification. For each patient we collected: age, Antimöllerian Hormone (AMH) level, Body Mass Index (BMI), Androstenedione, Testosterone, estrogen, stimulation protocol, ratio between estrogen and number of recovered oocytes. After oocytes thawing we evaluated these possible confounding factors: thawing embryologist, progressive spermatozoa motility, number of spermatozoa after capacitation, embryo quality and number of transferred embryos. All parameters have been compared with oocyte survival, fertilization, total and on-going pregnancy in oocytes thawing cycle.

Main results and the role of chance: None of the considered factors can be predictive of oocyte survival after thawing.

About fertilization rate we observed that a BMI >30 kg/m^2 has a negative affect: only 20% of patients had a fertilization rate more than 80%. Moreover, the number of patients that had a fertilization rate higher than 80% was superior in the group of tubal infertility. These results are influenced by progressive sperm motility and concentration, and the thawing operator. The subsequence multivariate analysis of these data shows that none of these factors influence the fertilization rate.

Considering the pregnancy rate after oocytes thawing, it was not influenced by any of the factors in fresh cycles but it was higher when we transferred more than one embryo and at least one top quality embryo. In the subsequent multivariate analysis we included the data showing a P < 0.1: stimolation with GnRH antagonist, BMI 18-25 kg/m^2 and BMI< 18 kg/m^2 to obtain positive βhCG; stimolation with GnRH antagonist and BMI 18-25 kg/m^2 to obtain of an evolutionary pregnancy.

This analysis demonstrates that none of the pre-freezing factors has a statistically significant impact on the pregnancy rate. Only the transfer of at least one grade 1 embryo influenced the outcome.

Limitations, reasons for caution: Patients having cryopreserved oocytes for fertility preservation or that didn't accept the cryopreservation of supernumerary embryos have been excluded from this study.

Wider implications of the findings: Further studies and a wider group of patient would be necessary to establish if the considered factors can influence the negative outcome of the thawing cycles in terms of failed oocytes survival, fertilization and embryo cleavage.

Trial registration number: Not applicable.

P-341 Vitamin D can inhibit proliferation of uterine fibroid cells in vitro through the Wnt/β-Catenin signalling pathway

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Study question: Does Vitamin D act to inhibit uterine fibroid growth in vitro through the Wnt/β-Catenin signalling pathway?

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Summary answer: Vitamin D inhibits in vitro proliferation rates in uterine fibroid cells that express vitamin D receptor through Wnt/β-Catenin signalling pathway.

What is known already: Uterine fibroids (UF) are the most common benign tumour in women during the reproductive years. Several studies demonstrated that gonadotropin-releasing hormone (GnRH) activates the Wnt/β-catenin signaling pathway promoting the proliferation and differentiation of UF. Although the main treatment for UF is surgery, inhibition of estrogen and progesterone by GnRH agonists (GnRHa) is also widely used. However, GnRHa administration cannot be continued more than 6 months due to adverse effects. As an alternative, Vitamin D (VitD), with its well-described role inhibiting Wnt/β-catenin signalling in cancer cells, has been proposed as a long-term treatment option for UF.

Study design, size, duration: This study was approved by the Clinical Research Ethics Committee at the Hospital Univeristario y Politécnico La Fe de Valencia (Spain) (2014/0691). Our pilot study was carried out in the Hospital Univeristario y Politécnico La Fe de Valencia and Fundación Instituto Valenciano de Infertilidad (FIVI). Intramural UF were collected from patients undergoing surgery due to UF pathology (n = 14) without any previous treatment. All participants provided written informed consent.

Participants/materials, setting, methods: UF tissue (n = 14) was analysed by Western blot to determine the presence of Vitamin D receptor (VDR) and WISP1 (Wnt1 inducible signalling pathway protein 1) as well as proliferation (PCNA; proliferating cell nuclear antigen) at protein level. UF was disaggregated with collagenase-II and cells were cultured with and without 1,25-dihydroxyvitamin D3 (VitD, 100 mM) treatment. Subsequently, in vitro UF cell proliferation was measured by flow cytometry using propidium iodide.

Main results and the role of chance: We observed that 92.8% of UF tissue showed greater proliferation than its corresponding myometrium. In addition, not all UF tissues presented the VDR. Interestingly, however, the 80% of UF with VDR also overexpressed WISP1, suggesting a correlation between the Wnt/β-Catenin signalling pathway and VDR in this pathology. In addition, following in vitro treatment with VitD, 75% of UF cells with VDR had inhibited proliferation. These findings suggest that VitD could inhibit UF proliferation through its receptor and via the Wnt/β-Catenin signalling pathway.

Limitations, reasons for caution: Limitations of this pilot study include the small sample size.

Wider implications of the findings: UF tissues show greater proliferation in comparison with myometrium. However, not all UF have altered Wnt/β-Catenin pathway. This study demonstrated that UF expressing VDR have upregulated Wnt/β-Catenin signalling. Moreover, treatment with VitD in UF cells expressing VDR decreases cell proliferation, indicating the potential role of this receptor in UF pathology.

Trial registration number: No clinical trial.

P-342 Do not discard! Significant live birth rates from immature, denuded, non-GV oocytes collected after conventional controlled ovarian stimulation

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Study question: Do denuded, immature oocytes (non-GV) collected after conventional ovarian stimulation give rise to live births after maturing in vitro?

Summary answer: Denuded immature oocytes (non-GV) that completed meiosis in vitro (IVMC) gave rise to 5% of live birth in transfers where all embryos proceed from IVMC

What is known already: On average, 15% of all oocytes collected in conventional IVF cycles do not reach the MII stage at the time of the ovum pick up (OPU). Having undergone the LH surge, however, their cumulus is most often expanded, and cumulus-oocyte connections irremediably severed. These immature, non-GV oocytes are therefore often discarded, especially when a sufficient number of MII oocytes are also retrieved. There is scarce information available in the literature on either the most appropriate maturation protocol for immature oocytes, or the reproductive potential of embryo generated from IVMC oocytes.

Study design, size, duration: Retrospective study including 4,022 IVF cycles from 3779 patients between January 2011 and December 2015; 4450 immature non-GV oocytes were collected from 1440 of these cycles and matured in vitro; 2365 reached the MII stage (IVMC) and were inseminated. Overall, 3,933 ETs were performed: 3,579 included embryos from MII oocytes; 264 included embryos derived from MII + IVMC oocytes, and 90 with embryos from IVMC only; 399 IVMC embryos in total were transferred fresh.

Participants/materials, setting, methods: Immature, non-GV oocytes retrieved at OPU strictly 36 h after the ovulation trigger were matured in vitro during 2-8 h, in either medium IVF™PLUS (n = 671 cycles; 2421 oocytes) or G-2™PLUS (n = 769 cycles; 2029 oocytes). Maturations were all confirmed visually by the extrusion of the first PB. A strict control of laboratory times was guaranteed by an automated electromagnetic tracking system (RI Witness® System). Mature oocytes were inseminated with either partner or donor sperm.

Main results and the role of chance: Overall, the maturation rate for immature, non-GV oocytes was 53.1%; importantly, significantly higher maturation rate was registered when using G-2™PLUS (1304/2029; 64.7%) rather than G-IVF™PLUS (1061/2421; 43.8%; p<0.001). Fertilization rate of the IVMC group was 64.3% (71% in MII group, p<0.001). Transferred embryos from IVMC oocytes had an average score of 6.8 out of 10 (7.7 for MII group; p<0.001). Regarding reproductive outcomes, the IVMC group gave overall 11.1%, 10.0%, 7.8% and 5.6% biochemical, clinical, ongoing pregnancy and live birth rates, respectively, versus 31.8%, 24.6%, 18.6% and 15.3% in the MII +IVMC group and 36.9%, 30.1%, 24.4%, 18.9% in the MII group. Importantly, single embryo transfer was the mostly transfer within IVMC only group (72.2%), while transfer of 2 embryos was more common among the MII group (61.0%). Interestingly, IVMC matured in G2 medium seemed to give a higher clinical pregnancy rates when transferred (12.1% vs. 6.3%, p>0.05).

Limitations, reasons for caution: Most embryo transfers that included IVMC derived embryos were mixed transfers, making it difficult to identify the full capacity of IVMC oocytes to give live births. Nevertheless, data from 90 transfers with embryos strictly derived from IVMC oocytes are encouraging, and might even underestimate the developmental potential of these embryos.

Wider implications of the findings: The maturation in vitro of denuded, non-GV immature oocytes should be performed at the very least when few MII are collected, and likely in all patients, as there is an acceptable maturation rate and a significant potential to give rise to live birth from these oocytes.

Trial registration number: NA.

P-343 Transcriptomic signatures in follicular cells associated with failure of in vitro fertilization in humans

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Study question: Can transcriptomic analysis of human follicular cells from failed in vitro fertilization (IVF) cycles reveal potential reasons for failure?

Summary answer: These findings provide potential biomarkers to identify the profile of different types of failure where proper control of inflammation appears to be a key element.

What is known already: Hormonal stimulation prior to IVF influences the ovarian environment and therefore impacts oocytes and subsequent embryo quality. The response to hormonal stimulation is highly variable among patients, but there is currently no way to characterize the patient's response and to know in which way its oocytes were affected by the treatment. Few studies have analysed what went wrong in failed IVF cycles. Having such information would allow clinicians to adapt and personalize the treatment, which would potentially improve success rate.

Study design, size, duration: Follicular cells principally composed of granulosa cells were obtained from a total of 200 consenting patients undergoing IVF

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treatment in 4 Canadian fertility clinics. Samples were analyzed according to the outcome of the IVF cycle. Positive group refers to patients for which pregnancy (heart beat) was confirmed and negative group refers to the failed IVF cycles (no pregnancy after the used of all embryos from the stimulation cycle).

Participants/materials, setting, methods: Using microarray, 32 samples (16 negative group vs 16 positive group) were compared to determine how gene expression is affected in follicles from failed cycles. Functional analysis of the differentially expressed genes was performed using ingenuity pathway analysis. In a larger cohort of patients (n = 97), quantitative RT-PCR was used to validate the microarray results and to analyze potential markers of failure. Hierarchical clustering was then used to segregate the negative patients based on gene expression.

Main results and the role of chance: A total of 165 genes were differently expressed ($P < 0.05$, fold change >1.5) in the negative group compared to the pregnancy group, including many pro-inflammatory cytokines or other factors related to inflammation. Several factors, some of which act upstream from vascular endothelial growth factor (VEGF), were also overexpressed in the non-pregnant group. The functional analysis highlighted the importance of the immune system and inflammatory reactions and showed an imbalance between pro-inflammatory and anti-inflammatory mediators which may account for the failure to conceive following ovarian stimulation. In addition, other differentially expressed genes appear related to abnormal differentiation and increased apoptosis. After hierarchical clustering, 3 distinct subgroups were identified and characterized according to the genes changes associated with these groups. This analysis resulted in a panel of 3 different diagnostic outcomes: 1) Follicles still in the growing mode; 2) hyper inflammation response; 3) follicular heterogeneity including overgrown follicles.

Limitations, reasons for caution: These analyses were performed in a single cohort of patients coming from 4 Canadian IVF clinics. Further analyses are needed to select the best biomarkers which will require further validation in a larger set of patients to ensure their global applicability.

Wider implications of the findings: Our results suggest that failure to conceive following ovarian stimulation could be associated with an imbalance between pro-inflammatory and anti-inflammatory mediators or by the abnormal differentiation profile of the pool of follicles. The findings point to potential biomarkers of failure causes and thereby suggest means of adapting subsequent stimulation protocols.

Trial registration number: This project was approved by Université Laval REB (2014-102/08-09-2014) for the collection and use of human tissues.

P-344 Andrographolide disrupts meiotic maturation by blocking cytoskeletal reorganization and decreases the fertilization potential of mouse oocytes

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Study question: The study aimed to explore the potential mechanism by which Andrographolide may cause infertility in females.

Summary answer: Andrographolide disrupts mouse oocyte meiotic maturation by blocking cytoskeletal reorganization and decreases the fertilization potential, which has an adverse effect on female fertility.

What is known already: Andrographolide(AG), a natural diterpenoid lactone isolated from Andrographis paniculata, has been widely used in Asian countries like China, India, and Thailand for its anti-inflammatory, antibacterial, antiviral, and immunostimulatory properties. Several animal studies have reported a negative effect of AG on male fertility. However, evidence regarding the effects of AG on female fertility is limited and inconsistent. Although an early study found no change in fertility in female mice fed a diet supplemented with AG, others reported poor pregnancy outcome of mice received AG. The mechanisms underlying the effects of AG on female reproduction have not been investigated to date.

Study design, size, duration: Germinal vesicle stage mouse oocytes were matured in vitro in the presence or absence of AG at various concentrations and for different periods, after which germinal vesicle breakdown (GVBD) and metaphase II (MII) rates were assessed. Furthermore, the effects on spindle formation and actin polarization of the AG-treated oocytes were analyzed by immunostaining. Finally, we examined the apoptosis rates and fertilization potential of the oocytes treated with AG.

Participants/materials, setting, methods: Immature germinal vesicle stage oocytes from female Kun Ming Bai mice were incubated in KSOM medium containing 0, 5, 10 or 20 μM AG and assessed the oocytes maturation at 6, 14, or 24 h of incubatuion. The spindle morphology and actin formation were examined by Immunofluorescence staining of β-tubulin and phalloidin. Apoptosis was detect by TUNEL assay. intracytoplasmic injection of sperm was performed to analyze the fertilization rate.

Main results and the role of chance: Immature oocytes incubated for 6, 14, or 24 h in medium containing 5, 10, or 20 μM AG showed time- and dose-dependent decreases in maturation rates as compared to the control group. Immunostaining revealed that AG exposure disrupted spindle organization and migration as well as actin cap formation and cytokinesis. Furthermore, most oocytes exposed to 20 μM AG underwent apoptosis when compared to control group, and the few oocytes exposed to 5 or 10 μM AG reaching metaphase II exhibited lower fertilization rates than that of control group after intracytoplasmic sperm injection. Our results suggest that AG may disrupt mouse oocyte meiotic maturation by blocking cytoskeletal reorganization and decreases the fertilization potential, which may thus have an adverse effect on female fertility.

Limitations, reasons for caution: The effects of AG on oocyte during in vivo maturation were not assessed. Only mouse oocytes were analyzed. Further studies using in vivo mouse experiments as well as clinical trials in humans to examine the drug safety are required.

Wider implications of the findings: This study firstly presented an important and uninvestigated aspect of the potential adverse effects of AG on oocyte maturation, providing a plausible explanation for the reported negative effects of AG on female fertility in mice. Based upon our findings, AG should be prudently consumed by women attempting to achieve conception.

Trial registration number: The trial registration number is not required for this study.

P-345 New trial of dydrogesterone regimen as an effective oral alternative for suppression of premature luteinizing hormone surges during controlled ovarian stimulation of assisted reproductive therapy

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Study question: Can a dydrogesteron with human menopausal gonadotropin (HMG) protocol suppress premature LH surges in women undergoing controlled ovarian stimulation (COS) for IVF/ICSI?

Summary answer: The dydrogesterone with HMG protocol can be effective for suppression of premature LH surges and this protocol is not inferior to GnRH antagonist protocol.

What is known already: It is widely known that GnRH antagonist is effective for rapid and reversible LH suppression to prevent premature LH surge during COS. However, the GnRH antagonist protocol is expensive and some patients experience premature LH surges. Previous studies demonstrated that medroxyprogesterone acetate suppressed premature LH surges during COS, and ovarian stimulation during luteal-phase with high progesterone level doesn't have a negative impact on oocyte/ embryo quality. Dydrogesterone, which has been widely used for luteal insufficiency, is structurally and pharmacologically similar to natural progesterone and lacks androgenic activity. It has good oral bioavailability and few side effects, compared with other progestins.

Study design, size, duration: This was a prospective observation study in 271 women (aged <41, AMH level >1.0) undergoing COS for IVF/ICSI at our private infertility clinic between July 2016 and December 2016. The patients

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were allocated alternately into two groups, one with the dydrogesterone and HMG protocol (study group, n = 145) and the other with the GnRH antagonist protocol (control group, n = 126).

Participants/materials, setting, methods: In the study group, dydrogesterone (20 mg/day) with HMG (150-225IU) was administered simultaneously beginning on day 2 or 3. In the control group, a GnRH antagonist protocol was given. In both groups, ovulation was triggered with GnRH agonist and HCG (1000-2500IU), when the leading follicle matured. All high quality embryos were cryopreserved for later transfer. Statistical analysis of the outcomes of cycles was performed using the unpaired t-test, Mann-Whitney U test and Chi-square test.

Main results and the role of chance: Patient characteristics: the age, body mass index, basal hormone profile, duration of infertility, and AMH level were similar in two groups. No statistically difference was found between the study group and the control group for the number of oocytes retrieved (10.23 ± 6.94 vs. 10.13 ± 6.03), the number of mature oocytes (8.1 ± 5.8 vs. 7.8 ± 4.8), the rate of fertilization (76.7% vs. 77.7%) and the viable embryos rate (66.0% vs. 66.8%). The study group received slightly higher HMG doze than the control group (2039.7 ± 833.6 IU vs. 1591.3 ± 695.2 IU, P < 0.001), but the duration of COS is statistically similar between the study group and the control group (14.9 ± 2.5 vs. 14.3 ± 1.9, P = 0.064). Premature LH surge occurred in each one patient of both groups, but they continued to perform luteal-phase ovarian stimulation and several follicles were retrieved from these patients. During the following up of frozen embryo transfer (FET), total 166 FET cycles were completed and no significant difference was seen between the study group and the control group for the clinical pregnancy rate (50.8% vs. 49.5%), implantation rate (50.7% vs. 48.6%), and on going pregnancy rate (43.1% vs. 42.6%) and early pregnancy loss rate (15.2% vs. 14.0%).

Limitations, reasons for caution: This study was not a randomized control study and was performed using women with normal ovarian reserves. For further research, live-birth outcomes following this protocol are needed.

Wider implications of the findings: Dydrogesterone has a more tolerability profile than other progestins and the advantages of oral administration, user convenience and cost reduction. This first study showed that the dydrogesterone with HMG protocol could be a new regimen as an oral alternative for prevention of premature LH surges during COS.

Trial registration number: Not applicable.

P-346 Effect of vitamin D supplementation on preconception immune tolerance in infertile women

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Study question: What is the relationship between vitamin D (VD) level and T-helper (Th) cells in infertile women as well as the effect of VD supplementation on maternal immune tolerance?

Summary answer: Most of the infertile women were deficient of VD. VD supplementation in infertile women with VD deficiency decreased Th1 cells associated with immune rejection.

What is known already: Th1 and Th2 cells play significant roles in immune rejection and tolerance. Pregnancy is Th2 dominance, and Th1 immune response is related to embryonic rejection. VD has been reported to play an important role in the modulation of the immune system through inhibition of Th1 cell proliferation and Th2 cell promotion. VD deficiency is associated with implantation failure, recurrent miscarriage and pregnancy complications, such as preeclampsia and gestational diabetes mellitus. However, the relationship between VD level and Th cells and the effect of preconception VD supplementation on the immune tolerance of infertile women with low VD are not clearly understood.

Study design, size, duration: This study was approved by the Local Ethics Committee. 25-hydroxyvitamin D (storage form) and Th1/Th2 cell levels were measured in infertile women from 2014 to 2016. We evaluated the alteration of immune levels during 3 months of VD supplementation (1,000IU/d) in women with VD deficiency.

Participants/materials, setting, methods: Out of 585 infertile women who underwent immunological examination, we excluded 309 patients with a history of recurrent miscarriage or repeated implantation failure due to the possibility of immune abnormality. In 276 generally infertile patients, we categorised VD deficiency(< 20 ng/ml), insufficiency(21–30 ng/ml) and sufficiency (>30 ng/ml) and compared Th1($CD4^+$/IFN-γ^+) and Th2($CD4^+$/IL-4^+) cell levels and Th1/Th2 cell ratio among the 3 groups. We additionally performed a detailed immunological test pre- and post-VD supplementation in 8 patients with VD deficiency.

Main results and the role of chance: The numbers of patients with VD deficiency, insufficiency and sufficiency were 130 (47.1%), 111 (40.2%) and 35 (12.7%), respectively. The infertile women have an extremely high proportion of low VD level. In addition, the infertile women with VD deficiency, insufficiency and sufficiency had 21.0 ± 6.8%, 21.0 ± 6.7% and 19.6 ± 5.6% Th1 cell levels, 2.3 ± 0.8%, 2.4 ± 1.2% and 2.5 ± 1.2% Th2 cell levels and 10.5 ± 5.5, 10.6 ± 6.0 and 8.6 ± 4.5 Th1/Th2 cell ratios, respectively. In the VD-sufficient group, there were relatively lower Th1 cells and Th1/Th2 cell ratio, but there were no significant differences among the 3 groups. In the patients who were supplemented with VD for VD deficiency, levels of Th1 cells were significantly decreased from 21.9 ± 6.2% to 19.6 ± 5.0% post-VD supplementation (p = 0.008). However, no significant changes in levels of Th2 cells, Th1/Th2 cell ratio, regulatory T cells and Th17 cells were detected in the patients with VD deficiency. No complications or adverse effects were identified in all patients.

Limitations, reasons for caution: VD is affected by exposure to sunlight. Therefore, the limitation of this study is that VD levels may be varied according to seasonal changes.

Wider implications of the findings: Appropriate Th1/Th2 cell balance is important during implantation and maintenance of pregnancy. An aberrantly high population of Th1 cells is associated with embryonic rejection, leading to repeated reproductive failure and complications during pregnancy. Preconception VD supplementation in women with VD deficiency may sufficiently suppress maternal immune rejection against the embryo.

Trial registration number: None.

P-347 In vitro fertilization outcomes in relation to adherence to the Mediterranean diet among women from a fertility clinic

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Study question: Is adherence to the Mediterranean diet (MedDiet) associated with better in vitro fertilization (IVF) performance in women attempting fertility?

Summary answer: Higher adherence to the MedDiet was associated with higher odds of achieving clinical pregnancy and live birth among women undergoing infertility treatment.

What is known already: A-posteriori dietary pattern approaches have revealed that dietary patterns with some of the characteristics of the MedDiet (i.e. high intakes of fruits, vegetables, legumes, whole grains, fish and low intake of meat) are associated with increased chances of pregnancy in couples undergoing IVF treatments. Yet, whether adherence to the MedDiet is associated with better reproductive performance of women attempting fertility remains largely unexplored.

Study design, size, duration: The study population comprised a total of 244 women who underwent a first IVF treatment in an Assisted Conception Unit in Athens, Greece, between June 2013 and September 2016. The study was designed to evaluate the influence of habitual dietary intake and lifestyle on fertility outcomes.

Participants/materials, setting, methods: Women aged 22-41 years (24.2% overweight) with complete dietary data were analyzed. Diet was assessed before the IVF treatment via a food-frequency questionnaire and adherence to the MedDiet through the validated MedDietScore (range: 0–55;

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higher scores indicating greater adherence to MedDiet). Study outcomes included oocyte yield, fertilization rates, embryo quality measures and clinical outcomes (implantation, clinical pregnancy and live birth rates). Associations were tested using multiple logistic regression controlling for various potential confounders.

Main results and the role of chance: Overall, 141 women (61.8%) had a successful implantation (elevation of b-hCG levels above 20 IU/l after embryo transfer), 104 women (45.6%) had intrauterine pregnancy confirmed by ultrasound, first performed at 6 weeks of gestation, and 98 women (43.2%) had a live birth. No association was found between MedDietScore and any of the intermediate IVF outcomes. However, a statistically significant association was observed between MedDietScore and clinical pregnancy and live birth rates ($p < 0.05$). Compared to women in the lowest tertile of the MedDietScore (≤30, N = 71), those in the highest tertile of the score (≥36, N = 83) exhibited higher clinical pregnancy rates (51.8 vs. 32.4%, p = 0.03) and corresponding live birth rates (49.4% vs. 29.6%, p = 0.02). In the multivariable adjusted models, women in the highest tertile of the MedDietScore had 2.2 times higher likelihood of having a clinical pregnancy (p for trend =0.03) compared to women in the lowest tertile of the score, and 2.5 times higher likelihood of achieving a live birth (p for trend =0.02), after adjusting for age, BMI, ovarian stimulation protocol, smoking, physical activity, anxiety levels, total energy intake, and cause of infertility.

Limitations, reasons for caution: The main limitation of the study stems from to its cross-sectional nature, so causal inference is limited. Moreover, results may not be extrapolated to women without a history of infertility.

Wider implications of the findings: The results suggest that diet modifications and adherence to the MedDiet may help increase the chance of achieving a clinical pregnancy and a live birth in women attempting fertility.

Trial registration number: Not applicable.

P-348 Increased peripheral blood natural killer cell fraction is associated with lower level of anti-Müllerian hormone

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Study question: Is natural killer (NK) cell fraction associated with ovarian reserve in reproductive-age women?

Summary answer: There was an inverse relationship between peripheral blood NK cell fraction and measured-to-reference anti-Müllerian hormone (AMH) ratio.

What is known already: High peripheral blood NK cell percentage is associated with adverse fertility and pregnancy outcomes such as recurrent pregnancy loss (RPL) and repeated implantation failure (RIF). Recent studies suggest a potential cytotoxic effect of NK cells on ovarian tumors. Yet, current knowledge of the effect of NK cell fraction on ovarian reserve in reproductive-age women is limited.

Study design, size, duration: This was a retrospective cohort study using data of women who were tested for NK cell activity and AMH at a university-affiliated fertility center from 2013 to 2015. Women with a history of bilateral/unilateral oophorectomy or chemotherapy and ≥ 1 year interval between NK cell and AMH tests were excluded. A total of 667 women who were tested for NK cell fraction in total lymphocytes and AMH were identified.

Participants/materials, setting, methods: Peripheral blood levels of NK cells were determined using flow cytometry and analyzed as a percentage of lymphocytes (%). To consider women's age, measured-to-(age-appropriate) reference AMH ratio ($AMH_{measured}/AMH_{age\text{-}appropriate\ reference}$) was used for the analysis. Age-appropriate reference level of AMH was calculated from a previously described quadratic model ($logAMH = -1.442 + 0.225 \times age - 0.004 \times age^2$). The association between NK cell activity and measured-to-reference AMH ratio was explored with Spearman correlation test.

Main results and the role of chance: The mean age of study population was 36.4 years. 37.2 % had histories of PRL and 59.7% experienced RIF. Mean level of peripheral blood NK cell fraction was 14.14 % (range: 2.50 - 41.60 %) and mean AMH was 3.51 ng/mL (range: 0.14 – 19.32 ng/mL). There was an inverse relationship between peripheral blood NK cell fraction and serum AMH (rho = -0.11, P = 0.007) which does not considered women's age. There was no significant correlation between NK cell fraction and women's age. Correlation test revealed a decreasing tendency of measured-to-reference AMH ratio according to increasing level of NK cell fraction (rho = -0.09, P = 0.026).

Limitations, reasons for caution: Since the data was collected in a single center cohort, a study of large general population is warranted.

Wider implications of the findings: The finding of this study suggests higher NK cell fraction in the peripheral blood may have a harmful effect on ovarian reserve. The potential role of NK cell fraction in women with decreased ovarian reserve should be explored to improve fertility outcomes.

Trial registration number: Not applicable.

P-349 Advanced glycation end-products accumulation affects assisted reproductive technology outcomes

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Study question: Do the accumulation of advanced glycation end-products (AGEs) affect the clinical outcomes in ART program?

Summary answer: Accumulation of AGEs has been associated with poor clinical outcomes in ART program.

What is known already: AGEs are produced endogenously in the body or exogenously by intake of diet containing high levels of AGEs. AGEs are known to play a role in the pathogenesis of several diseases, and recently female infertility by causing oxidative stress, altering enzymatic activities, affecting cytotoxic pathways, or damaging nucleic acids.

Study design, size, duration: Prospective study in a clinical-based cohort of 148 women who agreed with measurements after the new patient visit, from November 2015 until December 2016 inclusive.

Participants/materials, setting, methods: We measured skin autofluorescence non-invasively by AGE-Reader in female infertility patients (n = 148) seen between November 2015 until December 2016 at HORAC Grand Front Osaka Clinic. To evaluate the cumulative effect of AGEs, we compared cycle outcomes between patients with high-AGEs (HA) and low-AGE (LA). Primary outcome measures were infertility factors and clinical outcomes.

Main results and the role of chance: Accumulation of AGEs were higher in fertility patients than in patients whose infertility was attributed to male factors. The AMH levels of patients with HA were significantly lower than in patients with LA (2.1 vs. 3.7 ng/mL, respectively; $p < 0.05$). No correlation was observed between AGEs and other hormones (basal FSH, DHEA-S, testosterone and prolactin). There were no significant differences between the patients with HA and LA in the fertilization rate, rate of good quality embryos, blastocyst rates, rate of good quality blastocysts. Accumulation of AGEs were higher in patients with no pregnancy than in patients with pregnancy (195.3 vs. 175.1 AU, respectively; $p < 0.05$)

Limitations, reasons for caution: AGEs were measured three times at the volar side of the arm. Exposure to sunlight is a possible confounding factor in skin AGE accumulation, but the similarity between AGEs measurements taken at the volar side suggests that this effect is probably limited. Clinical outcomes included both ICSI and conventional IVF.

Wider implications of the findings: High AGEs levels are a contributing potential biomarker for diminishing ovarian reserve. AGEs accumulations correlated with a lower likelihood of pregnancy. The AGE-Reader is useful as a non-invasive clinical tool for assessment of increasing risk for infertility and may provide information for the development of more effective therapeutic strategies.

Trial registration number: Not applicable.

P-350 Vaginal microbiota and IVF outcomes - Development of a simple diagnostic tool to predict patients at risk of a poor reproductive outcome

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Study question: Could 16 S rRNA gene sequencing improve the diagnosis of abnormal vaginal microbiota (AVM) to predict patients at risk of a poor reproductive outcome?

Summary answer: 16 S rRNA gene sequencing did not add substantial diagnostic value compared to a quantitative PCR (qPCR) assay targeting *Gardnerella (G.) vaginalis* and *Atopobium (A.) vaginae*.

What is known already: Female reproductive tract microbiota might have an important impact on the implantation of the embryo and subsequently, the reproductive outcome. However, only a few studies have investigated this emerging issue. Previously, a qPCR assay for AVM diagnosis that targets *G. vaginalis* and *A. vaginae* above threshold levels suggested a poorer reproductive outcome in IVF patients with AVM. In the present study, qPCR was compared to 16 S rRNA gene sequencing of the vaginal microbiota to develop a diagnostic approach that could predict a poor reproductive outcome in IVF patients.

Study design, size, duration: The present study included a cohort of 111 IVF patients. Patients were followed with the primary outcome of clinical pregnancy at 7 weeks gestation and followed-up until birth.

Participants/materials, setting, methods: Patients were assigned to IVF treatment and prospectively enrolled from two centers in Denmark. Vaginal swabs were taken during speculum examination from the posterior fornix. 16 S rRNA gene sequencing of the V4 region was performed on the specimens from the same DNA extraction as was used for qPCR.

Main results and the role of chance: 16 S rRNA gene sequencing provided further insight into the vaginal microbiota of IVF patients. In a principal component analysis comparing qPCR and 16 S rRNA, a total of 28/32 AVM positive samples were clustered together. The 16 S AVM group had an age adjusted OR 0.18 (95%CI 0.03-0.98) for clinical pregnancy which was similar to the qPCR AVM group. Furthermore, a broader panel of tentative pathogenic bacteria did not seem to predict reproductive outcomes better than the qPCR approach. The only AVM patient (1/17) gave birth in gestational week 35 and the child had a low birth weight of 2320 grams.

Limitations, reasons for caution: The results from specific qPCR analysis suggest that the species selection is valid. However, it is a limitation that only the V4 region was used for 16 S rRNA gene analysis. Furthermore, due to the small number of patients, one should be cautious when interpreting the reproductive outcome results.

Wider implications of the findings: Although the number of detected bacterial species increases with 16 S rRNA gene sequencing, the simpler and less expensive qPCR diagnostic approach seems non-inferior compared to16S sequencing in the prediction of IVF patients at risk of a poor reproductive outcome.

Trial registration number: The project was registered at clinicaltrials.gov (file number NCT02042352).

P-351 Producing a polyclonal antibody specific to embryonic poly (A)-binding protein (EPAB) and characterizing EPAB protein expression in mouse somatic tissues, oocytes and early embryos

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Study question: What are the spatial and temporal expression patterns of EPAB and poly(A)-binding protein, cytoplasmic 1 (PABPC1) proteins in mouse somatic tissues, oocytes and early embryos?

Summary answer: We produced a polyclonal antibody specific to EPAB. EPABP and PABPC1 exhibit different spatial and temporal expression patterns in mouse tissues, oocytes and early embryos.

What is known already: Embryonic poly(A)-binding protein (EPAB) functions in translational control of maternal mRNAs during oocyte maturation, fertilization and early embryo development in mammals. EPAB is expressed in mouse and human germ cells and early embryos as well as in the ovary and testis tissues. It is revealed that female mice lacking Epab gene are infertility. Since there is no EPAB-specific antibody, there is no study aimed to analyze spatial and temporal expression pattern of the EPAB protein in mouse somatic tissues, oocytes and early embryos. Notably, PABPC1 shows a high homology with EPAB protein and can compensate the EPAB's functions.

Study design, size, duration: The study is designed as follows: 1) Polyclonal antibody production for EPAB protein, 2) Analyzing EPAB and PABPC1 protein expression in the 5-week old Balb/C mouse (n = 5) somatic tissues including ovary, testis, stomach, kidney, small intestine, heart, spleen, brain, and lung, 3) Characterizing expression of the EPAB and PABPC1 proteins in the germinal vesicle, metaphase II oocytes and 1-cell, 2-cell and 4-cell early embryos obtained from 5-week old Balb/C female mice (n = 5) using superovulation.

Participants/materials, setting, methods: The competitive enzyme-linked immunosorbent assay (ELISA) has been used to determine the immune specificity of the produced EPAB polyclonal antibody in rabbit. To determine the EPAB and PABPC1 protein expression in the mouse somatic tissues, we have used immunohistochemistry. Also, the spatial and temporal expression the EPAB and PABPC1 proteins in the mouse germinal vesicle and metaphase II oocytes and in the 1-cell, 2-cell, and 4-cell embryos have been characterize by using immunofluorescence staining.

Main results and the role of chance: The competitive ELISA test revealed that produced EPAB polyclonal antibody is highly specific to the EPAB synthetic peptide. Then, we have observed that EPAB protein is only expressed in the mouse ovary and testis tissue samples; however it is not detected in the somatic tissues including stomach, kidney, small intestine, heart, spleen, brain, and lung. On the other hand, PABPC1 is expressed in both gonadal and somatic tissues. It is important to note that Epab mRNA expression had been characterized only in the mouse gonadal tissues. The immunostaining on the mouse oocytes and early embryos showed that EPAB protein is expressed in the nuclear and cytoplasmic regions of the GV and MII oocytes and early embryos and its expression exhibit remarkably differences during oocytes maturation and early embryo development ($P < 0.05$). Similarly, PABPC1 expression has been determined in the nuclear and cytoplasmic regions of the oocytes and early embryos, and its expression exhibit predominant changes during oocyte maturation and early embryo development ($P < 0.05$).

Limitations, reasons for caution: There is no limitation for this study.

Wider implications of the findings: We have tested firstly produced EPAB antibody and analyzed EPAB and PABPC1 protein expression in the mouse tissues, oocytes and early embryos. This is a preliminary study to determine the critical functions of EPAB protein in the translational control of the maternal mRNAs in the mouse oocytes and early embryos.

Trial registration number: As this study is a basic scientific research, there is no trial registration number.

P-352 Time to pregnancy and birth during fertility treatment – A Delphi consensus

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Study question: Does time to pregnancy/birth (TTP/B) play a significant role for fertility treatment and if so, how could it be shortened?

Summary answer: Overall, TTP/B was considered an important factor that should be included in any decision making related to fertility treatment.

What is known already: TTP/B has been suggested to be of importance when considering treatment choices for assisted reproductive technologies (ART). Although TTP/B is applicable to all patients, it is of particular importance for ageing women due to the decline in fertility observed with age. Until now there is no consensus as to how TTP/B could be taken into account during fertility treatment.

Study design, size, duration: This Delphi consensus process comprised three steps.

Step 1: A panel of experts developed 12 statements.

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Step 2: A larger panel of experts voted on these statements, giving motivations for their vote. Consensus was reached if ≥66% of participants agreed/disagreed with the statement(s). Any statement(s) without consensus would be revised and voting repeated until consensus was reached.

Step 3: Details on any statements reaching consensus was communicated to participants.

Participants/materials, setting, methods: The core scientific panel for Step 1 comprised 12 experts. The larger panel for Step 2 included the core scientific panel plus 15 experts recommended by the core panel (total of 27 experts). Discussions (Steps 1&3) were held by WebEx and voting for consensus was conducted by an online poll. Each statement was accompanied by references to provide a context for the statement and these were considered part of the statement that was agreed/disagreed with.

Main results and the role of chance: During Step 1, 12 statements were developed, focussing on the following topics related to TTP/B:

– Managing fertility treatment in a timely manner, avoiding over- or under-treatment is crucial;
– In all subfertile women <40 years old, IVF outcomes could be optimized by performing 3–6 embryo transfers;
– A number of procedures might reduce TTP/B, including preimplantation genetic screening, and the use of frozen replacement cycles immediately after failed fresh cycles, as clinical pregnancy rates are similar to those for frozen cycles postponed to a later time. Moreover, use of gonadotropin-releasing hormone (GnRH) antagonist protocols shorten the treatment period, and reduce OHSS cycle cancellation rates;
– The number of oocytes retrieved should be maximised to increase the cumulative live birth rate;
– Double stimulation (follicular and luteal phase) could be used in patients with reduced ovarian reserve to accumulate oocytes/embryos.

Consensus (agreement) was reached on all statements after the first round of voting; four statements reached 100% consensus. These four statements related to an increased live birth rate with a larger number of oocytes retrieved, patient age not affecting donor cycles if donors were aged 18-34 years, and the use of GnRH antagonists (two statements).

Limitations, reasons for caution: This consensus does not represent an exhaustive list of statements on TTP/B and is aimed at healthcare professionals, not patients. The views represent the collective opinion of the experts and not all statements reached 100% agreement. Some statements reached consensus even though a minority of participants absolutely disagreed with them.

Wider implications of the findings: As the population seeking ART is ageing, TTP/B is of increasing importance when selecting treatment strategies. Some approaches, e.g. GnRH antagonist co-treatment or frozen-thaw transfer immediately after a failed fresh cycle, are related to reduced treatment duration, however, more research is needed exploring the impact of treatment choices on TTP/B.

Trial registration number: N/A.

P-353 In vitro proliferation of uterine fibroids is related with vitamin D receptor and patient´s age

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Study question: Could Vitamin D be a potential uterine fibroid treatment for women of all ages?

Summary answer: Vitamin D, acting via its receptor, is a potential treatment for uterine fibroids, which occur in women at reproductive ages.

What is known already: Uterine fibroids (UF) (also known as leiomyomas or myomas) are benign tumours occurring in an estimated 20%-40% of women during their reproductive years. UF affect a patient's quality of life, as well as her fertility and obstetrical outcomes. Recent studies have proposed Vitamin D (VitD) as an effective treatment for UF. Diverse functions for VitD have been confirmed by the presence of vitamin D receptor (VDR) in a wide range of human tissues, including the myometrium of the human uterus, but its specific role in UF remains unclear.

Study design, size, duration: This study was approved by the Clinical Research Ethics Committee at the Hospital Univeristario y Politécnico La Fe de Valencia (Spain) (2014/0691). Our pilot study was carried out in the Hospital Univeristario y Politécnico La Fe de Valencia and Fundación Instituto Valenciano de Infertilidad (FIVI). Intramural UF were collected from patients undergoing surgery due to UF pathology (n = 14) without any previous treatment. All participants provided written informed consent.

Participants/materials, setting, methods: UF tissue (n = 14) was analysed by Western blot to determine the presence of Vitamin D receptor (VDR) (Cell Signaling antibody) protein. Subsequently, UF tissue was disaggregated with collagenase-II (Sigma-Aldrich) and cells were cultured in DMEM-F12 medium. In vitro cell proliferation assays were performed by flow cytometry using propidium iodide. Data were normally distributed; parametric linear regression and Student's t-test were conducted with R commander software.

Main results and the role of chance: We corroborated the statistically significant negative correlation between in vitro proliferation of UF cells and patient age (ranging from 22 to 52 years, p-value = 0.028). Additionally, cells from UF with VDR showed greater cell growth compared with cells without VDR (p = 0.008). Surprisingly, we observed a trend in which the VDR was absent in patients ranging from 44-52 years old (p = 0.07). These findings were supported by the statistically significant correlation between in vitro proliferation, presence of VDR, and patient age (p = 0.049), suggesting a strong interaction among the three.

Limitations, reasons for caution: Limitations of this pilot study include the small sample size.

Wider implications of the findings: Active uterine fibroid cells appear during the reproductive years and regress around pre-menopause. These active cells exhibit an in vitro proliferation rate that correlates with the presence of vitamin D receptor and the patient´s age. These findings provide a foundation for exploring vitamin D treatment in the clinical setting.

Trial registration number: No clinical trial.

P-354 Why do follicles rupture earlier in poor ovarian response: The role of Cyclooxygenase-2 expression in the follicular micro-environment

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Study question: Does the expression of Cyclooxygenase-2 (Cox-2) mRNA in oocytes and surrounding cumulus cells or serum and follicular fluid Cox-2 concentration has an effect on oocyte maturation and follicular rupture?

Summary answer: High follicular Cox-2 concentration could be the reason of premature follicular rupture before oocyte maturation, especially in poor responders.

What is known already: Cox-2 plays an important role via enzymatic activity on follicle rupture and cumulus expansion induced by the LH surge. Animal studies on Cox-2 inhibitor application have confirmed that Cox-2 activity is required as a key factor for ovulation, follicle rupture and oocyte competence.

Study design, size, duration: This was a prospective clinical trial of a total of 26 oocytes and 52 cumulus cells, and 52 samples of folliculer fluid retrieved from infertile patient underwent intracytoplasmic sperm injection (ICSI) cylcles at the Department of Gynecology and Obstetric ART Center, Uludag University School of Medicine, Bursa, Turkey, between September 2015 and May 2016.

Participants/materials, setting, methods: The samples were divided into three groups according to the stage of oocyte maturity and fertilization: Group1 included 16 immature oocytes with their micro-enviroments, Group2 included 10 mature-unfertilised oocytes with their micro-enviroments and Group3

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consisted from 26 micro-enviroments of mature fertilized oocytes as a control group. Oocyte and cumulus cell Cox-2 mRNA expression was detected by Real Time-PCR. Serum and follicular fluid Cox-2 concentrations were measured by the ELISA assay.

Main results and the role of chance: Compared with patients baseline characteristics, serum AMH concentration and follicular Cox-2 concentration had a significant negative correlation (CF: -0.310; p = 0.036). When follicular Cox-2 concentration compared in terms of stimulation protocols, a significant difference was observed also. In poor ovarian response patients whom Letrozole co-administrated to gonodotropins, higher follicular concentration of Cox-2 was detected (p = 0.026). Follicular Cox-2 concentration and follicle diameter showed positive correlation in only Group 3 (mature and fertilized oocytes) (CF: 0.414; p = 0.035). The Cox-2 concentration in serum samples showed no significant difference with regard to oocyte maturation stage (p = 0.426, p = 0.504 respectively) and also relative Cox-2 mRNA expression in cumulus cells had no effect on stage of oocyte maturity (p = 0.394) or fertilization (p = 0.143).

Limitations, reasons for caution: The cumulus cells and oocytes were obtained from patient who underwent ovarian stimulation, which in comparison with natural cycles, may have affected gene and protein expression. Also due to the ethical restriction and patient rights, fertilized oocytes regarded as potential embryos and were not analyzed.

Wider implications of the findings: Our data suggest that, in patient with diminished ovarian reserve for preventing premature follicular rupture and retrieving more mature oocyte, co-administrating Cox-2 inhibitors in COH protocols could be a novel clinical approach.

Trial registration number: We do not have clinical trials registration number.

P-355 Targeted mutant mZP1 results in defective zona pellucida and reduced fecundity in female mice

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Study question: Does a homozygous frameshift mutation in mZP1, cause a defect on zona pellucida (ZP) development and fertility in vivo same as human?

Summary answer: The mutant mZP1 exerts defects on ZP development and fertility results in a thin and looser weave ZP of most of eggs and reduced fecundity.

What is known already: The pervious reported research identified a novel 8 bp frameshift deletion (I390fs404X) in ZP1 gene in a consanguineous Chinese Han family. This deletion co-segregates with infertility and absence of ZP in the family, with an autosomal recessive inheritance mode. Importantly, the co-localization of truncated ZP1 and normal ZP3 proteins were detected throughout the cytoplasm but not at the cell surface in eggs from the affected patients, suggesting the different relationship between the ZP1 proteins and fertility. Nevertheless Zp1 knockout female mice ovulate the eggs surrounded by loosely organized and swollen zona pellucida, with reduced fecundity.

Study design, size, duration: The fertility of the mice was assessed by breeding $Zp1^{tm/wt}$, $Zp1^{tm/tm}$ and normal female mice. Each group contained 15 female mice, fixed with normal fertile male mice (1:1) after 6 weeks age, and got the live births after 3 weeks.

Participants/materials, setting, methods: We used CRISPR/Cas 9-mediated genomic editing technology to develop a mouse model carrying the homologous 8 bp deleted mutation in Zp1 gene($Zp1^{tm/tm}$, tm: targeted mutation). In silico analysis was done for the sequence variants followed by molecular assays to examine the functional effects of the sequence variants in mouse. The sanger sequencing and western analysis were used to detect the mouse model edited result. Light microscope visualizing the oocytes, ovarian histology and immunocytochemistry of $Zp1^{tm/tm}$.

Main results and the role of chance: We determined that an 8 bp-deletion at nucleotides 1145-1152 (TCTTCTCA) of mouse ZP1 CDS (CCDS37918.1), which will result in a premature stop codon (I390fs411X) that leads to a truncated mouse ZP1 protein of 411 aa with the terminal recoding sequence of 22 aa bearing no homology to the corresponding aa sequence of normal mouse ZP1 protein. Examining all oocytes, the zona matrix was multitudinous, the most of zonae of Zp1tm/tm mice thinner with a poorly defined peripheral border compared to normal zonae, while Zp1tm/wt female did not have significant difference. Additionally, In some oocyte-cumulus complexes, cumulus cells were observed between the zona matrix and the oolemma, a phenomenon not observed in normal zonae. These inner cells had undergone mucification and a granular matrix was observed surrounding them and infiltrating the zona matrix. Eggs lacking zonae is not a rare phenomenon.The number of litters of $Zp1^{tm/tm}$ was reduced, in contrast with infertility of the patients.

In each group, the mean and arithmetic Average ± standard error(SE) were calculated. Student's t-test or chi-square test was used for statistical analysis of measurement data or enumeration data, respectively; significance was assumed for $P < 0.01$. The calculations were made using SPSS 19 software.

Limitations, reasons for caution: Although the in vitro assays demonstrated the causative effect of mutant zp1 on on zona pellucida (ZP) development and fertility.further studies need to validate its long-term effects on folliculogenesis.

Wider implications of the findings: These results will aid both researchers and clinicians in understanding the molecular pathology of mutant ZP1 causing a defect on zona pellucida (ZP) development and fertility in vivo and to develop diagnostic assays or therapeutics approaches.

Trial registration number: none.

P-356 Do the Bologna criteria assist in predicting ART outcome for women aged over 38?

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Study question: What is the live birth rate (LBR) for advanced aged ART patients and do the Bologna criteria assist in predicting the treatment outcome?

Summary answer: A LBR of 14.1% was found in the study population. The LBR was not significantly lower for women fulfilling the Bologna criteria.

What is known already: Postponing parenthood has steadily increased during past decades. Women delay childbearing reassured by the belief that ART can compensate for age-related decline in fertility. Growing portions of ART patients are women in their late 30 s and older. Many of them present with poor ovarian response. In an attempt to standardize the definition of POR the Bologna criteria were set. The combination of advanced maternal age with additional features of poor ovarian response may be connected to minimal chance of achieving genetic parenthood. Unveiling these chances is important to patients and clinicians in making rational decisions about medical treatment.

Study design, size, duration: A database containing clinical and laboratory information on ART treatment cycles carried out at an academic hospital between December 2010 and February 2016 was analyzed. This data was collected prospectively and recorded in the database of the infertility unit. All women 38 years or older were included. Autologous fresh and frozen thawed cycles were included if the patients' age at the pickup was at least 38 years. The main outcome was the live birth rate.

Participants/materials, setting, methods: Two hundred and fifty four women had 696 ART cycles (571 fresh and 125 frozen thawed). To establish the diagnosis of poor ovarian response according to the Bologna criteria at least 2 of the following had to be present: 1. Advanced maternal age (>40 years), endometriosis, or previous chemotherapy. 2. A previous POR cycle (≤3 oocytes retrieved with a conventional stimulation protocol). 3. Abnormal ovarian reserve tests: FSH > 12. Fifty-eight patients fulfilled these criteria.

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Main results and the role of chance: Overall, 36 women had a live birth (14.1%). In comparison to the patients who failed, these patients were younger (40.5 vs 41.4, p = 0.004), had more oocytes aspirated (5.9 vs 4.3, p = 0.005), more embryos transferred (2.1 vs 1.5 p = 0.002) and frozen (1 vs 0.4, p = 0.03). A higher proportion of the patients who gave birth used donor sperm (28 vs 15% p = 0.05) and a lower proportion had 3 or less oocytes aspirated in a former cycle (38 vs 60%, p = 0.02). The patients who fulfilled the bologna criteria were less likely to have a previous delivery (34 vs 56%, p = 0.003), had lower maximal estradiol (738 vs 1220 pg/ml, p < 0.0001) and progesterone (0.78 vs 0.94 ng/ml, p = 0.007) levels, and a lower number of oocytes (2.3 vs 5.1, p < 0.0001) and mature oocytes (1.5 vs 3.2, p = 0.02) aspirated. They had fewer embryos transferred (1.2 vs 1.8, p = 0.007) and cryopreserved (0.007 vs 0.7, p = 0.014).Overall 6.9% from the patients with poor ovarian response according to the bologna criteria delivered as compared to 16.3% in the non-poor responders. This difference was not statistically significant (p = 0.07).

Limitations, reasons for caution: This is an observational study with a limited sample size. Although no statistically significant difference was seen in the primary outcome, the study may have been underpowered to a difference. No definite cutoff values can be drawn regarding the probability of live birth.

Wider implications of the findings: The public should be aware of the low pregnancy rates in advanced maternal age. It should be emphasized that advanced reproductive technologies can't overcome this age related decline in fertility and that there are no absolute values in predicting the prognosis.

Trial registration number: NA.

P-357 Does a rise in LH levels during artificial frozen–thawed embryo transfer (FET) cycles affect pregnancy rate?

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Study question: To evaluate the association between high serum luteinized hormone (LH) levels during artificial FET cycles and positive pregnancy test

Summary answer: Increase in LH level in artificial FET cycles does not affect pregnancy rate.

What is known already: Supplementation of estrogen (E2) in the early follicular phase in FET cycles for endometrium preparation can lead to a rise in LH level, similar to that observed before ovulation. Such a rise of LH might interfere with endometrial receptivity during a FET cycle. According to previous studies, it was demonstrated that the likelihood of clinical pregnancy is not associated with absolute serum LH levels on day 14 of an artificial FET cycle. The association between a rise in LH level during artificial FET cycle compared to early follicular phase level and pregnancy rates, has not been studied.

Study design, size, duration: A retrospective cohort study. A total of 1685 FET cycles, between 01/2007 to 12/2016 were included in the analysis

Participants/materials, setting, methods: Women undergoing artificial FET cycles.

Setting-Assisted Reproductive Technology unit at Bnai-Zion medical center

Methods- we compared cycles in which LH double/triple itself from early follicular phase and further, to cycles without rise in LH. Endometrium preparation was achieved by administration of 2 mg*3\day estradiol valerate tablets. Embryo transfer (ET) was conducted after achieving endometrial thickness >7 mm and vaginal progesterone supplementation was added according to the embryo's age. A beta hCG was measured 13-14 days after ET.

Main results and the role of chance: Data from one thousand six hundred and eighty-five FET cycles were retrieved between the years of 2007 and 2016. LH values, E2, progesterone values, endometrial thickness and pregnancy outcomes were available in all patients. We calculated the LH rise between LH on early follicular phase in each cycle, to LH on the ET day. Moreover, we calculated a rise in E2 level and endometrial thickness during the cycle. 44.6% of 1685 FET cycles had doubling LH level until ET day, 27.24% had tripling LH level. Mean patient age was 30 years (range, 20–45 years), similar in cycles with and without rise in LH level. Overall pregnancy rate was 30.6%. Pregnancy rates per ET were similar between cycles with and without doubling or tripling LH level, (31.8% Vs. 29.8%, P = 0.38 and 31.2% Vs. 30.3%, P = 0.79, respectively). Multivariate logistic regression was performed with the dependent variable positive pregnancy test and independent variables including; doubling LH level with doubling E2 level, or with endometrial thickness above 7 mm, on ET day. In this model, there was no association between doubling LH values with doubling E2 values during the cycle or with endometrial thickness above 7 mm on ET day and pregnancy rates.

Limitations, reasons for caution: The main limitation of our study is its retrospective nature. Nevertheless, our study represents one of the largest series addressing the association between LH rise in FET cycles and Pregnancy rates.

Wider implications of the findings: Though the pregnancy rate was higher among cycles with doubling and tripling LH values, it was not statistically significant. Therefore, LH rise during FET cycles does not alter pregnancy rates. Apparently, hormonal monitoring of LH levels does not yield useful information in FET cycle, and should therefore not be conducted.

Trial registration number: 0101-16-bnz

P-358 FMR1 CGG-genotype influences FMR1 expression in human granulosa cells

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Study question: Does the CGG repeat-length (RL) of *FMR1 (*Fragile X Mental Retardation 1 gene) influence its expression in granulosa cells of women with different ovarian response?

Summary answer: Poor responder (POR) with a heterozygous low/norm CGG repeat demonstrate significantly elevated *FMR1* expression profiles in their granulosa cells during IVF/ICSI cycles.

What is known already: CGG RL of *FMR1* varies about 30 in general. >200 (Full Mutation) a Fragile-X-Syndrome can occur. Ranges of 54-200 are called premutation (PM) and show instable heredity. ~20% of the female PM-carriers suffer from premature-ovarian-failure (POF). *FMR1*-expression in peripheral blood cells, as well as female granulosa cells (GC) increase with increasing RL in PM-carriers. But variable *FMR1*-expression-profiles independent of PM in POF-women are also reported. The influence of aberrant *FMR1*-CGG-RL before PM on ovarian reserve and response is controversially discussed and an analysis of *FMR1*-expression and its potential dependence on ovarian response in womens´ GC has not yet been performed.

Study design, size, duration: 229 women undergoing IVF/ICSI-treatment were included and divided into three ovarian-response-subgroups: POR using "Bolognia Criteria", polycystic ovary syndrome (PCO) using "Rotterdam Criteria" and normal responder (control group) (. A CGG-RL of 26-34 was considered normal. Women were subdivided into 3 haplotypes (normal [both alleles in range], heterozygous [one allele outside range] or homozygous [both alleles outside range]) and 6 genotypes ("low" [<26 repeats], "normal" [26-34 repeats] or "high" [35-55 repeats]) according to their be-allelic CGG-RL.

Participants/materials, setting, methods: Clinical data were collected from a questionnaire and medical reports. CGG repeat length was determined using ALF express sequence automat or ABI 3100/ 3130xl sequencer.

RNA was extracted from granulosa cells after follicular aspiration. Quantitative RNA-expression was analysed using specific TaqMan gene expression assays and compared using ΔΔCT method. Statistical analyses were performed with SPSS (*Statistical Package for the Social Sciences V. 22.0*) and statistical significance was set at $P \leq 0.05$.

Main results and the role of chance: We here analyzed the *FMR1*-gene-expression in the female germline directly *in vivo* in human GC and evaluated

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there a potential association with the different *FMR1* geno- and haplotypes, as well as ovarian response and reserve parameters. Women with POR with a normal/low genotype compared to either high/normal, or normal/normal genotypes in our study showed a significant higher *FMR1*-mRNA-level in their GC ($p = 0.008$, $p = 0.027$, respectively). So low allels putatively negatively affect female ovarian response or vice versa low response is caused by impaired folliculogenesis prior to stimulation due to a low-allel-effect.

Women with high/high and high/normal genotypes of all response groups demonstrated a tendency of lower AMH level, and patients of low/low and high/high genotypes presented the lowest number of total and MII oocytes.

Aberrations of the CGG repeat outside the normal range of 26-34 may be causal for elevated *FMR1*-gene-expression-levels in POR and also influence outcome after follicular aspiration in all response groups. Results may help to offer an optimized, risk adjusted treatment for future patients during IVF/ICSI-treatment depending on their *FMR1*-genotype and *FMR1*-expression-profile by for example better clarification of their expected outcome and potentially optimizing their stimulation protocol adapted to their elevated risk of limited response or outcome.

Limitations, reasons for caution: Study population especially of the subgroups was low. Further studies of *FMR1* expression in granulosa cells with bigger sample sizes are needed.

Wider implications of the findings: Norm/low genotype in poor responders is associated with elevated granulosa cellular *FMR1*-expression. Herewith the hypothesis of *FMR1* as relevant regulator of folliculogenesis gets further substantiated. A better understanding of *FMR1*-expression in GC and its regulation in future may help to elucidate pathomechanisms of different folliculogenesis disorders.

Trial registration number: not applicable.

P-359 Dipeptidyl peptidase-IV and Adenosine deaminase enzyme activity levels in Polycystic Ovary Syndrome

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Study question: Is there any relationship between Dipeptidyl peptidase-IV and Adenosine deaminase enzyme activity levels in Polycystic Ovary Syndrome?

Summary answer: DPP-4 activity levels, ADA, AMH, HOMA-IR and insulin levels were found to be increased in PCOS patients when compared to control grup.

What is known already: Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in reproductive age women around the world. Insulin resistance and hyperinsulinism play a critical role in the syndrome pathogenesis. Glucagon-like peptide-1 (GLP-1) promotes insulin secretion, inhibits glucagon secretion. GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4). DPP-4 also known as adenosine deaminase (ADA) binding protein. An increase in ADA activity in type 2 diabetic patients has been reported. Reduced levels of adenosine increase glucose uptake into cells.It was proved that Polycystic ovary syndrome is realeted with insulin resistance. Therefore, insulin resistance may have an important relationship with ADA activity.

Study design, size, duration: This study was made by prospective, among april of 2015 and september 2016.

Participants/materials, setting, methods: The subjects enrolled in this study were recruited from 44 patients with PCOS and 44 infertile controls which were revealed with USG and Anti-Müllerian Hormon (AMH) levels. Serum ADA and DPP-4 activities and AMH levels were measured by ELISA methods. Homeostasis model assessment of insulin resistance (HOMA-IR) method was used for evaluating insulin sensitivity (HOMA-IR=Fasting Insulin (μu/ml) x Fasting Glucose (mg/dl) / 405). All results were compared.

Main results and the role of chance: There was no difference between the PCOS and control groups in terms of age and BMI. ADA and DPP-4 activity levels and AMH, HOMA-IR ($p<0.05$) and insulin levels ($p<0.01$) were found to be increased in PCOS patients when compared to control. Glucose levels were similar in both groups.

Considering all study participants AMH levels positively correlated with ADA (r:0.734) and DPP-4 (r:0.449) activity levels. Also ADA was positively correlated with DPP-4(r:0.472), insulin (r:0.216) and HOMA-IR (r:0.223) parameters.

Limitations, reasons for caution: Since small sample size. Future functional studies are essential to define relation of between insulin intolarance and PCOS.

Wider implications of the findings: Defining of insuline resistans for PCOS is essential in view of treatment of infertility. Oral DPP-4 inhibitor drugs and GLP-1 analogues may be used for the treatment of insulin resistance in PCOS.

Trial registration number: 1.

P-360 Abnormal ratio of CD57+ cells to CD56+ natural killer cells in women with recurrent implantation failure

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Study question: Are altered levels of endometrial and peripheral natural killer cells (NKs) and regulatory T cells (Tregs) present in women with recurrent implantation failure (RIF)?

Summary answer: An increased ratio of endometrial and peripheral $CD57^+$ cells to $CD56^+$ NKs and a decreased percentage of peripheral $Foxp3^+$ Tregs in women with RIF.

What is known already: NKs and Tregs perform important immune regulatory function in embryo implantation. Although the detection of $CD56^+$ uterine NKs using immunohistochemistry has been developed extensively, the debate regarding the predictive value of the measurement may reflect a lack of understanding of the subtypes of NKs and the relation between NKs and Tregs.

Study design, size, duration: A case control study was conducted on 32 women with RIF and 23 normal controls (regular menstrual cycle, no oral contraceptives) during the period between June 2015 and June 2016.

Participants/materials, setting, methods: Endometrial biopsies were obtained during the mid-luteal phase (LH+ 7–9 of the menstrual cycle) followed with immunohistochemistry, and the percentages of stromal cells positive for CD56, CD57, and Foxp3 were calculated. Blood samples were obtained on days 12–14 of the menstrual cycle to determine $CD45^+CD56^{bright}CD57^+$ NKs and $CD4^+CD25^+Foxp3^+$ Tregs using multi-color fluorescence flow cytometry.

Main results and the role of chance: In endometrium, lower percentage of $CD56^+$ NKs and $Foxp3^+$ Tregs, while higher percentage of $CD57^+$ cell were found in the women with RIF compared to normal controls ($p<0.05$). In peripheral blood,lower percentage of $CD45^+CD56^{bright}$ cells in $CD45^+$ cells was found in women with RIF compared to normal controls ($p<0.05$), while none difference was found in the percentage of $CD45^+CD56^{bright}CD57^+$ cells in $CD45^+$ cells ($p = 0.29$) and the percentage of $CD4^+CD25^+Foxp3^+$ cells in $CD4^+$ cells ($p = 0.30$) between two groups. Compared to normal controls, the ratio of $CD57^+$ cells to $CD56^+$ NKs was significantly increased in women with RIF, both in the endometrium [median (range)/ 29.8% (1.8–94.6%) vs 13.3% (1.4–88.2%), $p<0.01$] and peripheral blood [46.9% (24.7–77.8%) vs 34.1% (11.1–71.8%), $p<0.05$]. There was a negative correlation between the ratio of $CD57^+$ cells to $CD56^+$ NKs and the percentage of $Foxp3^+$ Tregs in the peripheral blood (correlation=-0.32, $p<0.05$), but none significant correlation between them was found in the endometrium (correlation=-0.20, $p = 0.15$).

Limitations, reasons for caution: We used nonparametric test to compare the CD56, CD57 and Foxp3 positive ratio of cells due to non-normal distribution and non-homogeneous variances which may result from the limited cases. Another limitation was the absence of double staining of CD56 and CD57 in endometrium limited to the existing test-bed condition.

Wider implications of the findings: Our study provides insights into evaluation of the subtypes of NKs as well as a clue for further understanding the relation between NKs and Tregs in women with RIF.

Trial registration number: Not applicable.

P-361 Maternal aging affects mechanisms regulating Ca2+ homeostasis in mammalian oocytes

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Study question: We wished to examine whether mechanisms regulating Ca2+ homeostasis in mammalian oocytes change with female age, leading to age-dependent decline in female fertility.

Summary answer: Maternal aging affects Ca2+ homeostasis, including fertilization-induced Ca2+ oscillations and their association with ATP production, but not the overall developmental potential of mouse oocytes.

What is known already: Maternal aging (aging of oocytes in vivo in a female's organism) has been reported to have an adverse effect on the oocyte quality, leading to an age-dependent decline in female fertility. Changes associated with maternal aging include both malfunctions of the nuclear apparatus and imbalance of cytoplasmic processes. Among other cytoplasmic regulatory processes, homeostasis of Ca2+ ions combined with energy metabolism has crucial importance for the further embryo development.

Study design, size, duration: In our study we used mouse oocytes isolated from young (2-3 months old) and aged (12-15 month old) mouse females of different genetic backgrounds, which were equivalents to women in early and late (>35 yrs old) reproductive age.

Participants/materials, setting, methods: Oocytes recovered from young and aged mice were labelled with fluorescent Ca2+ and /or Mg2+ markers, fertilized in vitro and subjected to time-lapse fluorescence imaging in order to follow Ca2+ oscillations induced by fertilization and/or ATP production (associated with the Mg2+ level). Additionally, amounts of Ca2+ present in young and aged oocytes were compared, as well as expression of genes involved in Ca2+ homeostasis. TMRE staining was used to assess mitochondrial membrane potential in oocytes.

Main results and the role of chance: We show that maternal aging alters the pattern of fertilization-induced Ca2+ oscillations in a manner depending on the genetic background. These changes, as our results indicate, are caused likely by altered expression of genes coding proteins involved in maintaining the Ca2+ homeostasis and/or by different levels of available Ca2+ ions in the oocyte. We also find that maternal aging changes dynamics of ATP production accompanying Ca2+ oscillations. Interestingly, this does not associate with a significant modification of mitochondrial functionality, as a mitochondrial membrane potential is similar in young and aged oocytes. Importantly, although we notice differences in Ca2+ homeostasis in young and aged oocytes, the oocytes display the same ability to undergo fertilization and further pre- and post-implantation development.

Limitations, reasons for caution: Our study was performed on mouse oocytes, which although widely used as a model of human gametes, differ in some aspects from human counterparts. Therefore our results should be treated as an indicator of certain tendencies possibly present in other mammalian species, but should not be directly translated to humans.

Wider implications of the findings: Our findings implicate that genetic background alters oocyte susceptibility to maternal aging, at least on the Ca2+ homeostasis level. Moreover, even though maternal aging can significantly affect mechanisms involved in Ca2+ homeostasis, it does not necessarily diminish developmental potential of the oocyte.

Trial registration number: not applicable.

P-362 The effect of Liuwei Dihuang Granules on ovarian granulosa genomics of IVF patients with Kidney-yin deficiency

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Study question: To study the differences of granulosa cell gene expression pretherapy and post-treatment and to analyze the mechanism of Chinese herbs on the infertility patients with Kidney-yin deficiency by genomics.

Summary answer: After treating with Chinese herbs, patients' gene expression differences were detected, cell proliferation and apoptosis were affected, leading to the improvement of their pregnancy outcomes.

What is known already: The ovarian granulosa cell has a close relationship with the growth of the oocyte and embryo. In traditional Chinese medicine, Kidney Governing Reproduction Theory is a crucial element of Visceral Manifestation Theory. According to our former subject research supported by Chinese National Natural Science Fund, Chinese herbs for tonifying Kidney-yin can improve the quality of oocyte and embryo. However, the underlying mechanism is not known. In this work, we explore the scheme that how the Chinese herbs for tonifying Kidney-yin improve the quality of the oocyte and embryo, and hence improve pregnancy outcomes based on the ovarian granulosa cell genomics.

Study design, size, duration: Sixty-six infertility patients aged from 25 to 40 with Kidney-yin deficiency syndrome, were randomly assigned to either a treatment group or a control group according to a random number table, 33 cases in each group. Besides Western routine therapy, Chinese herbs Liuwei Dihuang Granule (LDG) were given 3 menstrual cycles before IVF to the treatment group, and placebo granules to the control group.

Participants/materials, setting, methods: The scores of Kidney-yin deficiency symptoms were assessed, the rates of high quality oocytes/embryos and clinical pregnancy rate were recorded, and the granular cell was collected on the day when the ovum was picked up. With high-throughput gene sequencing technology, the differential gene expression was detected by Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Main results and the role of chance: The syndrome score in the treatment group decreased significantly(from 17.36 ± 2.41 to 9.06 ± 2.47)($P < 0.05$), while it changed insignificantly in the control group(from 16.79 ± 2.61 to 16.55 ± 2.53) ($P > 0.05$). The high quality rates of oocytes and embryos and clinical pregnancy rate were all higher in the treatment group than the control group (83.76%&74.44%,77.89%&68.44%,54.55%&30.30%,all $P < 0.05$). After Chinese herbs' treatment, 280 differential expressed genes were detected, of which 156 up-regulating genes and 124 down-regulating genes. These differentially expressed genes were, according to GO functional, mainly related to the defense response, inflammatory response, cytokine activity, regulation of cell proliferation, and regulation of apoptosis; according to KEGG classification, involved multiple signal transduction pathways, including immune function, apoptosis, metabolism, and human cytokine receptor interaction.

Limitations, reasons for caution: The sample size was small and all cases were collected at our reproductive center. The patients with Kidney-yin deficiency syndrome were collected from those undergoing in vitro fertilization-embryo transfer (IVF-ET) and these results may not reflect patients in the general population.

Wider implications of the findings: Genes expression differences were detected after treated with Chinese herbs, providing a proof for the mechanism of action on Chinese herbs. This research looks for the targets of Chinese medicines, applying to clinical diagnosis, treatment and drug development, to seek for theoretical breakthrough in Kidney Governing Reproduction Genomics Mechanism.

Trial registration number: ChiCTR-IOR-17010516

P-363 Perinatal outcomes following stimulated versus unstimulated IVF: a systematic review and meta-analysis

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Study question: Does ovarian stimulation affect perinatal outcomes of preterm birth (PTB) and low birthweight (LBW) following *in vitro* fertilisation (IVF) treatment?

Summary answer: There was a higher risk of PTB and LBW following stimulated compared to unstimulated IVF.

What is known already: Women with infertility have an increased risk of adverse perinatal outcomes. The incidence of adverse perinatal outcomes is also higher following assisted reproductive treatments (ART) compared to spontaneous conceptions. It is an on-going debate whether underlying infertility or the various procedures involved during ART contribute to these risks. It is a matter of interest if ovarian stimulation routinely used during IVF affects perinatal outcomes. We conducted a systematic review and meta-analysis of

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studies comparing stimulated versus unstimulated IVF, to overcome the current inconsistencies in literature and provide an objective answer

Study design, size, duration: We conducted a systematic review to evaluate the risk of PTB, early PTB, LBW and very LBW following stimulated versus unstimulated IVF. The following electronic databases were searched from 1980 to September 2016: Medline, EMBASE and Web of Science. Study selection and data extraction were conducted independently by two reviewers. A meta-analysis was carried by pooling eligible studies.

Participants/materials, setting, methods: We included cycles involving stimulated IVF and unstimulated IVF (natural cycle IVF or modified natural cycle IVF). Only studies which reported singleton live births following a fresh IVF cycle were included. Perinatal outcomes were LBW (birth weight <2500 grams), PTB (live birth <37 weeks gestation), very LBW (birth weight <1500 grams) and early PTB (live birth <32 weeks gestation). Data synthesis and analysis was done using the Rev Man 5.3 software

Main results and the role of chance: Four studies with 97 702 participants were included in meta-analysis including one RCT and three cohort studies. The risk of PTB (risk ratio RR 1.25, 95% CI 1.01-1.56; 4 studies, I^2 0%), LBW (RR 1.95, 95% CI 1.03 -3.67; 4 studies, I^2 44.3%), early PTB (RR 4.42, 95% CI 1.61-12.13; 3 studies, I^2 0%) were significantly higher in stimulated IVF compared to unstimulated IVF. The RR for very LBW was 5.18 (95% CI 1.00-26.87; 2 studies, I^2 0%).

Limitations, reasons for caution: We were unable to perform age or BMI adjusted comparisons within the meta-analysis framework due to lack of information.

Wider implications of the findings: The increased risk of PTB and LBW following stimulated compared to unstimulated IVF could suggest a possible contributory role of ovarian stimulation. However, the effect size is likely to be small.

Trial registration number: Not applicable.

P-364 Digital-holographic-microscopy as a potential method for fertility diagnostic

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Study question: Do sperm motility parameters change in the presence of oocytes with or without the cumulus cells in a four dimensional way?

Summary answer: Motility characteristics of capacitated and uncapacitated sperm close to the oocyte and related to cumulus cells was identified with digital-holographic-microscopy in a four dimensional way.

What is known already: The fusion of egg and sperm is the basis for a successful fertilization. Therefore only sperm with modifications of their swimming behavior in response to fluid flow (rheotaxis) and temperature (thermotaxis) are capable of penetrating the oocyte. Another parameter of sperm selection is the episodic rolling and transient attachments of sperm. Only motile, hyperactivated and acrosome-intact sperm are able to penetrate the cumulus oophorus and bind to the zona pellucida in special to the zona pellucida protein 2 (ZP2). In most studies the focus on the sperm movement and the oocyte is limited to a two dimensional way.

Study design, size, duration: In this trial a difference in swimming behavior and other motility parameters like curvilinear velocity (VCL), straight-line velocity (VSL) and also the averaged path velocity was analyzed. The influence of the cumulus cells on these parameters was studied. With these results sperm should be identified with all qualifications for a successful fertilization.

Participants/materials, setting, methods: For the study we analyzed mouse sperm and oocytes of NMRI mice. After superovulation via hCG-injection, oocytes were dissected and in some cases cumulus cells were removed using a hyaluronidase-containing medium. The sperm were dissected out of the ductus deference via swim-out procedure in a physiological buffer. Both, sperm and oocyte were collected to a dish and analyzed under the digital holographic microscope directly. With the use of numerical algorithm the swim trajectory was analyzed.

Main results and the role of chance: Four dimensional analysis of sperm movement on their way to the oocyte was measured with the use of digital-holographic-microscopy. Different motility parameters of capacitated and uncapacitated sperm were measured. Capacitated sperm showed a higher amplitude, asymmetrical flagellar bending, which was compared with higher rates in their curvilinear velocity (VCL). These changes lead capacitated sperm to penetrate the cumulus mass easier and faster. Also larger XY-excursions of the tracked head were detected, which seemed to qualify these sperm to overcome an obstacle like the cumulus mass. We performed a comparative study of swimming behavior in the presence of cumulus mass and without to show the importance of this specific cluster of cells.

Limitations, reasons for caution: All results were generated in-vitro, which could be different in-vivo. Until now, the results were generated only with one species (mouse).

Wider implications of the findings: This method provides conditions which lead to specific changes in the sperm movement. Digital-holographic-microscopy could become a method for selecting sperm with optimal parameters for in-vitro-fertilization.

Trial registration number: None.

P-365 GnRH agonist for final oocyte maturation trigger and hCG as exclusive luteal support in normal responders, a prospective observational study

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Study question: Does GnRH agonist trigger result in comparable oocyte maturation rates and allows good cycle results in comparison to hCG trigger in normal responders?

Summary answer: GnRHa trigger and hCG trigger result in comparable oocyte maturation rates in normal responders treated with the GnRH antagonist protocol

What is known already: GnRHa trigger induces FSH as well as LH surge. Previous studies suggest that this more physiologic surge may promote oocyte maturation. A drawback of GnRH agonist trigger is the luteolysis which follows, necessitating modification of luteal support. Clinical studies regarding cycle results thus far resulted in conflicting results.

Study design, size, duration: This prospective, observational study was conducted in a single center from January 2012 to December 2015. 168 patients were enrolled in the study, at a 2:1 ratio between the control and the study groups

Participants/materials, setting, methods: Patients were stimulated using a GnRH-antagonist protocol. Each patient in the study group (n = 56) was matched to 2 controls (n = 112) based upon age and number of previous cycles. Final oocyte maturation in the study group was achieved with 0.2 mg triptorelin followed by two doses of 1500 IU hCG on the day of oocyte retrieval and 4 days later. The control group was triggered with 250 mcg hCG followed by luteal support with progesterone suppositories.

Main results and the role of chance: Demographic parameters of the study group and control group did not differ. A maturation rate of 70% was found in both groups. No significant difference was found in the number of oocyte retrieved (8.2 ± 4.3 vs 7.3 ± 4.2), fertilization rate in IVF (60 ± 20 vs 60 ± 30) or ICSI (80 ± 20 vs 70 ± 30) and the number of available embryos (4 ± 2.7 vs 3.2 ± 2.3). Similar results were also seen in positive pregnancy test (33.9% vs 32.%), implantation rate (19.3 ± 32 vs 21.7 ± 39) and live birth rate (25 vs 21.4). Pregnant patients from the study group had significantly higher mean progesterone levels (ng/ml) compared to pregnant patients in the control group (55.37 ± 12.86 vs 42.9 ± 22.21, p = 0.02).

Limitations, reasons for caution: This study is limited by its non-randomized design and small sample size. It has insufficient power to conclude that the live birth rate in both protocols is equivalent. Future randomized controlled trails are needed to establish its results

Wider implications of the findings: GnRH triggering with hCG luteal support in normal responder may be a valid alternative to the traditional hCG triggering with progesterone luteal support. This protocol combines the potential

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advantages of a physiological trigger with a simple, patient friendly, luteal support

Trial registration number: NCT01638026.

P-366 Prediction of tubal damage by using new serological markers for chronic Chlamydia trachomatis infection

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Study question: Does the presence of serum antibodies against *Chlamydia trachomatis* proteins TroA and HtrA expressed in chronic chlamydial infection predict tubal damage in subfertile women?

Summary answer: Subfertile women with serum IgG antibodies against *C. trachomatis* TroA and HtrA have tubal pathology more often than seronegative women.

What is known already: The association between serum antichlamydial antibodies and tubal factor infertility (TFI) is well known. However, positive chlamydial antibody testing (CAT) is a marker of previous exposure to *C. trachomatis*, but does not reflect the persistence of infection and the extent of tubal damage. TroA (YtgA) and HtrA are proteins expressed in chronic chlamydial infection *in vitro*. In addition, patients with ascending infection are shown to generate antibodies against these proteins. To identify the subgroup with persistent *C. trachomatis* infection and the highest risk for tubal pathology, we studied the antibody response against *C. trachomatis* TroA and HtrA among subfertile women.

Study design, size, duration: This is a prospective study consisting of subfertile couples referred to Helsinki University Hospital for infertility investigations. Between July 2007 and December 2010, a total 258 women were enrolled and 213 were followed to June 2014 or until the first successful pregnancy. Clinical data and the results of infertility examinations, treatments and subsequent pregnancies were collected.

Participants/materials, setting, methods: Serum specimens of the participants were tested for immunoglobulin G (IgG) antibody response to recombinant *C. trachomatis* proteins TroA and HrtA by enzyme immune assay (EIA). IgG antibodies for *C. trachomatis* major outer membrane protein (MOMP) and chlamydial heat shock protein (CHSP60) were analyzed by commercially available EIA kits. Chi-squared test and Mann-Whitney U-test were used for statistical analysis.

Main results and the role of chance: Altogether 55 (21.3%) of 258 women had anti-TroA IgG antibodies and 39 (15.1%) had anti-HtrA IgG antibodies. 26 (10.1%) women were positive for both anti-TroA and anti-HtrA antibodies. Tubal patency was examined in 209 (81%) women either by hysterosalpingosonography (HSSG) or laparoscopy. Ten out of the 209 women were excluded because of severe endometriosis or previous abdominal surgery with pelvic adhesions. The result of tubal examination was unclear in 14 women (6.7%). Tubal pathology was found in 24 of 185 women (13.0%). One tube was occluded in 19 (10.3%) women and both tubes in 5 (2.7%). Women with tubal occlusion were more likely to have anti-TroA and anti-HtrA antibodies than women without (41.7% versus 16.8%, $p = 0.011$ for anti-TroA; 33.3% versus 11.8%, $p = 0.011$ for anti HtrA and 25.0% versus 7.5%, $p = 0.016$ for both antigens). In addition, the seroprevalence rate increased with the severity of tubal damage. However, live birth rate did not differ by the serological status (86.8% versus 86.8%, $p = 1.000$ for anti-TroA; 82.8% versus 87.4%, $p = 0.553$ for anti-HtrA and 84.2% versus 87.0% for both antigens).

Limitations, reasons for caution: The participants were followed only in the Helsinki University Hospital area. Thus, we did not have data on patients who moved to a different locality or were treated in private clinics. Consequently, the group sizes followed in this study were relatively small.

Wider implications of the findings: Non-invasive methods are needed to identify the patients with chronic *C. trachomatis* infection and TFI. CAT traditionally used in infertility work-up does not discriminate the clearance or persistence of a *C. trachomatis* infection, while our results are more specific in evaluating the persistence of infection with higher risk for TFI.

Trial registration number: -.

P-367 Thioredoxin and Glutaredoxin systems are differently expressed in the oocytes of low responder patients compared with donors

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Study question: Do Thioredoxin (TRX) and Glutaredoxin (GRX) systems play a role in the redox regulation of the ovary?

Summary answer: TRX-GRX system is differently expressed in granulosa cells of low responders compared with donors suggesting that it plays a role in the ovarian redox regulation.

What is known already: Excessive Reactive oxygen species (ROS) production or oxidative stress have been associated with several diseases, including infertility. Oxidative stress causes ageing and dysfunction of the ovary, which is accompanied by gradual depletion of ovarian follicles and poor quality oocytes. To prevent ROS-induced disorders, cells are equipped with effective antioxidant systems. The thioredoxin (TRX) and glutaredoxin (GRX) systems are two central systems upholding the sulfhydryl homeostasis by reducing disulfides and mixed disulfides within the cell and thereby protecting against oxidative stress. Moreover, TRX is likely to be involved in the optimal growth and maturation of ovarian follicles and responsiveness to hyperstimulation.

Study design, size, duration: The mRNA expression of Thioredoxin reductase 1 (TXNRD1), Thioredoxin reductase 2 (TXNRD2) and Glutathione-disulphide reductase (GSR) were measured in oocyte-cumulus (CCs) and oocyte-granulose (CGs) complexes retrieved from 4 healthy fertile oocyte donors (<35 y.o. with at least a previous cycle with pregnancy) and compared with 4 low responders patients (<35 y.o. who had ≤ 5 oocytes retrieved after gonadotropic stimulation) whom took part in a prospective observational study realized from July to December 2016.

Participants/materials, setting, methods: All patients were stimulated with the same protocol (FSHr and triggering with GnRH analogues). GCs and CCs were collected from each patient and immediately fresh-frozen. mRNA was extracted using commercial kits and the mRNA expression of TXNRD1, TXNRD2, GSR and endogenous controls was measured by qRT-PCR. Relative changes in gene expression were calculated using the 2-ΔΔCT method. No parametric tests were used to identify any significant difference between groups. Statistical significance was set at $p<0.05$.

Main results and the role of chance: No significant differences were observed in the TXNRD1 gene expression of oocyte donors compared with low responders patients both in CGs ($p = 0.79$) and CCs ($p = 0.87$); whereas the TXNRD2 gene expression of donors was significantly higher than the one observed in the low responders patients both in CGs ($p = 0.031$) and CCs ($p = 0.015$). GSR gene expression was significantly reduced in low responders patients compared with donors, but this difference was only evident in CGs ($p = 0.121$) (CCs $p = 0.94$). TXNRD2 is predominantly localised in mitochondria, and seems to be important for the maintenance of mitochondrial function, presumably due to scavenging of mitochondrial ROS. Hence, its alteration in both CGs and CCs suggests the presence of a mitochondrial dysfunction that may contribute to oxidative stress.

Limitations, reasons for caution: The main limitation of this study was the relatively small sample size. Further studies are needed to elucidate the molecular and cellular mechanisms underlying the regulation of follicular TRX and GRX systems and their biological role in follicular function and activity.

Wider implications of the findings: To observe significant differences in the TRX system of low response patients compared with donors suggests that, in these patients, the antioxidant defenses are diminished. Consequently, oxidative damage occurs in the ovary. These findings could open up to new therapeutic strategies for the treatment of low ovarian reserve.

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Trial registration number: This study received no external funding and there were no competing interests.

P-368 What has changed for the definition of poor responders since the introduction of the Bologna criteria?

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Study question: Are the Bologna criteria (Bc) to define poor ovarian responders (POR) embraced by the IVF community?

Summary answer: Researchers seem reluctant to incorporate the Bc into clinical research. Since 2011, less than half of the published studies on POR has used the Bc.

What is known already: In an effort to standardize the definition of POR, the ESHRE Working Group on poor responders defined a set of variables named as the Bc in 2011.

Study design, size, duration: Systematic review of published and unpublished/ongoing trials on POR between September 2011 and September 2016.

Participants/materials, setting, methods: By using the relevant terms including 'poor response', decreased response', diminished ovarian response' and/or 'Bc', MEDLINE, PubMed, Ovid®, Google Scholar, and Scopus® electronic databases were searched for relevant trials. Ongoing trials were searched through www.clinicaltrials.gov. The references of the included studies were cross-searched for potentially missed articles. Only clinical trials providing an evidence level ≥II were included. Extracted studies were reviewed and divided into two groups; studies in which the BC was used or not.

Main results and the role of chance: Ninety-four published clinical studies analyzing a total of 19853 women and 107 unpublished/ongoing trials were identified. The Bc was used to define poor responders in 42 (44.6%) of the published and 40 (37%) of the unpublished trials. The vast majority of the published studies on poor responders were retrospective cohort or case control studies and 55% utilized the criteria. Randomized controlled trials, 29.4% of which preferred the Bc constitute only a small fraction (18%) of all published studies. The number of retrieved oocytes after ovarian stimulation was the most commonly preferred primary outcome regardless of the Bc used or not used. Since 2011, the practice of the Bc has gradually increased from 33% to 42% by the end of 2016 ($p = 0.98$). The majority of the published studies on POR were reported from Asia. European researchers were more likely to use the criteria compared to Asian and American researchers (62%, 48% and 24%, respectively). Neither the design of the study nor the impact factor of the publishing journal was related with the utilization of the Bc.

Limitations, reasons for caution: Interpretation of the data should take into account the descriptive nature of the data based on a literature search.

Wider implications of the findings: The definition of POR continues to be vague. It seems that the problem has not been resolved by the Bc, since many of the recently published trials as well as the registered trials keep on employing other, somewhat arbitrary definitions.

Trial registration number: None.

P-369 Can high markers of ovarian reserve overcome the usual age-related decline in euploidy in IVF-PGS cycles in women over age 40

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Study question: Are patients over 40 with average or above-average baseline markers of ovarian reserve protected against the typical age-related increase in aneuploidy?

Summary answer: Women with high markers of ovarian reserve and those with polycystic ovary syndrome (PCOS) retain a chance of obtaining a euploid embryo even at ≥44.

What is known already: Advanced reproductive age increases the likelihood of poor ovarian response and aneuploidy. Prior data suggests that there may be an age threshold beyond which above-average markers of ovarian reserve no longer increase viable pregnancy rates in in-vitro fertilization (IVF) cycles without preimplantation genetic screening (PGS).

Study design, size, duration: Retrospective cohort study of IVF-PGS cycles at an urban academic fertility center from 2013-2016.

Participants/materials, setting, methods: IVF-PGS cycles for women aged 40 and above using autologous oocytes were included. The cycle protocol was determined by the individual physician. PGS was performed using qPCR and Next Generation Sequencing of trophectoderm biopsies on expanded blastocysts. Descriptive statistics, Pearson's correlation, Mann-Whitney U and Kruskal-Wallis tests were computed as appropriate using Stata v12.1 (College Station, TX).

Main results and the role of chance: Of 990 IVF-PGS cycles included, 45.8% resulted in at least one euploid embryo; only about 5% yielded more than 2. The numbers (%) of cycles resulting in no euploid embryo were as follows: 129/330 (39.1%) for 40yo, 113/236 (47.9%) for 41yo, 118/194 (60.8%) for 42yo, 97/130 (74.6%) for 43yo, and 80/100 (80.0%) for 44yo and above. In each age category, the median anti-Müllerian hormone (AMH) level for those with at least one euploid embryo was substantially higher than age-based averages, ranging from 1.2-1.8 ng/mL; the median antral follicle count (AFC) in these women ranged from 8-10. AMH ($p<0.001$) and AFC ($p<0.001$) were higher, and day 3 follicle-stimulating hormone (FSH) ($p<0.02$) lower, in those women with a euploid embryo. However, AMH had the strongest correlation ($\rho=0.39$, $p<0.001$) with the number of euploid embryos and was most consistent across age groups, followed by AFC ($\rho=0.31$, $p<0.001$) and then FSH ($\rho=-0.10$, $p = 0.003$). Body mass index did not correlate with count of euploid embryos. Women with PCOS had a significantly higher median number ($p = 0.01$) of euploid embryos, 1 [0,2] versus 0 [0,1] for non-PCOS women.

Limitations, reasons for caution: The study was retrospective in nature, and certain confounders, such as ethnic background, may remain unaccounted for. As we move toward exclusive testing via a next-generation sequencing platform, it is possible that more embryos will be found to have some degree of aneuploidy, potentially changing the results reported here.

Wider implications of the findings: The age-related increase in aneuploidy is inevitable. However, women with ovarian reserve markers exceeding age-based averages retain a substantial chance of obtaining a euploid embryo despite advanced reproductive age. IVF-PGS in this population allows for identification and selective transfer of embryos with increased potential to develop into viable pregnancies.

Trial registration number: not applicable.

P-370 Is diagnostic hysteroscopy more useful than transvaginal ultrasound prior to IVF?

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Study question: Is diagnostic hysteroscopy prior to in vitro fertilization (IVF) useful in infertile females?

Summary answer: Hysteroscopy (HSC) combined with endometrial biopsy is superior than transvaginal ultrasound (TVS) alone in diagnosing unsuspected intrauterine anomalies interfering with fertility.

What is known already: IVF-workup focusses on the assessment of ovulation and visualisation of the female anatomy. Uterine structural abnormalities can contribute to subfertility in 10% of all cases. Visualisation can be achieved by: transvaginal ultrasound, hysterosonography, hysterosalpingography or hysterosalpingo foam sonography and diagnostic hysteroscopy with or without combination with laparoscopy. IVF success rates have been improved recently. Failure to implant is one of the biggest causes of IVF failure. Determining factors are the embryo's quality, endometrial receptivity and the uterine cavity's integrity. The latter is the most accessible for investigation and improvement. Most teams prefer HSC, without any justification for this choice.

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Study design, size, duration: This retrospective cross-sectional observational monocentric study was conducted at the Centre for reproductive medicine (CRG) at the Brussels University Hospital. 3087 cases were included in the study; all were recruited from the CRG between 2003 and 2012.

Participants/materials, setting, methods: Patients between 18 and 48 years old presenting with primary or secondary infertility were included. They all underwent a transvaginal ultrasound and, independent of the results, they all received an hysteroscopical examination and an endometrial biopsy. The examinations were performed by multiple gynaecologists at the University Hospital Brussels (UZB). The hysteroscopy examinations were performed on an outpatient basis.

Main results and the role of chance: In our study, we implemented 3087 infertile patients. They all underwent a transvaginal ultrasound to detect possible uterine anomalies interfering with their fertility. Of these, we found 2740 cases with a normal TVS (88.8%) and 347 cases with an abnormal TVS (11.2%). Independently to the outcome of the TVS, all patients received a hysteroscopical examination. In 2246 cases the hysteroscopy was normal (72.8%) and 841 cases with an abnormal hysteroscopy (27.3%). In 2179 cases both TVS and HSC were normal (79.5%). Both showed abnormal results in 280 cases (80,7%). However, in 67 cases TVS showed intrauterine abnormalities in absence of abnormalities on HSC (19.3%). In contrast HSC showed abnormalities in 561 cases in absence of abnormalities on TVS (20.5%). We calculated a correlation between TVS and HSC of 79.7%. The expected agreement expected by chance alone is 67.7%. Finally, for the agreement between TVS and HSC, we became a Kappa value of 0.37. This implied a low agreement.

Limitations, reasons for caution: The weakness is our retrospective study design. A prospective and cohort or case-control study would be able to implement the effective pregnancy outcomes and support the use of HSC and endometrial biopsy prior to IVF much stronger.

Wider implications of the findings: We showed that the prevalence of certain pathologies was not as expected by the literature and that TVS as a screening tool is lesser than the HSC combined with endometrial biopsy. Diagnostics but also early therapeutic interventions could be implemented at the same time and thereby rise IVF pregnancy rates.

Trial registration number: not applicable.

P-371 Can we turn back the clock? The effect of melatonin on expression of clock genes in the endometrium of women with recurrent implantation failure (RIF)

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Study question: Does the hormone melatonin alter the expression of clock genes in the decidualised and undifferentiated endometrium of fertile women and those with RIF?

Summary answer: The rhythmicity of clock genes is influenced by pathology within the endometrium of women with reproductive pathology. This expression may be altered with melatonin.

What is known already: Clock genes are found in stromal cells of the endometrium. Many aspects of reproductive function are strongly regulated by circadian rhythms and the expression of clock genes is different in RIF compared with healthy fertile women.

Endometrial dysfunction may occur if regulatory stimulation fails. If this dysregulation could be ameliorated, implantation might occur where it would have otherwise failed. The hormone melatonin, which regulates sleep and wakefulness, is also involved in modulating circadian rhythms and its cyclical release has pleiotropic effects. It is thought to be critical to reproductive functioning and this could also be mediated via the endometrium in humans.

Study design, size, duration: A case-control gene expression study (August 2013-2015) was performed in vitro to quantify mRNA levels of clock genes in human endometrial tissue. The level of gene expression was measured every 4 hours over a 36-hour period (in decidualised and non-decidualised endometrial stromal cells) to investigate how expression level changes over time. The affect of adding different concentrations of melatonin was compared with untreated cells in healthy endometrial tissue and in those women with RIF.

Participants/materials, setting, methods: Women between 25-45 years were recruited and baseline demographics and fertility characteristics were collected. Women were included if they suffered RIF or were fertile controls. Endometrial biopsies were taken by suction curette, the stromal cells were isolated and cultured in vitro before decidualisation was commenced by the addition of progesterone. Cells were sampled at baseline and four-hourly intervals. Total RNA extraction, cDNA synthesis and PCR (normalisation to ENOX2/PRDM4) of the 6 clock genes was performed.

Main results and the role of chance: Cyclical expression of the six clock genes (*Clock, Bmal1, Per1, Per2, Cry1* and *Cry2*) varied with the addition of melatonin. It appears to alter the relative expression of these genes and this effect continues over time (up to 36 hours) without resynchronising the peripheral 'clock' and independently of central control.

Previous studies have shown that the endometrium from fertile women displays a different rhythmicity of these genes compared with those women who have suffered poor reproductive outcomes. This suggests that any underlying pathology in RIF women may be related to dysynchrony at the level of the gene regulation within the endometrium.

The timing of events at the embryo-endometrial interface during the reproductive cycle may be altered by Clock-controlled genes, which in turn may be modified by melatonin. Future work should be aimed at demonstrating the level of this control and the mechanism of the effect as well as investigating the downstream pathways involved. Melatonin is inexpensive and widely used for entrainment of circadian rhythms but its potential therapeutic role in reproduction needs further experimentation.

Limitations, reasons for caution: Our results may be confounded by a small sample size within a cell-culture environment; a larger scale study in this specific subgroup of women is further needed to confirm our findings.

Wider implications of the findings: The expression of clock genes within the endometrium are modulated by the addition of melatonin. It is not known whether cellular functions of clock genes might have a role in the process of embryo implantation but if proven by further studies, melatonin might have a beneficial therapeutic effect.

Trial registration number: Regional ethics committee number 12/SC/0568.

P-372 The effect of body mass index on IVF outcome within a polycystic ovarian syndrome population

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Study question: Does high body mass index (BMI) in patients with polycystic ovarian syndrome (PCOS) affect ART outcomes?

Summary answer: PCOS patients with low BMI had better ovarian response to COH compared to patients with higher BMI but showed no significant difference in ART outcomes.

What is known already: It has been established that 35-65% of PCOS patients are obese. Obesity, overweight and elevated BMI are clinical features of PCOS that may constitute a major impediment for successful fertility treatment in women diagnosed with PCOS. Reduction of weight could improve fertility in PCOS patients who attempted spontaneous conception. Current evidence suggests that, regardless of PCOS, pregnancy achievement and maintenance is adversely affected by obesity, overweight or elevated BMI. However, few studies have examined the relationship between BMI and IVF treatment success in women with PCOS.

Study design, size, duration: A retrospective study was conducted using existing records on a consecutive sample of PCOS women who received IVF treatment between January 2012 and December 2016. A total of 181 cycles were included in this study.

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Participants/materials, setting, methods: BMI was calculated according to the standard formula: BMI = weight/height^2 (kg/m^2). All participants were stratified by BMI calculated from height and weight recorded at cycle start: group A (non-obese group: BMI < 24.9 kg/m^2) and group B (obese group: BMI > 25.0 kg/m^2). Of the total 181 cycles, 102 cycles were group A (non-obese group) and 79 cycles were group B (obese group).

Main results and the role of chance: During controlled ovarian hyperstimulation, obese group required more FSH dose than non-obese group [A:1240.2 IU vs. B:1653.8 IU, p<0.05]. Also, obese group needed more stimulation duration than non-obese group [A:9.1 days vs. B:10.5 days, p<0.05]. Non-obese group retrieved more oocytes than obese group [A:20.3 vs. B:14.9, p<0.05], but proportion of mature oocyte [A:55.1% vs. B:57.4%, p = 0.163] and fertilization rate [A:62.3% vs. B:65.5%, p = 0.264] were not different in both groups. Freeze all cycle rate was higher in non-obese group than in obese group due to large number of retrieved oocytes [A:44.1% (45/102) vs. B:20.3% (16/79), p = 0.0013]. Pregnancy rate did not differ between both groups [A:71.9% (41/57) vs. B:65.1% (41/63), p = 0.650]. Also, miscarriage rate had no significant difference between both groups [A:3.5% (2/57) vs. B:9.5% (6/63), p = 0.143]. For patients with PCOS, elevated BMI did not affect obvious poor IVF outcomes but required more FSH dose and stimulation duration.

Limitations, reasons for caution: The retrospective study design may have limited our ability to obtain accurate data on other potentially confounding factors such as pre-existing health conditions, smoking, alcohol and coffee consumption. Large prospective cohort studies are needed to confirm our study findings.

Wider implications of the findings: We did not observe a significant effect of BMI on final ART outcomes in PCOS patients. But, PCOS patients with low BMI had better ovarian response compared to patients with higher BMI. Therefore in PCOS patients with elevated BMI, weight reduction is an effective way to reduce cost and time.

Trial registration number: None.

P-373 The effect of intramural fibroids without an intracavitary component on in-vitro fertilization outcomes in single fresh blastocyst transfer cycles

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Study question: Does the presence of non-cavity deforming intramural fibroids alter the clinical pregnancy rates and live birth rates in single fresh blastocyst transfer cycles?

Summary answer: The presence of intramural fibroids reduces the chances of clinical pregnancy in fresh single blastocyst transfer cycles, even after adjusting for possible confounders.

What is known already: Submucosal fibroids are associated with lower clinical pregnancy rates (CPR) and live birth rates (LBR) from IVF. However, the current evidence for the impact of other fibroids is controversial. Literature bias exists because patients with large fibroids are likely to undergo myomectomy prior to IVF, multiple embryo transfer complicates analysis of results, as does repeated sampling of individual patients. A unique situation existed in Quebec; a long wait list for myomectomy (>1year), and the government funded 3 IVF cycles, resulting in all subjects with fibroids attempting at least one IVF cycle prior to surgery.

Study design, size, duration: A retrospective cohort study performed at a single academic reproductive center between January 2012 and June 2015. All single fresh blastocyst transfer cycles in women less than 43 years of age were included. Cycles with donor oocytes, multiple embryo transfers, submucosal fibroids, cavity distortion, and genetic screening were excluded.

Participants/materials, setting, methods: 929 fresh single blastocyst transfer cycles were included; 165 with and 764 without, fibroids. Only intramural fibroids were included in the analysis. Subjects with submucosal or subserosal leiomyomas were excluded. Cleavage embryo transfers were excluded to reduce bias based on embryo quality. T and chi square tests were used for demographic data, stepwise logistic regression was used to evaluate the effect of fibroids on outcomes.

Main results and the role of chance: In patients with intramural fibroids, the mean age was 36.3 ± 3.2 vs. 34.2 ± 3.9 years in patients without fibroids (p<0.001). There were no differences in; gravity, parity, body mass index or smoking in patients with and without fibroids. Women with intramural fibroids had lower antral follicle counts (AFC) (15.3 ± 9.2 vs.18.9 ± 12.3, p = 0.007) and higher doses of gonadotropins used (2350 ± 1368 IU vs. 2653 ± 404 IU, p = 0.04) than women without fibroids. The number of mature oocytes collected and the quality of embryo transferred were comparable. 63% of cases had 2 fibroids, 25% had 3 fibroids, and 12% had 4 or more fibroids. The mean largest fibroid diameter was 2.7 ± 1.7 cm with 44% of cases having a fibroid of ≥3 cm. Interestingly, CPR (47% vs. 32%, p = 0.005) and LBR (37.8% vs. 25.5%, p = 0.02) were lower in patients who had intramural fibroids compared to those who did not. Miscarriage rates were similar (15.8% vs. 19.4% p = 0.83). After adjusting for the confounding effect of age, AFC, dose of gonadotropins, and the quality of embryo transferred, CPR were significantly lower in patients with intramural fibroids compared to those without (OR 0.6, CI 0.37-0.96). Live birth rates also trended lower (OR 0.67, CI 0.41-1.11).

Limitations, reasons for caution: Although fibroids may reduce pregnancy rates, it remains unclear if a myomectomy will improve outcomes. The retrospective nature of this study raises the possibility of an undetected bias, in spite of the ideal population situation with 100% of women undergoing care prior to myomectomy, due to unique circumstances.

Wider implications of the findings: Women with intramural fibroids should be made aware that their chances of pregnancy are reduced regardless of the size of the fibroid, despite having a good quality embryo to transfer. Clinical pregnancy rates are still reassuring, but surgery could be discussed at the initial visit prior to starting IVF.

Trial registration number: not applicable.

P-374 Molecular networks underlying ovarian aging for clinical applications

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Study question: Our objective is to determine molecular pathways of ovarian aging that result in pregnancy failure and other complications in women health to develop treatment strategies.

Summary answer: Candidate molecular networks and medicines (chemicals) for targeting were proposed to develop treatment strategies or delay ovarian aging.

What is known already: Due to modern trends with postponing childbearing and getting worse living environment in women, an ovarian aging increased pregnancy failure and other complications with menopause or premature ovarian failure. Although several theories have been suggested such as mitochondrial malfunction, DNA damage/repair/methylation, caloric restriction, studies regarding ovarian aging-related molecular mechanisms for development of therapeutic methods are insufficient so far.

Study design, size, duration: This is an experimental study that is consisted of two parts: in Phase I stage, we analyzed distinct gene expression profile between young and aged mouse ovaries, and in Phase II stage several preferentially expressed genes in both ovaries were selected and analyzed their physiological functions and involved molecular networks related to ovarian aging for development of diagnostic markers and therapeutic methods.

Participants/materials, setting, methods: Ovaries from 10 week and 11 month-old FVB/NJ female mice with synchronized estrus cycle were collected for this study. A half of each ovary was used for RNA preparation and the other half for histological analysis. Using the Illumina HiSeq 2000 System, preferentially expressed genes were identified. Functional annotation database-based gene-set enrichment analyses and Pathway Studio® were employed to evaluate aging-related molecular networks. These findings were confirmed through qRT-PCR and immunohistochemistry.

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Main results and the role of chance: To validate RNA-Seq data, we examined expression patterns of marker genes (Amh, Bmp15 and Nobox) that were well-known to be decreased in ovarian aging process. In young or aged ovary, preferentially expressed 876 genes were identified and extracellular matrix (ECM; $p < 0.001$) and chromatin/nucleosome-related ($p<0.001$) protein-coded genes have the majority in these genes by GOTERM analysis. Among them, we selected several candidate genes and confirmed their expression profiles by qRT-PCR and immunohistochemistry followed by molecular network analysis. Expression levels of male enhanced antigen 1(Mea1), serum amyloid A3 (Saa3), peroxiredoxin 1 (Prdx1) and S100 calcium binding protein A9 (S100a9, calgranulin B) were significantly increased in aged ovary, whereas those of endothelin 1 (Edn1), seizure related gene 6 (Sez6), zinc finger protein 182 (Zfp182), a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 19 (Adamts19) and solute carrier family 18, member 2 (Slc18a2) were decreased. Regarding molecular interactions in these genes, Pathway Studio® was employed to predict aging-involved molecular networks in mouse ovary. Here we report a couple of candidate molecular networks and medicines (chemicals) for targeting these preferentially expressed genes/proteins. Further analyses are scheduled to produce transgenic animal models and with human ovarian tissues and cell lines.

Limitations, reasons for caution: This study does not include results from human materials, therefore it will be needed to verify their roles in ovarian aging using human ovarian tissues or cell lines for future clinical applications.

Wider implications of the findings: This study will provide new tools and enable formulation of new strategies to diagnose causes of ovarian aging and improve treatment options. It will be also providing new biomarkers useful to predict ovarian ages and reserve to care or rejuvenate aged ovaries.

Trial registration number: NA.

P-375 Link between cesarean section (CS) delivery and secondary infertility. Uterine cavity evaluation in women undergoing fertility treatment using three dimensional (3D) saline infusion sono-hysterogaphy (SIS)

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Study question: To determine the incidence of uterine cavity anomaly in women presenting with sub-fertility of at least two-years duration after successful pregnancy and birth by CS.

Summary answer: A high incidence of abnormal intrauterine morphology related to CS scar defect in association with intrauterine adhesion's with future fertility and obstetric implications were noted.

What is known already: Trauma to the basal layer of the endometrium from surgery or infection can lead to residual fertility impairing changes. This may occur from previous pregnancy or delivery. CS birth rate is increasing worldwide and remains the most invasive pregnancy related procedure on the uterus. It carries risk of endometrial trauma, infection and scarring resulting in intrauterine pathology associated with adverse pregnancy and obstetric outcome. There is a lack of systematic studies evaluating the incidence of uterine cavity anomalies in women presenting with sub-fertility following successful pregnancy and birth by cesarean section.

Study design, size, duration: This was a prospective observational study. Women presenting with sub-fertility (n = 510) of more than two years duration undergoing investigations prior to fertility treatment were included in the study for routine uterine cavity evaluation by 3D and SIS over a period of 18 months.

Participants/materials, setting, methods: Women seeking fertility treatment following previous CS who failed to conceive again after two years of trying or who returned for frozen embryo transfer FET (Group1, n = 108), were compared to age matched controls with no previous history of uterine surgery (Group 2, n = 402). Baseline 3D ultrasound scan followed by SIS was carried out between days 6 to 10 of the menstrual cycle using GE Voluson Logiq S8 ultrasound system.

Main results and the role of chance: In Group 1, 18/108 (16.6%) a defect of the CS scar line was demonstrated. In 6/18 (33.3%) of these, endometrial bands of aadhesions were associated with CS scar defect. While significantly ($p<0.01$) less number 4/90 (3.6%) of adhesions bands were observed in women who showed intact scar line. This was comparable to the 17/402 (4.2%) bands of adhesions observed in Group 2.

Ten of the 18 women (55.5%) who had defective scars in Group 1 demonstrated intrauterine fluid collection at the baseline 3D scan. This was significantly higher ($p<0.05$) than the 8/402 (1.8%) in Group 2 who had adhesion bands.

Total number with adhesion bands in Group 1, 10/108 (9.2%) was higher than in Group 2, 17/402 (4.2%) the difference was significant ($p<0.05$).

Endometrial polyp(s) or submucosal fibroid distorting endometrial cavity alone or together were observed in 12/108 (11.1%), 5/108 (4.6%), and 17/108 (15.7%) respectively in Group 1. These were comparable to 57/402 (14.1), 21/402 (5.2%) and 78/402 (19.4%) observed in Group 2

Limitations, reasons for caution: This study demonstrates a high incidence of potential fertility impairing observations secondary to CS delivery. Delivery by CS is increasing world-wide the implications of these findings for future fertility, pregnancy and delivery in the wider population must be interpreted with caution and awaits further studies.

Wider implications of the findings: CS delivery is associated with a high incidence of abnormal intrauterine morphology. These anomalies are a precursor of impaired endometrial receptivity and a risk factor not just for future fertility but obstetric outcome. The implications of scar defect for future obstetric performance is unknown and requires further investigations

Trial registration number: none.

P-376 Effect of regular exercise on reproductive function of aged female mice

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Study question: Can regular exercise improve the expression of ovarian anigogenic factors, oocyte quality and fertility potential in aged female mice

Summary answer: Regular exercise induced by illumination with incandescent lights in aged mice improves their reproductive outcomes by improving ovarian function and oocytes quality.

What is known already: Some studies have reported the relation between exercise and angiogenesis (Gustafsson et al., 2001; Prior et al., 2004). Previously it has been reported that a single bout of moderately intense treadmill running upregulates VEGF expression of skeletal muscle in rat (Breen EC et al., 1996) and upregulation of VEGF mRNA also occurs during exercise in humans in both normal healthy individuals (Gustafsson et al., 1999; Richardson et al., 1999) and patients with heart failure (Gustafsson et al., 2001).

Study design, size, duration: Forty female mice of 30-32 weeks were divided into the two groups. One group (n = 20) were induced to stimulate exercise (physical activity) by illuminating incandescent lights (60 Watts, 220 V), placed on the top of the cage, starting at 11:00 a.m. for 30 minutes daily. The other group (n = 20) were served as control without the induction of exercise.

Participants/materials, setting, methods: C57BL inbred mice were used and purchased from Korea Experimental Animal Center (Daegu, South Korea). Mice were maintained on a light-dark cycle, with light on at 5:00 AM and off at 7:00 PM, and with food and water available ad libitum.

Main results and the role of chance: The total number of pregnant mice was 15 (75%) in the exercise group, which was significantly higher than 5 (25%) in the control group ($P < .05$). The mean number of offspring was also significantly higher in the exercise group (9.2) than the control group (6.3) ($P <.05$). The mean number of one-cell embryos retrieved and blastocyst formation rate were 12.6 and 43.8% in the exercise group and 10.8 and 8.1% in the control group with a significant difference ($P < .05$). Ovarian VEGF and eNOS expression was increased, but ovarian apoptosis was decreased in the exercise group.

Limitations, reasons for caution: First, this study did not use a general exercise equipment, such as treadmill. Second, this study did not examine the effect of exercise on implantation because a lot of studies have shown that physical activity can affect implantation of endometrium

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Wider implications of the findings: This study did not clearly elucidate the mechanism that exercise treatment results to improve ovarian function and oocyte quality. However, this study showed the increased ovarian VEGF and eNOS expression and decreased ovarian apoptosis.

Trial registration number: none.

P-377 Single human spermatozoon freezing technique for cryptozoospermia or non-obstructive azoospermia patients

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Study question: Is it possible to freeze a single spermatozoon and keep a high recovery rate to improve the clinical outcome of cryptozoospermia or non-obstructive azoospermia patients?

Summary answer: Our data indicates that it is highly possible to freeze a single spermatozoon with a high survival rate of about 80%.

What is known already: Clinical outcome of non-obstructive azoospermia or cryptozoospermia has increased after ICSI when using fresh spermatozoa. When oocyte collection cannot be performed on the same day of the Micro-TESE or in the case of cryptozoospermia, cryopreservation of collected spermatozoa becomes crucial. However, conventional freezing procedures are not appropriate for very low numbers of spermatozoa with poor motility.

Study design, size, duration: We performed a retrospective analysis of the clinical outcome of 79 ICSI cycles using our novel cryopreservation procedures for ejaculated spermatozoa from 27 cases of cryptozoospermia in 52 cycles and testicular spermatozoa from 20 cases of non-obstructive azoospermia in 27 cycles from January, 2012 to December, 2014.

Participants/materials, setting, methods: This study dealt with 27 men with cryptozoospermia and 20 men with non-obstructive azoospermia. Moving spermatozoa were carefully aspirated into a pipette one by one and put into a micro-drop of medium. About 2 µl of freezing medium was put on the tip of the CRYOTOP. 1-10 sperms were aspirated into an injection pipette and inserted into the medium and left in liquid nitrogen vapor for 2 min before being stirred in liquid nitrogen.

Main results and the role of chance: Five healthy babies (5 ongoing) from 47 patients were born following this freezing method. Clinical outcome is listed in the following table.

Clinical outcomes	cryptozoospermia	Non-obstructive azoospermia
No. of patients	27	20
No. of treatment cycles	52	27
No. of sperm frozen [per Cryotop®]	543	248
Sperm recovery rate after thawing (%)	81.03 (440/543)	78.23 (194/248)
Motile sperm rate after thawing (%)	57.27 (252/440)	40.72 (79/194)
No. of ICSI oocytes	215	98
Fertilization rate (%)	33.02 (71/215)	37.76 (37/98)
No. of embryos cultured for 5 days	42	19
Blastocyst rate (%)	45.24 (19/42)	36.84 (7/19)
No. of cycles transferred embryos	28	13
Clinical pregnancy rate (%)	28.57 (8/28)	38.46 (5/13)
Miscarriage rate (%)	25.00 (2/8)	20.00 (1/5)
No. of ongoing pregnancies	2	3
No. of births	4	1

Limitations, reasons for caution: This freezing procedure needs a special apparatus (Inverted microscope equipped with a micromanipulator) and requires a high level of technique for gathering and expelling 1-10 spermatozoa correctly and swiftly. So this procedure can only be performed by specialists, precious sperm can easily die with poor technique.

Wider implications of the findings: Once this procedure is mastered, the clinical outcome of severe male infertility is definitely improved. Especially in the treatment of azoospermia, precious spermatozoa surgically collected from epididymis or testicles can be cryopreserved to avoid repeating a biopsy. This procedure can lessen the patients' physical, mental and financial burdens.

Trial registration number: None.

P-378 Water- and oil-based tubal flushing for subfertility, alone, both or none? Systematic review and network meta-analysis

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Study question: Can tubal flushing with oil-based contrast media increase pregnancy rates in women with subfertility compared with water based media and no tubal flushing?

Summary answer: Tubal flushing with oil-based contrast media increases pregnancy rates in women with subfertility, compared to tubal flushing with water-based contrast media and no tubal flushing.

What is known already: The use of tubal flushing with either water- or oil-based contrast media as a therapeutic intervention has been controversial. In pairwise meta-analysis, the effectiveness of these two interventions cannot be compared to no treatment in a single statistical model and indirect evidence in these comparisons has not been used.

Study design, size, duration: We searched electronic databases including MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials as well as reference lists to identify eligible studies. We performed a systematic review and network meta-analysis of relevant randomised controlled trials (RCTs).

Participants/materials, setting, methods: We included RCTs comparing the following interventions with each other or no treatment in women with subfertility: tubal flushing with water-, oil-based contrast media, or in combination. The primary outcome was clinical pregnancy rate. Network meta-analyses were conducted within a random effects multiple regression model in Stata 14 (StataCorp, USA).

Main results and the role of chance: We included 14 RCTs reporting on 3,680 women. Network meta-analyses showed that tubal flushing with oil-based contrast media led to significantly higher pregnancy rates, compared to tubal flushing with water-based contrast media (odds ratio [OR] 1.48, 95% confidence interval [CI] 1.12-1.96) and no tubal flushing (OR 2.46, 95% CI 1.48-4.10). Oil- and water-based contrast media combined were superior to water-based contrast media alone (OR 1.56, 95% CI 1.02-2.39), while this

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combination was not better than oil-based contrast media alone (OR 1.05, 95% CI 0.66-1.68). It is not certain whether water-based tubal flushing is more effective than no flushing (OR 1.67, 95% CI 1.00-2.78) in terms of clinical pregnancy.

Limitations, reasons for caution: The overall quality of the evidence was low in comparisons other than water- versus oil-based contrast media. The heterogeneity should also be taken into consideration in decision making. The diagnostic methods of pregnancy were not specified in all included studies and live birth rates were reported in only six studies.

Wider implications of the findings: Since oil-based tubal flushing during tubal patency test improves pregnancy rates as compared no treatment, hysterosalpingography with oil-based tubal flushing should be offered to women with subfertility at an early stage of the infertility work-up.

Trial registration number: Not applicable.

P-379 Two-year continuation rates of long-acting reversible contraceptive methods: A cohort study in the French national healthcare insurance database

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Study question: Are the Long-Acting Reversible Contraceptives (LARCs) associated with higher continuation rates than Oral Contraceptives (OCs) in real life practice?

Summary answer: This real-world study shows that the two-year continuation rates for LARCs were more than two-fold higher than OCs.

What is known already: The number of unintended pregnancies and abortions remains high despite efficient contraceptive methods. Poor continuation rates associated with contraceptive methods have been identified as a risk factor for unintended pregnancy. Long-acting reversible contraceptives (LARCs), such as contraceptive implants and copper or hormonal intrauterine devices (IUDs), are among the most effective birth control methods because they do not require users' action after insertion, but continuation rates in real life practice need to be investigated.

Study design, size, duration: This is a retrospective cohort study based on the EGB ("*Echantillon Généraliste des Bénéficiaires*") database which is a representative sample of the general population of subjects affiliated with the French health insurance system. The EGB database includes approximately 600,000 beneficiaries. Continuation rates were assessed in a cohort of women having used any reimbursed contraceptives from 2009 to 2012.

Participants/materials, setting, methods: Women aged 15 years and over and initiating contraception during the period 2009–2012 were included. The start of contraception was the date of the first dispensation for OCs and the date of the insertion for LARCs. The end of contraception was presumed if there was a removal for LARCs, a dispensation of a new type of contraception, a pregnancy, sterilization or the absence of dispensation at the end of the covered period.

Main results and the role of chance: A population of 42,635 women with a reimbursed contraceptive was identified in the EGB. Among the 42,635 women, 74.6% used oral contraceptives (OCs), 22.0% intrauterine device (IUD) and 3.5% subdermal contraceptive etonogestrel implant (Nexplanon®). The 24-month continuation rate was highly variable according to the method with 28.6% (95% confidence interval, 27.9–29.3) for 1st-2nd generation combined OCs, 34.7% (33.6–35.9) for 3rd generation combined OCs, 9.5% (8.4–10.6) for progestative-only OCs, 88.6% (87.3–89.7) for copper IUD, 91.4% (90.5–92.3) for hormonal IUD and 84.6% (82.0–86.8) for the etonogestrel implant.

Limitations, reasons for caution: For OCs, only the dispensation date was available in the database. The discontinuation date was estimated according to the number of delivered pills. For LARCs, insertion and removal were often not recorded in the database, and hypotheses were made based on recorded physician consultations.

Wider implications of the findings: This real-world study shows that among French reimbursed contraceptives, LARCs had the highest continuation rates thus indicating their good acceptability. The use of LARCs remains, however, too limited and training measures for health care professionals could help to initiate LARCs more largely.

Trial registration number: 0.

P-380 Quantitative assessment of endometrial volume and uterine vascularity and pregnancy outcome in frozen thawed embryo transfer cycles

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Study question: to investigate the usefulness of the endometrial volume and vascular indicies in endometrial region as an effective predictor for pregnancy outcome in frozen-thawed embryo transfer (FET).

Summary answer: This study suggest that endometrial volume and the vascular indices measured in endometrial region are a useful predictor for pregnancy outcome.

What is known already: Kim et al. measured the vascular indices at endometrial region and subendmetrial region within in 5 mm from the endometrial border. They reported the pregnant group had higher vascular indices in endometrial region, not in subendometrial region than non-pregnant group.

Study design, size, duration: We evaluated 131 embryo transfer cycles in 73 infertile women. After controlled ovarian stimulation all embryos were cultured to blastocyst stage, and the blastocysts with good quality were vitrified for elective FET.On the day of FET, endometrial thickness, endometrial volume, pulsatility index (PI), and resistance index of uterine artery and endometrial-subendometrial vessels (ESVs) with zonal discrimination were evaluated by transvaginal ultrasonography in each patient. These variables were compared between pregnant and nonpregnant cycles.

Participants/materials, setting, methods: We prospectively conducted this study from October 2013 to December 2014 in Infertility Center, Pusan National University Hospital. A total of 73 infertile women were recruited for this study and agreed with informed consent.

Main results and the role of chance: The endometrial volume was significantly higher in pregnant group (2.32 ± 0.86, 1.96 ± 0.62 mL, P = 0.007). Also, PI of endometrial-subendometrial vessels (ESV) was significantly higher in pregnant cycle (2.58 ± 1.32 and 2.05 ± 1.08, P = 0.016). The other variables were not different between two groups.

Limitations, reasons for caution: the number of FET cycles was relatively small. Second, the samples were collected over one year. It was relatively long period and the possibility of intra-observer variation was increased. Though these limitation, this study has several strong points. All cases were elective FET cycles and collected prospectively without omission.

Wider implications of the findings: this study suggest that endometrial volume measuring by 3D ultrasonography and the vascular indices measured in endometrial region are useful tools for predicting pregnancy in FET. If further studies with standard measuring technique are conducted, the useful prognostic protocol with ultrasonography could be established.

Trial registration number: none.

P-381 The effect of strict sperm morphology and activity/motility on IUI clinical outcome

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Study question: This study was intended to evaluate whether the value of Kruger strict sperm morphology (SSM) and sperm motility (SM) could predict pregnancy after IUI

Summary answer: The values of SSM have affected IUI pregnancy rates. When the SSM is 1-3% then the IUI pregnancy rate may increase based on the SM.

What is known already: Sperm analysis is the primary method of evaluating the fertility potential of the male partner. Parameters include sperm concentration, motility and morphology. This retrospective chart review evaluated whether isolated abnormal sperm morphology in men with otherwise normal concentration and motility affected pregnancy rates for IUI procedure.

Study design, size, duration: The study was conducted in prospective observational manner since 2016 with 252 IUI cycles. IRB consent was obtained from each participant.

Participants/materials, setting, methods: The couples attending IUI were recruited for the study at a single fertility clinic. IUI was performed after spontaneous LH surge or hCG induced ovulation in induction cycle. IUI was prepared using swim- up method. Kruger strict sperm morphology value was calculated from 300 sperms according to 2010 WHO manual and criteria.

Main results and the role of chance: The average ages of wife and husband, number of IUI procedure, pre-washed semen volume, total sperm concentration, motility were (mean±SD): 33.6 ± 2.9, 35.9 ± 3.4, 2.9 ± 1.7 ml, 80.1 ± 52.2 ×106, 50.7 ± 24.3%, respectively.

There were 43/252 (17.1%) positive clinical pregnancy were observed among known outcomes. The pregnancy rate increased as its near to the normal SSM range. When SM is in 1~3% range and SM is 40%, the pregnancy rate has increased.

The relationship between SSM values and IUI outcomes is summarized as below:

Kruger Stric morphology value	1%↓	1%	2%	3%	4%↑
Cycle numbers	18	59	87	61	27
Clinical pregnancies	1	9	16	11	6
Clinical pregnancy rate	5.5%	15.3%	18.4%	18.0%	22.3%

	SM: 1-3%	> 20×106	> 40%
Cycle numbers	207	189	138
Clinical pregnancies	36	33	29
Clinical pregnancy rate	17.3%	17.5%	21.0%

Limitations, reasons for caution: Non-randomized, non-controlled nature of the study. Heterogenous population with various infertility factors, method of ovulation induction and treatment order.

Wider implications of the findings: The clinical outcome may be increased when SM is 1~3% with sperm count (20×106 over) motility(40% over) in IUI and consequently this criteria can be used in counselling of infertility couples to accelerate their decision for ART.

Trial registration number: Not applicable.

P-382 High risk of preterm birth and low birth weight following oocyte donation compared to autologous IVF: a systematic review and meta-analysis

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Study question: Is oocyte donation-recipient IVF treatment associated with a higher risk of adverse perinatal outcomes of preterm birth (PTB) and low birth weight (LBW)?

Summary answer: There is a higher risk of PTB and LBW following oocyte donation (OD) compared to autologous IVF.

What is known already: The trend towards delayed childbearing has been reflected in a steady increase in the average age of women undergoing IVF. Advances in the field of IVF have proven insufficient to compensate for the decline in fertility resulting from female reproductive ageing. Oocyte donation has been offered as a more successful alternative for these women contributing to a steady increase in the number of OD cycles worldwide. Although available studies have indicated higher incidence of poor obstetric outcomes following OD in comparison with spontaneous conceptions, evidence comparing perinatal outcomes following OD versus autologous IVF pregnancies has not been consistent.

Study design, size, duration: We conducted a systematic review to evaluate the risk of PTB, early PTB, LBW and very LBW following OD versus autologous IVF. The following electronic databases were searched from 1980 to December 2016: Medline, EMBASE and Web of Science. Study selection and data extraction were conducted independently by two reviewers. A meta-analysis was carried by pooling eligible studies.

Participants/materials, setting, methods: A total of 6 studies including 19,784 and 187,964 singleton live births following OD and autologous IVF respectively met the inclusion criteria for the various outcomes. Six studies reported data for PTB (live birth at <37 weeks gestation), three studies for early PTB (live birth at <32weeks gestation), five studies for LBW (<2500 grams) and three studies for very LBW (<1500 grams).

Main results and the role of chance: Meta-analysis of the included studies showed a significantly higher risk of PTB, early PTB, LBW and very LBW with OD pregnancies compared to autologous IVF: Relative risk (RR) 1.45; 95% CI 1.24-1.71 for PTB, RR 2.14; 95% CI 1.40 -3.25 for early PTB, RR 1.31; 95% CI 1.10-1.56 for LBW and RR 2.08; 95% CI 1.13-3.84 for very LBW.

Limitations, reasons for caution: We were unable to perform age-adjusted comparison within the meta-analysis framework, although individual studies included our meta-analysis had performed adjustment for confounding factors including.

Wider implications of the findings: These findings are useful for fertility clinicians in informing their patients and for obstetricians whilst managing OD-recipient pregnancies.

Trial registration number: Not applicable.

P-383 Clinical outcomes of frozen transferred blastocysts derived from monopronucleated (1PN) zygotes after conventional in vitro fertilization (cIVF) and intracytoplasmic sperm injection (ICSI) cycles

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Study question: To examine the clinical outcomes of frozen transferred blastocysts derived from monopronucleated (1PN) zygotes after conventional in vitro fertilization (cIVF) and intracytoplasmic sperm injection (ICSI) cycles.

Summary answer: Pregnancy rate was 31.4% after transfer of 1PN blastocysts in cIVF cycles. After transfer of 1PN blastocysts in ICSI cycles, pregnancy rate was 5.3%.

What is known already: The blastocyst formation rate of 1PN embryos was lower than 2PN embryos in cIVF and ICSI cycles. 1PN blastocysts in cIVF, and not from ICSI, demonstrated an adequate ongoing pregnancy rate. 1PN blastocysts in cIVF are available for clinical use and may lead to an increase in the chance of pregnancy in patients receiving assisted reproductive technology with 1PN embryos.

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Study design, size, duration: This was a retrospective study at a reproductive center. To evaluate clinical outcomes, cIVF or ICSI cycles with 1PN embryos were compared with those with 2PN embryos. This study included single frozen blastocyst transfer cycles performed between January, 2007 to December, 2014 at our clinic.

Participants/materials, setting, methods: Women's age were limited between 30 to 40 years old at the time of freezing. We compared clinical outcomes between 3,576 cycles of 2PN (IVF-2PN,1,863 couples) in cIVF, 35cycles of 1PN (IVF-1PN,34 couples) in cIVF, 4,793cycles of 2PN (ICSI-2PN, 2,242 couples) in ICSI, and 19 cycles of 1PN (ICSI-1PN,18 couples) in ICSI.

Main results and the role of chance: Clinical pregnancy rate achieved with 1PN blastocysts in cIVF did not show any significant difference compared with 2PN blastocysts in cIVF (IVF-1PN;31.4% vs IVF-2PB;42.8%). Live birth rate also did not show any significant difference (IVF-1PN;25.7% vs IVF-2PB;31.8%) Miscarriage rate did not show significant difference(9.1% inIVF-1PN vs 21.6% in IVF-2PB). On the other hand, ICSI-1PN produced significantly low clinical pregnancy rate (ICSI-1PN;5.3% vs ICSI-2PN;42.8%, $p<0.05$), live birth rate (ICSI-1PN;5.3% vs ICSI-2PN;30.7%, $p<0.05$). Miscarriage rate of ICSI-2PN was 24.7%. One healthy infant was born with no newborn malformations after transfer of 1PN-ICSI cycle.

Limitations, reasons for caution: Confirmation of our preliminary data on a large population should be required.

Wider implications of the findings: 1PN-IVF blastocysts can produce adequate pregnancy rate. Although pregnancy rate after transfer of 1PN-ICSI blastocysts was very low, one healthy infant was born with no newborn malformations after transfer of 1PN-ICSI cycle.

Trial registration number: Not applicable.

P-384 Does endometrial scratching change the biological age of the endometrium? -A paired prospective study

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Study question: To determine if the epigenetically determined biological age of the human endometrium correlates with chronological age, and investigate if endometrial scratching changes endometrial biological age.

Summary answer: The biological age of timed endometrium samples correlates significantly with chronological age. However, any fertility effects of scratching is unlikely a result of epigenetic rejuvenation

What is known already: An algorithm that integrates specific DNA methylation sites in the genome can be used to predict biological age of individual tissues. Furthermore, a negative discrepancy between chronological and biological age is a predictor for adverse conditions.Several studies indicate, that endometrial scratching may enhance implantation in assisted reproductive technology. The molecular mechanisms behind this possible improvement are yet unknown. One attractive hypothesis is that scratching rejuvenates the methylation profile and thus the biological age. Although a tissue independent predictor of biological age has been developed, it was previously shown inconclusive in sex-hormone responsive tissues, including the endometrium.

Study design, size, duration: Nine female volunteers with a regular menstrual cycle (28-32 days) were included in a paired prospective study during the period of 2015. The volunteers were recruited through adverts in the municipality.

Inclusion criteria were: No prior use of intrauterine device or anticonception pills, age of 18-40 years, and a normal BMI (18-32 kg/m2). Women with uterine abnormalities, as well as women with suspected hydrosalpinges, adenomyosis and with pregnancy wish were excluded from the study.

Participants/materials, setting, methods: We obtained endometrial scratching samples in two consecutive cycles strictly timed to day 7 after the LH surge (LH+7), corresponding to the window of implantation, and a peripheral whole blood sample.

We analyzed the samples using the Illumina 450 K methylation array and Horvath's DNA methylation age algorithm, calculated as proxy for the biological age in endometrial samples and peripheral whole blood samples.

Main results and the role of chance: When using carefully timed endometrial sampling, the biological age of the endometrium was found to correlate significantly with chronological age ($r = 0.73$, $p = 0.025$) and with biological age of the peripheral blood ($r = 0.81$, $p = 0.005$). The biological age of the endometrium was determined to (mean 35.35 ± 4.74 years), two years higher than the corresponding whole blood biological age (mean 33.88 ± 5.90 years), and four years higher than the chronological age (mean 31.0 ± 6.56 years). No change was found in the biological age between the first (mean 35.35 ± 4.74 years) and second biopsies (35.55 ± 3.58 years; $p = 0.85$), suggesting that the biological age of the endometrium is not affected by scratching.

Limitations, reasons for caution: Despite using a paired design, low sample size (nine women) limits the ability to detect small effect sizes. Further, the study used homogenized tissue, composed of various cell types with potentially unique methylation profiles. We therefore cannot exclude that scratching affects biological age of specific cell types of the endometrium.

Wider implications of the findings: As an increasing number of women postpone their motherhood, it is important to assess biological age in terms of fertility prognosis and optimizing fertility treatment individually. The present study demonstrates that epigenetically based endometrial biological age can be determined reliably, providing an important novel tool in fertility research and treatment.

Trial registration number: NCT02219425.

P-385 Does night shift work increase the risk of urogenital defects after ART treatment?

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Study question: Does infertility related to maternal exposure to night shift work increase the risk of urogenital defects after ART?

Summary answer: Maternal night shift work was associated with urogenital anomalies among ART first births (aOR = 1.89,CI 1.13-3.38, particularly for multiples (aOR = 3.11,CI 1.33-7.28), but not natural conceptions.

What is known already: Urogenital anomalies are among the most commonly diagnosed congenital anomalies, affecting up to 16 per 1,000 births per year, with significantly higher prevalence among births conceived using assisted reproductive technologies.

Infertility may also increase the risk of urogenital anomalies by various pathways.

Circadian perturbation is a potent endocrine disruptor, with higher rates of menstrual disturbance, endometriosis and miscarriage among shift workers.

Circadian disruption of melatonin secretion is implicated in reproductive function, particularly during pregnancy, and is important in regulating the fetal circadian rhythm. Through its effects on estrogen levels, circadian disruption may also have a sex-specific role in sexual development.

Study design, size, duration: Whole of population data-linkage cohort for the state of South Australia, comprising 302,811 births, from Jan 1986 to Dec 2002, with congenital anomalies detected until children reached age 5 years. Analysis was restricted to 98,359 nulliparous women who were in paid employment at the time of their first birth.

Participants/materials, setting, methods: The South Australian Birth Cohort is based on all registrations of birth and terminations of pregnancy, linked to all cycles of ART, and to all congenital anomalies notified to the 5th

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birthday (ICD-9 British Paediatric Association codes). Logistic regression was used to investigate associations between night shift imputed from maternal occupation, treatment modality and the presence of urogenital defects. Occupation recorded at birth was used to calculate probability of shiftwork.

Main results and the role of chance: Nulliparous women employed in occupations involving night shift were older, more likely to be Caucasian, more likely to reside in wealthy areas and less likely to smoke. ART treatment received did not differ by night shift exposure status.

ART first births showed that the risk of urogenital anomalies was significantly higher among multiple births to night shift workers, OR = 3.11 (95% CI 1.33-7.28) compared to singletons without maternal exposure to night shift work. The risk of urogenital defects was elevated for singleton births to night shift workers (OR=1.85 (95% CI 0.96-3.59)), although this did not reach statistical significance. There was no difference in the risk of urogenital anomalies for multiple and single births conceived using ART where the mother was not exposure to night shift work. These results were adjusted for sex of the baby, maternal age, ethnicity, socioeconomic status, infertility diagnosis, ART treatment type, pregnancy induced hypertension, pre-existing diabetes and asthma.

A significant interaction indicated that the effect of ART conception on the risk of urogenital anomalies for night shift workers is 1.9 times higher than that of non-shift workers.

Limitations, reasons for caution: Shift work was based on a job exposure matrix which calculated the probability of exposure, whereas a more precise measures of exposure using detailed occupational records may yield a more robust result.

A caveat is that shift workers may have different patterns of exposure to unrelated occupational hazards.

Wider implications of the findings: The interaction between maternal shift work and use of ART suggests that individual susceptibility to circadian disruption, and the impact of this on the severity of infertility, are important factors in predicting adverse outcomes, such as urogenital anomalies.

Trial registration number: not applicable.

P-386 Progesterone ratio as a predictor in the ART Cycles with premature progesterone elevation on the day of hCG trigger

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Study question: To evaluate the progesterone ratio as a predictor for implantation in women with a premature progesterone rise (>1.5 ng/ml) on the day of hCG trigger.

Summary answer: Serum progesterone ratio is a prediction and diagnosis tool for improvement of implantation in women with a premature progesterone rise on the day of hCG.

What is known already: Several studies suggested that poorer implantation rates occurred in IVF cycles with elevated progesterone at the end of follicular phase in ovarian stimulation. Much evidence supports the negative impact on endometrial receptivity of elevated progesterone concentrations on the day of hCG trigger. A suggested cause is a premature progesterone elevation which impairs endometrial receptivity. Freeze-all embryos and frozen embryo transfer (FET) in these patients is the other chose for improving clinical outcomes. Whether all of the woman with increased progesterone on the day of hCG trigger should undergo frozen embryo transfer and the diagnostic criteria remains to be explored.

Study design, size, duration: This is a retrospective study involving patients who underwent ICSI/IVF cycles in Lee's Women Hospital, Taiwan in the period from Jan. 2013 to Sep. 2016. One hundred and sixty-nine patients who fulfilled the inclusion criteria were recruited. Patients age range from 20 to 38 years with AFC more than five follicles per ovary and FSH level that is below 10 mIU/L were enrolled. Women with polycystic ovarian syndrome (PCOS) were excluded in this study.

Participants/materials, setting, methods: The progesterone levels were recorded until hCG trigger during ovarian stimulation cycles. The ratio of progesterone was calculated using the following formula: progesterone ratio= progesterone level on the day of hCG trigger / progesterone level on 2 days before hCG trigger. The patients were divided into fresh or frozen embryo transfers (ET) after oocyte retrieval. The embryo vitrification was underwent on day 1 (pronuclear stage) and all embryo transferred on day 3.

Main results and the role of chance: Receiver operating curve (ROC) analysis was used to evaluate the cutoff value of progesterone ratio with maximal sensitivity and specificity to differentiate between viable and non-viable pregnancies in fresh ET cycles. The AUC was 0.701, (95% CI: 0.589–0.812). A cut of level is 1.445 that corresponded to a maximum sensitivity and (0.875) specificity (0.645). In fresh ET group, women with progesterone ratio ≤ 1.445 has significantly lower implantation (IR: 4.8%, 4/83) and pregnancy (PR: 15.4%, 4/26) rates than that in women with progesterone ratio ≤ 1.445 (IR: 22.5%, 46/204 and PR: 41.2, 28/68, respectively, $p<0.05$). The frozen ET in patients with progesterone ratio ≤ 1.445 has significantly higher implantation (21.3%, 20/94) and pregnancy (48.4%, 15/31) rates than that in fresh ET cycles with progesterone ratio ≤ 1.445 ($p = 0.001$ and 0.008, respectively). However, the implantation and pregnancy rates between fresh (IR: 22.5%, 46/204 and PR: 41.2%, 28/68) and frozen ET (IR: 27.9%, 36/129 and PR: 56.8%, 25/44) cycles with progesterone ratio > 1.445 were no significantly difference ($p = 0.265$ and 0.106, respectively). This study suggested that monitoring progesterone ratio can be a predictor of clinical outcomes for women with elevated progesterone on hCG day and further a diagnosis tool for FET.

Limitations, reasons for caution: This finding is only applied in normal responder but not in advanced age women or PCOS.

Wider implications of the findings: The fresh or frozen ET both can be choice in women with progesterone ratio higher than 1.445, although they have elevated progesterone level on hCG day.

Trial registration number: The retrospective data analysis was approved by the Institutional Review Board of Chung Shan Medical University, Taichung, Taiwan (CS-14124).

P-387 Number of follicles ≥15 mm and previous childbirth, but not ovarian reserve tests, are predictors of clinical pregnancy rates of ovarian stimulation and intra-uterine insemination cycles

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Study question: Is there any predictor of clinical pregnancy rates (CPR) of ovarian stimulation and intra-uterine insemination (OS-IUI) cycle in couples with unexplained, chronic-anovulatory, and male factor infertility?

Summary answer: Number of follicles ≥15 mm on the day of oocyte maturation triggering and presence of previous childbirth were only significant predictors of CPR of OS-IUI cycles.

What is known already: One-third of subfertile couples with unexplained and mild male factor infertility achieving a live birth after several OS-IUI cycles and remaining requires in-vitro fertilization (IVF) cycle. Since the differences at live birth rate of per started cycle between OS-IUI and IVF (10-15% vs. 35-40%), identification of patient and OS related predictors of treatment success after OS-IUI may provide more effective counseling and treatment planning for the physician. There is inconclusive and limited data whether ovarian reserve test [antral follicle count (AFC) and anti-Müllerian hormone (AMH)] or OS cycle characteristics could predict ovarian response and pregnancy.

Study design, size, duration: In this retrospective cohort study a total of 323 consecutive couples with unexplained (n = 204), chronic anovulatory (n = 75) and mild male-factor (n = 44) infertility undergoing OS-IUI at tertiary university clinic in-between January-2015 and November-2016 were enrolled with their chronologically first cycle. Inclusion criteria were >5 million/ml total motile sperm count, and confirmation of tubal patency at least in one side with hysterosalpingography. Clinical pregnancy was defined as visualization of gestational sac/fetal-heart-beat by trans-vaginal ultrasound (TV-US).

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Participants/materials, setting, methods: In all patients follicle stimulating hormone (FSH) starting dose was in-between 75 and 150IU and dose were adjusted according to TV-US on stimulation Day6. In logistic regression model female age, body mass index (BMI), previous childbirth, AFC, AMH, duration of OS, number of follicles ≥15 mm, FSH starting dose, total FSH dose, type of subfertility, concentration and percentage of total and progressive motile sperm count after preparation, and luteal-phase support (LPS-vaginal-gel) were studied as predictor.

Main results and the role of chance: Clinic pregnancy rates per inseminated cycle were comparable among the couples with unexplained, chronic anovulatory, and mild male-factor infertility (12.7%, 18.7%, and 11.4%, p = 0.774, respectively), with an overall rate 13.9%(45/323). Female age (29.2 ± 5.3 vs. 30.0 ± 4.5, p = 0.320), BMI (25.9 ± 4.6 vs. 24.6 ± 4.6, p = 0.103), AFC (18.3 ± 11.0 vs. 19.0 ± 10.8, p = 0.783), AMH (4.4 ± 4.0 vs. 4.2 ± 3.5, p = 0.885), duration of OS (13.4 ± 5.2 vs. 13.0 ± 4.7, p = 0.646), FSH starting dose [75.0IU(50-150) vs. 75.0IU(50-150), p = 0.624], total FSH dose (879.8 ± 369.3 vs. 883.0 ± 484.8, p = 0.961), number of follicles ≥15 mm (2.2 ± 1.4 vs. 1.9 ± 1.1, p = 0.187), percentage of total (67.1 ± 17.9% vs. 64.4 ± 16.7, p = 0.348) and progressive (73.1 ± 5.1% vs. 71.1 ± 15.6%, p = 0.431) motile sperm after preparation, patients with LPS (26.7%-12/45 vs. 39.9%-111/278, p = 0.121) were comparable among the CP-positive or CP-negative couples, respectively. However, in couples with CP-positive total (44.7 ± 25.5 vs. 35.0 ± 26.7, p = 0.027) and progressive (33.0 ± 21.5 vs. 23.8 ± 19.0, p = 0.011) motile sperm count and number of couples with previous childbirth (20/44.4% vs. 76/27.3%, p = 0.019) were significantly higher. In logistic regression model significant predictors of the CP were only the number of follicles ≥15 mm (b:0.412, 95%CI 1.107; 2.058, p = 0.009) and previous childbirth (b:0.412, 95%CI 1.151; 6.324, p = 0.022). When linear regression analysis was performed, to identify predictor for the number of follicles ≥15 mm, only BMI was significant (b:-0.039, 95%CI -0.073; -0.004, p = 0.029).

Limitations, reasons for caution: Retrospective design is a limitation. Cancelled cycles during this time period were not recorded and not included in the analysis. Thus may restrict the extrapolation of the results.

Wider implications of the findings: Number of follicles ≥15 mm and previous childbirth were only significant predictors of CPR of OS-IUI cycles. BMI was the only significant predictor for the number of follicles ≥15 mm, where as female age, AFC, AMH, FSH starting dose, duration of infertility, duration of OS, type of subfertility were not.

Trial registration number: None

P-388 Follicle stimulating hormone type is a risk factor for developing ovarian hyper stimulation syndrome during in vitro fertilisation as observed in vivo and in vitro

Abstract withdrawn by the author

P-389 Influence of male and female smoking habits on outcome of artificial insemination: results from a prospective cohort study

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Study question: Do female/male smoking habits influence the CPR (clinical pregnancy rate) after artificial insemination with donor or homologous semen?

Summary answer: Female smoking habits influence the CPR negatively after donor insemination, while male smoking and smoking of both partners significantly decreases the CPR after homologous insemination.

What is known already: The prevalence of smoking amongst women and men in their reproductive years remains high, despite the known negative influence of smoking on male and female infertility. Surprisingly, given the widespread studies of effect of smoking on outcome of IVF (in vitro fertilization), there is limited literature on influence of male and female smoking habits on outcome after intrauterine insemination (IUI). The few trials do not show a significant effect. IUI remains a technique with a rather low success rate, insights in predictive factors could therefore be useful.

Study design, size, duration: A prospective cohort study was performed from 01-07-2011 until 30-09-2015. Data from 1401 homologous and 1264 heterologous IUI cycles were collected through a questionnaire during 20 minutes of mandatory bed rest after insemination. 19 cycles were lost to follow-up (0.7%). The primary outcome parameter was CPR, confirmed with a gestational sac and fetal heartbeat on ultrasonography at 7-8 weeks of gestation.

Participants/materials, setting, methods: The study, performed in a tertiary referral center, included all women elective for IUI with homologous or donor semen. Firstly the univariate relationship between smoking and CPR was studied. Secondly taking into account dependency (possibly different cycles for same patient) and confounding factors, an alternative logistic regression model, GEE (Generalized Estimating Equations) was used.

Main results and the role of chance: In the homologous IUI group the mean age of women was 32.1 years (range 19-47 years), of whom 85% were non-smokers, 13% smoked 1-14 cigarettes daily and 2% more than 15 cigarettes a day. The mean age of men was 34.6 years (range 22-63 years), of whom 70% were non-smokers, 19% smoked 1-14 cigarettes daily and 11% more than 15 cigarettes a day. In the donor IUI group the mean age of women was 33.4 years (range 20-46 years), of whom 89.5% were non-smokers, 7.5% smoked 1-14 cigarettes daily and 3% more than 15 cigarettes a day.

Multivariate GEE analysis revealed male smoking to be predictive for clinical pregnancy in the homologous insemination group (p = 0.0079), secondly a trend towards a decreased CPR was discovered with both partners smoking. In the donor IUI group female non-smoking or smoking less than 15 cigarettes a day turned out to be significantly associated with a higher CPR (p = 0.047) compared to in women smoking more than 15 cigarettes daily.

Limitations, reasons for caution: As smoking is an exposure, randomization is not feasible. Nevertheless the data were collected prospectively by means of a questionnaire. Subjects may have underreported their smoking habits. The series of women smoking more than 15 cigarettes daily is rather small.

Wider implications of the findings: Given the significant effect of smoking on IUI outcome, smoking cessation or at least reduction should be advised to all women and their male partners before artificial insemination is performed.

Trial registration number: not applicable.

P-390 Reduced pregnancy rates after intra-uterine insemination (IUI) in Human Papillomavirus (HPV) positive couples

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Study question: Investigate the relationship between HPV infection in couples and pregnancy outcome after intra uterine insemination (IUI).

Summary answer: Presence of HPV virions detected in both male and female partner is associated with a negative IUI pregnancy outcome.

What is known already: Recent evidence has identified HPV virions as a possible cause of male and couple infertility. Serial measurements of type-specific HPV DNA can categorize HPV infections in two HPV-induced pathways, an infectious virion-producing and a non-infectious transforming cancer-inducing pathway. Because virions can bind two distinct sites on the spermatozoa's head, this may not only have a detrimental effect on sperm parameters but also on gamete interaction, since in vitro experiments have demonstrated that spermatozoa can not only transfer HPV virions to the oocyte, but the transferred HPV virions also induce stage-specific maturation arrest in infected embryos.

Study design, size, duration: Multi-centric non-interventional prospective study in which both partners of a couple undergoing IUI were tested for the presence of HPV types (6,11,16,18,31,33,35,39,45,51,52,53,56,58,59,66,67

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and 68) using quantitative real-time PCR. For male partners different sperm rest fractions (density gradient rest fraction, sperm pellet, swim-up and seminal plasma) were tested. For the female partners the HPV status was assessed on the last 6 cm of the insemination catheter used after insemination.

Participants/materials, setting, methods: HPV positivity was correlated with IUI outcome using following categories: no pregnancy, biochemical pregnancy, miscarriage (>7weeks) and ongoing pregnancy. During the last 2 years rest fractions from capacitation and insemination from both men and women attending for IUI at the AML intermediate structure body material, Antwerp Belgium and the Department of Obstetrics and Gynaecology, Ziekenhuizen Oost-Limburg, Genk, Belgium were tested anonymously for 18 different HPV types. A total of 1000 couple/cycles will be tested.

Main results and the role of chance: For 629 IUI cycles the HPV status of both partners was measured. In couples where both partners were HPV negative (n = 483), 61 pregnancies were achieved (12.6%; 18 biochemical/miscarriages and 43 ongoing). In couples where men were HPV positive (41 cycles) only 2 pregnancies were achieved (4.9% 1 biochemical/miscarriages and 1 ongoing). When the female partner was HPV positive (73 cycles) 6 pregnancies were recorded (8.2%; 5 biochemical/miscarriages and 1 ongoing). When both partners were HPV positive (31 cycles) only 1 pregnancy that ended in miscarriage was detected (3.2%). There was an significant reduction in ongoing pregnancies when HPV was detected (no HPV in couple>HPV+ male partner >HPV+ female partner >both partners HPV+) X^2 for trend p = 0.0043. In the group of biochemical pregnancies and miscarriages 7/22 (31.8%) significantly more couples were HPV positive compared to couples with an ongoing pregnancy with only 2/45 (4.42%)(p = 0.0069). The HPV prevalence per IUI cycle was 23.2%(146HPV+/629).

Limitations, reasons for caution: Only a limited number of intermediate and low risk HPV types was tested for. This could result in an underestimation of the global HPV virion impact on the % achieved biochemical pregnancies and miscarriages.

Wider implications of the findings: IVF or ICSI might be indicated in HPV positive couples to increase successful pregnancies, considering the low IUI success rates in HPV positive couples.

Trial registration number: not applicable.

P-391 Proteomic and peptidomic analysis of human follicular fluid by mass spectrometry as a potential tool for oocyte quality differentiation

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Study question: Are there significant differences in proteome profiles of follicular fluids (hFF) from separate ovarian follicles of healthy women?

Summary answer: Statistically significant changes in concentrations of proteins between hFF samples from separate ovarian follicles of a single patient concern 19 identified proteins.

What is known already: Due to its close relation to maturing oocyte, human follicular fluid is hypothesized to carry traces of information revealing the quality of the oocyte. Multiple studies on hFF composition have been conducted, focusing on hormones, growth factors, anti-apoptotic factors, proteins, peptides, amino acids, and sugars. However, a great majority of proteomic studies of hFF did not undertake analysis of multiple samples from the same healthy patient to unambiguously identify biomarkers of oocyte quality instead of patient-related features. Moreover, no previous study has focused on both proteomic and peptidomic analysis of hFF.

Study design, size, duration: Twelve samples of hFF from separate ovarian follicles of four women undergoing IVF procedure (3 hFF samples per patient) were acquired at the INVICTA Fertility Clinic in October 2015. All samples were free of blood contamination. Follicular fluids from individual follicles were analyzed separately and the proteomic results were compared between oocytes of a single patient and between patients.

Participants/materials, setting, methods: Patients that participated in the study were undergoing the IVF procedure due to male infertility factor. HFF material was further subjected to ultrafiltration on a 10 kDa membrane in order to divide it into high molecular weight fraction (HMWF) containing proteins and low molecular weight fraction (LMWF) containing endogenous peptides. Both fractions were analyzed in a qualitative and quantitative manner by SWATH-MS technique (Sequential Window Acquisition of All Theoretical Fragment Ion Spectra) and compared statistically.

Main results and the role of chance: In the course of the study we identified 108 proteins in HMWF and 92 proteins in LMWF with a confidence score higher than 95%. 62 of identified proteins were never reported before in hFF literature. We were able to quantitatively measure 73 proteins of HMWF and 24 proteins in LMWF in respective single runs. With $p < 0.05$ and fold change higher or equal to 2, we obtained sets of proteins specific for differences between hFF from separate ovarian follicles of a single patient (alpha-1-antitrypsin, hemopexin, retinol-binding protein 4, apolipoprotein A-I, vitronectin, complement factor I, ceruloplasmin, apolipoprotein A-IV, apolipoprotein A-II, leucine-rich alpha-2-glycoprotein, clusterin, complement factor B, protein AMBP, hemoglobin subunit beta, apolipoprotein D, inter-alpha-trypsin inhibitor heavy chain H4, plasma protease C1 inhibitor, Ig heavy chain V-III region TUR, phospholipid phosphatase-related protein type 3) and between hFF from separate patients (18 proteins). In peptidomic fraction 21 peptides from 15 proteins were present showing statistically significant changes in concentrations between patients.

Limitations, reasons for caution: The main limitation of the study is a small number of participants. Moreover, it is necessary to compare proteomic results with medical outcomes in order to unambiguously establish biomarkers of good oocyte quality.

Wider implications of the findings: Our study established possible biomarkers of oocyte quality differentiation that need to be verified in large-scale research and compared with medical outcomes to develop diagnostic biomarkers of good oocyte quality, which would allow for fertilization of only most promising oocytes in the ART treatment.

Trial registration number: N/A.

P-392 In vitro fertilization outcomes in women with mullerian anomalies: Revisiting the controversy

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Study question: Are uterine anomalies associated with decreased rates of embryo implantation and clinical pregnancy in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: When compared to women with normal uterine anatomy, women with uterine anomalies undergoing IVF/ICSI have comparable Implantation rates (IR) and clinical pregnancy rates (CPR).

What is known already: Mullerian anomalies are relatively uncommon. It is well known that uterine anomalies are associated with higher rates of obstetrical complications, such as preterm delivery and miscarriages, but only few studies have focused on IVF outcomes in these patients. In the past decades, rapid technological advance has led to diagnostic improvement in evaluating uterine anatomy and more anomalies are now identified in women undergoing assisted reproduction, making available literature data out of date. The existing studies are limited by very small sample sizes, so far showing conflicting results – reduced IR and CPR by some, and no significant differences by others.

Study design, size, duration: A retrospective cohort study performed at a single public academic reproductive center between January 2006 and December 2016. Computerized medical records of 11,415 IVF/ICSI patients during the study period were screened in order to identify patients with proven mullerian anomalies. Fertility preservation cycles, cycles using donor oocytes and frozen-thawed embryo transfers were excluded (n = 253, 2.2%).

Participants/materials, setting, methods: Anomalies diagnosis was confirmed by hysteroscopy or different imaging/surgical modality. Patients with no definitive diagnosis were excluded.

To prevent bias, only first IVF cycles with available fresh embryos to transfer were included.

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Septate uterus was excluded since most patients underwent resection before IVF or there was unclear data about correction.

IR and CPR were compared between the anomalies group (n = 34) to age matched controls (n = 34) with anatomically normal uteri from cycles carried out during the same period.

Main results and the role of chance: The incidence of mullerian anomalies in the screened infertile population during elven years of study period was 0.68% (76/11,162). After exclusion (4 indefinite diagnosis, 14 no fresh embryo transfer (ET), 24 septate), 34 patients with the following types of congenital uterine malformation were included at the final statistical analysis: 7 arcuate, 12 unicomuate, 12 bicomuate, 1 uterus didelphy and 2 T-shape uteri.

Mean patients age was comparable in both groups)35.26 vs 35.21). There were no statistically significant differences in Body mass index (p = 0.27) or follicular stimulating hormone (FSH) day 3 (p = 0.19) The mean number of retrieved oocytes was comparable - 7.91 in the anomlies group vs 8.76 in control group (p = 0.321).

IR was comparable between the anomalies and the control group (18% vs 12%, p = 0.417), with comparable number of embryos transferred (1.88 vs 2.06, p = 0.394).

CPR and live birth rate were also comparable between the groups: 41.2% vs 26.5% (p = 0.15) and 23.5% vs 11.8% (p = 0.17), respectively.

Limitations, reasons for caution: The main limitations were the retrospective nature of the study and the small sample size, which may limit it's statistical power.

Wider implications of the findings: This information may be of vital importance when providing preconceptional counseling to women with uterine anomalies about their expected reproductive success chances.

Trial registration number: N/A.

P-393 Could embryo banking be used as a treatment strategy in the management of poor responders?

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Study question: Does cumulative freezing day 3 embryos over two or more cycles improve the blastocyst conversion and pregnancy rates in poor responders?

Summary answer: Banking day 3 embryos improves blastocyst and pregnancy rates in poor responders. There is a positive linear relationship between cumulative embryos banked and pregnancy rate.

What is known already: Poor responders (≤ 9 oocytes collected) have been previously identified by the Bologna Criteria. These patients are also associated with increased aneuploidy in embryos. The POSEIDON group suggest that increased embryo numbers could improve chances of obtaining an euploid embryo and a subsequent successful clinical outcome. Cummulative embryo banking offers this opportunity. Recent data suggests frozen embryo transfers may improve pregnancy outcome compared to fresh embryo transfer.

Study design, size, duration: This is a retrospective analysis of 150 poor responder patients at Centre for Reproductive and Genetic Health (CRGH) who have undergone a minimum of twooocyte retrievals followed by day 3 vitrification. In a subsequent cycle, all embryos were thawed and culture to blastocyst with intention to transfer. Cycles were included between 2013 and 2015.

Participants/materials, setting, methods: Poor responder patients had at least two cycles of day 3 embryo vitrification. Embryos were required to be 6 cells or more at the time of vitrification. Slow embryos were not vitrified. We used Kitazato vitrification and warming kits and, post warming, embryos were cultured in Sage One Step Media. Outcomes reviewed were blastocyst formation (> equal to 1) and pregnancy outcome per cycle. Statistical analysis (SPSS) included logistic regression, ROC and Chi-Squared.

Main results and the role of chance: Logistic regression for the effect of total day 3 embryos on blastocyst outcome shows that the odds of blastocyst formation are 3.63 times greater as the number of day 3 embryos increases by one (OR = 3.63 (95%CI 2.17 to 5.79 p<0.001), which is significant. ROC analysis and a 2x2 Chi-squared table analysis indicate that, based on these patients, there is strong evidence of a difference between the chance of blastocyst with <equal 4 day 3 embryos (estimated by 30.4%) and > 4 day 3 embryos (estimated 97.8%), suggesting that a cohort of 4 or more day 3 embryos should result in at least one blastocyst per patient. Logistic regression for the effect of total day 3 embryos on pregnancy rate shows the odds of pregnancy increase by 15% as the total number of day 3 embryos increases by one (OR = 1.15, p = 0.017), which is also significant. Since the dataset is reasonably small the element of chance cannot be discounted and further studies will be required for validation.

Limitations, reasons for caution: Limitations of this study include the relatively small dataset, the fact that patients may have undergone different stimulation protocols and the objective nature if the term 'poor responder.'

Wider implications of the findings: We have shown that'poor responder' patients have an improved chance of blastocyst development and pregnancy by banking day 3 stageembryos and subsequently culturing the cumulative cohort during a frozen embryo replacement cycle.

Trial registration number: Not applicable.

P-394 Pituitary suppression protocol does affect clinical outcomes in egg donation cycles

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Study question: To compare different pituitary suppression protocols in endometrial priming of egg donation cycles based on clinical outcomes and cancellation rates.

Summary answer: The use of depot GnRH agonist in midluteal phase in endometrial priming protocol is associated with better implantation rates in egg donation cycles.

What is known already: In patients with ovarian function, it is possible that during endometrial priming for embryo transfer, spontaneous ovulation occurs leading to desynchronization of implantation window. To avoid this phenomenon there are different pituitary suppression techniques as use of depot GnRH agonist or GnRH daily dose antagonist during first 5-7 days of estrogen supplement. Not administering any medication is considered a valid option if the initial dose of estrogen is maximum so suppression would be obtained by negative feedback.

Previously reported studies refer no difference in clinical outcomes using different protocols.

Study design, size, duration: Retrospective analysis with common database from 11 private clinics of IVI group. We included 7690 women with preserved ovarian function who underwent egg donation cycle with endometrial priming for fresh embryo transfer from Jan 1st 2014 to Dec 31st 2015. Statistical analysis was performed by ANOVA and chi-square.

Participants/materials, setting, methods: Participants received increasing dose of oral estradiol from 2 to 6 mg or transdermal estrogen 75 to 150 μg every 3 days. Subjects in the agonist group received GnRH agonist 3,75 mg in midluteal phase of previous cycle. The group with GnRH antagonist received 0,25 mg daily during the first 7 days of estrogen supplementation. The third group received oral estradiol with initial dose of 6 mg. Cycles were supplemented with vaginal progesterone 400 mg twice/day.

Main results and the role of chance: Patients were distributed as follows: 47.9% (n = 3684) were prepared with GnRH agonist; 35.2% (n = 2706) were primed with GnRH antagonist; 16.9% (n = 1300) did not receive any medication for pituitary suppression. According to analyzed clinical variables, we found statistical differences among the study groups. Data were as follows for agonist, antagonist and without suppressive therapy group respectively; age (41.1 ± 0.1; 41.3 ± 0.1; 40.8 ± 0.2, p<0.001); endometrial thickness (mm) (7.8 ± 1.5; 7.8 ± 1.3; 8.9 ± 0.3, p<0.001).

When we considered clinical outcomes, we found a significant increase in implantation rate in the agonist group (53.2%) compared with the antagonist group (50.8%) and the women without pituitary suppression (49, 2%), p = 0.014. Similar results were observed in pregnancy rate in favor of the

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agonist group (64.7%) in relation with the antagonist group (62.8%) and women without GnRH analogues (58.3%), p = 0.001. Finally, miscarriage rates were comparable among groups being 10.2%, 8.8% and 9.1% (p = 0.346) for agonist, antagonist and no analogue administration.

Concerning cancellation rate due to endometrial impairment, data were identical for the three treatments (2.0%)

Limitations, reasons for caution: Despite the advantages that our data set confer the analysis, limitations still remain. One consequence of a retrospective study is that not all pertinent risk factors are likely to have been identified and subsequently recorded. So only association, and not causation, can be inferred from the results.

Wider implications of the findings: In the endometrial preparation for egg donation cycles, there is a statistically significant advantage regarding clinical outcomes with the use of depot GnRH agonist for pituitary suppression when compared with antagonist protocol or no pituitary suppressive protocol.

Trial registration number: Not applicable.

P-395 Real-life costs and effectiveness of contraceptive methods: A cohort study in the French national healthcare insurance database

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Study question: What are the failure rates and utilization costs of French reimbursed contraceptive methods in real life practice?

Summary answer: The study shows that etonogestrel implant and intrauterine devices are associated with significantly lower unintended pregnancy rates and costs.

What is known already: With nearly 220,000 abortions per year in France and a proportion of repeat abortions increasing steadily, enhancing the use of contraceptive method allowing a better adherence and acceptability is a public health priority.

Study design, size, duration: This is a retrospective study based on the EGB ("*Echantillon Généraliste des Bénéficiaires*") database which is a representative sample of the general population of subjects affiliated with the French health insurance system. The EGB database includes approximately 600,000 beneficiaries. Women having used any reimbursed contraception in 2012 were included.

Participants/materials, setting, methods: Unintended pregnancies were identified through delivery and abortion occurring at least one month after contraceptive initiation. The start of contraception was the date of the first dispensation for OCs; the date of the insertion for LARCs. The end of contraception was assumed if there was a removal for LARCs, a dispensation of another type of contraception, a pregnancy, sterilization or an absence of dispensation at the end of the covered period. Associated costs were estimated.

Main results and the role of chance: A sample of 48,090 women with a reimbursed contraceptive method in 2012 was identified in the EGB: 67.6% used oral contraceptives (OCs), 28.1% intrauterine devices (IUD) and 4.3% implant (Nexplanon®). This distribution was highly dependent on women ages, except for the implant that was evenly distributed among age groups. Unintended pregnancies ranged from 0.8% for the implant to 4.8% for 1st and 2nd generation combined OCs. The mean direct cost associated with an unplanned pregnancy was estimated at about € 2,500, a weighted average of normal delivery, abortion, miscarriage and extra-uterine pregnancy costs. When the costs of the unintended pregnancies were taken into account, the total cost for the first year was lower for progestin-only OC (€251) and copper IUD (€257) compared to Nexplanon® (€300) and hormonal IUD (€323; Mirena®). For the next years through the intended use of the product, Nexplanon® was associated with the lowest cost (€87) per year.

Limitations, reasons for caution: For OCs, only the dispensation date was available in the database. The discontinuation date was estimated from the number of delivered pills. For LARCs, insertion and removal were often not recorded in the database; hypotheses were made based on physician consultations. The costs may vary from one country to another.

Wider implications of the findings: This real-world study shows that the contraceptive implant and IUDs should be considered for first-line use to prevent unintended pregnancies and related abortions in France both from a public health and economic perspective.

Trial registration number: 0.

P-396 Number of oocytes needed to have a healthy baby born

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Study question: Which is the ideal number of oocytes needed to have a healthy baby born after preimplantation genetic testing?

Summary answer: In women 40 years and older, significantly more oocytes are needed to have a baby born compared to the younger categories.

What is known already: In conventional IVF, the total number of retrieved oocytes decreases significantly with maternal age. Accordingly, there is a significantly positive correlation with the number of usable embryos and the cumulative pregnancy rate. When adding chromosome analysis as an additional selection factor, these differences among the age categories are even higher confirming the association between euploidy and maternal age. It has been estimated that, although aneuploidy increases with age but not with the cohort size, the chances of having at least an euploid embryos depends on the number of oocytes and maternal age.

Study design, size, duration: Retrospective cohort study including 263 IVF cycles undergoing preimplantation genetic testing for aneuploidy (PGT) during the last 5 years. The cycles were arbitrarily divided in 5 groups according to the female age. The proportion of euploid embryos and the clinical outcome were related to the number of oocytes retrieved in each group of patients.

Participants/materials, setting, methods: Participants were divided in 5 groups: Group 1: ≤35 years old (n = 36), Group 2: 35-38 (n = 39), Group 3: age 38-40 (n = 51), Group 4: 40-43 (n = 106), Group 5: age > 43 (n = 31). For every group, the number of euploid embryos obtained after PGT was related to the number of oocytes retrieved. The number of oocytes needed to have a baby born was also calculated.

Main results and the role of chance: The mean number of retrieved oocytes decreased proportionally to maternal age (10.8, 10.3, 9.2, 8.7, and 8.2 respectively). The same trend was detected for the euploidy rate (20, 16, 16.6, 9.5 and 4.7% respectively), meaning that the number of oocytes needed to have a euploid embryo was 5 in Group 1, 6.25 in Group 2, 6 in Group 3, 10.5 in Group 4 and 21.3 in Group 5. Embryo transfer was performed in 176 cycles (89% of cycles in Group 1, 95% in Group 2, 88% in Group 3, 51% in Group 4 and 26% in Group 5) resulting in 66 term pregnancies. In all, the number of oocytes needed for a baby born was 33.3, 16, 29.4, 100 and 83 respectively. When restricting the analysis to the fertilized oocytes, for a birth to occur 20 were needed in Group 1, 11.2 in Group 2, 20.8 in Group 3, 66 in Group 4 and 66.6 in Group 5.

Limitations, reasons for caution: In the youngest group, the indication to PGT was primarily due to repeated IVF failures, while for the other groups age was the main indication.

Wider implications of the findings: In women 40 years and older, significantly more oocytes were needed to have a euploid embryo compared to the younger categories. The same trend was observed when calculating the number of oocytes required for a birth. These data can assist to assess couples' odds at producing a viable pregnancy.

Trial registration number: None.

P-397 BMI and outcome after artificial intrauterine insemination: results from a prospective cohort study

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Study question: Does BMI influence the CPR (clinical pregnancy rate) after artificial intrauterine insemination with donor or homologous semen?

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Summary answer: Female BMI does not significantly influence CPR in the donor semen group nor in homologous insemination group, even in a higher range (BMI ≥30 kg/m^2).

What is known already: Extreme weight and fat storage is known to be correlated with ovulation disorders and compromised fecundity. The value of obesity as a predictor of infertility treatment outcome is controversial.

Study design, size, duration: A prospective cohort study was performed from 01-07-2011 until 30-09-2015. Data from 1401 homologous and 1264 heterologous IUI cycles were collected through a questionnaire during 20 minutes of mandatory bed rest after insemination. 19 cycles were lost to follow-up (0.7%). The primary outcome parameter was CPR, confirmed with a gestational sac and fetal heartbeat on ultrasonography at 7-8 weeks of gestation.

Participants/materials, setting, methods: The study, performed in a tertiary referral center, included all women elective for IUI with homologous semen or with donor semen. Firstly the univariate relationship between smoking and CPR was studied. Secondly taking into account dependency (possibly different cycles for same patient) and confounding factors, an alternative logistic regression model, GEE (Generalized Estimating Equations) was used.

Main results and the role of chance: Mean age of patients was 33.5 years (range 20-46 years). 17.7% of women had a BMI ≤20 kg/m^2, BMI was 20-24.99 kg/m^2 in 52.5% of cases, in 19% of cycles BMI was situated between 25-29.99 kg/m^2 and in 10.8% of cycles BMI was 30 kg/m^2 or more. Univariate statistical analysis revealed female BMI in the homologous IUI group as a covariate significantly influencing CPR per cycle (p = 0,0319). Multivariate GEE analysis could not with hold this significant influence. Partner BMI did not significantly influence CPR per cycle (p = 0.4184). In the donor IUI group BMI was not found to be a significant factor influencing CPR in our study, although a slight decrease in CPR could be observed in women with a BMI of less than 20 kg/m^2 and 30 kg/m^2 or more.

Limitations, reasons for caution: BMI is an objective measurement; a randomized controlled trial to investigate the influence of BMI on IUI outcome is not possible. We noted that the series of women with extreme BMI (≤20 kg/m^2 or ≥30 kg/m^2) are rather small which makes it difficult to draw strong conclusions.

Wider implications of the findings: Our results indicate that a high BMI doesn´t affect the success rate in an IUI programme, neither after homologous or donor insemination. On the other hand it is generally known that extreme high BMI (≥35 kg/m^2) does affect obstetrical and perinatal outcomes.

Trial registration number: not applicable.

P-398 Should patients be advised to have treatments with Gonadotropins and Intra Uterine Insemination (IUI) before switching to IVF?

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Study question: To compare pregnancy rates in IUI and IVF in women aged over 30 using either partners or donor sperm.

Summary answer: In women aged >30,with unexplained infertility or male factor the chances to conceive by IVF treatment is 5.34 times higher than with IUI.

What is known already: Gonadotropins(GT) and IUI is a common fertility treatment, in patients with unexplained infertility or mild male factor before switching to In-Vitro Fertilization (IVF). The advantage of IUI treatments prior to switching to IVF in women aged >39 has been reported low and women are advised to go directly to IVF treatments. Although the practice of GT+IUI has been the standard of care, the UK NICE guidelines state that IUI is not beneficial in couples with unexplained infertility a low sperm count or poor-quality sperm.

Study design, size, duration: A retrospective study, IRB approved in women aged 30 and above, who underwent 307 IUI with either partners or donor sperm due to unexplained infertility or male factor and 589 IVF treatments at the IVF unit in Carmel medical center during the years 2011-2013. IVF cycles included patients with an indication for IVF as the primary treatment and patients who had IVF after 3 failed IUI cycles. All groups were compared.

Participants/materials, setting, methods: Data collected included: age, number of past pregnancies, indication for IUI, IVF treatment protocol, source of sperm concentration and Motility, number of oocytes collected and their maturity, fertilization rates number of embryos transferred.Clinical Pregnancy rates were calculated per IUI treatment with partners sperm and donor sperm and compared with pregnancy rates per embryo transfer of each IVF treatment. A SPSS 22 version software was used for Statistical analysis.

Main results and the role of chance: The data included 176 women who underwent 589 IVF cycles, which resulted in 104 clinical pregnancies and 307 IUI cycles performed prior to IVF yielding 12 pregnancies.

In any age above 30, outcomes with IVF were significantly higher than with IUI (P < 0.0001). The chances to conceive by IVF was 5.34 times higher compared with IUI.

No significant difference in pregnancy rates was found between fertility treatments using partner sperm in Unexplained or Male factor infertility compared to donor sperm.

Limitations, reasons for caution: Pregnancy rates per one cycle of IUI were low during the study period probably due to low cutoff of sperm used as indication for IUI.

Results may change if better selection of patients for IUI due to male factor infertility will be implemented.

Wider implications of the findings: Although treatment with GT+IUI prior to switching to IVF is a routine in fertility centers, our results are in line with the UK NICE guidelines demonstrating that IUI is not beneficial in couples with unexplained or mild-moderate male factor at any age above 30 using either partners' or donor sperm.

Trial registration number: not aplicable.

P-399 Characterization of age-related transcriptomic changes within the follicular unit: clues to the mechanisms underlying female reproductive ageing

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Study question: Age is arguably the most important factor influencing female fertility. Can new technologies reveal how molecular pathways in oocytes and cumulus cells alter with age?

Summary answer: Single cell RNA sequencing (scRNA-seq) provides a unique view of alterations in transcriptional activity in oocytes and their companion cumulus cells with advancing age.

What is known already: The well-recognized decline in female fertility with age is principally attributed to reduced oocyte quality. Oocyte growth and maturation is coordinated by bidirectional communication involving the surrounding somatic cells (cumulus cells - CCs). It is possible that deterioration in the function of these critical cells with age might play a role in declining oocyte competence. Despite the importance of oocytes and cumulus cells, characterization of changes to the transcriptome, related to age, has seldom been undertaken using modern molecular genetic methods. Additionally, most studies have involved pooled oocytes or CCs, which are likely heterogeneous in terms of competence.

Study design, size, duration: 24 individual oocyte cumulus complex (COC) were recovered from three different ages of female C57 mice (8 COC from each group). Mice were stimulated with PMSG 48 hours prior to the collection of ovaries. The ages were chosen based on timing points in the mouse reproductive life span: 1-year-old (similar to premenopausal women), 9 weeks old (prime reproductive age) and at 3 weeks (prepubertal).

Participants/materials, setting, methods: Polyadenylated RNA from single oocytes and their matching CCs (recovered from the same individual follicle) was separately prepared. cDNA was synthesized and sequencing libraries were generated using Nextera (Illumina), and processed for massive parallel sequencing. RNA sequencing data was assessed statistically using R package, revealing

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alterations in gene activity in the different age groups. Genes were considered differentially expressed if they had an adjusted p-value (false discovery rate - FDR) of less than 0.05.

Main results and the role of chance: 1642 genes were found to be differentially expressed in 1-year-old oocytes compared to those at 9 weeks. A diversity of pathways was affected, suggesting that aged oocytes may be compromised on multiple levels. Interestingly, significantly altered activity was seen for multiple genes associated with apoptosis and cell cycle control, DNA damage repair and nuclear stability, chromatin remodelling, lipid metabolism, and fatty acid and steroid hormone synthesis. Dysregulation of such important cellular processes could conceivably contribute to a deterioration in oocyte competence with age. 2713 genes were differentially expressed at 3 weeks of age compared to 9 weeks. These included a variety of genes encoding proteins involved in reproduction and FSH beta signalling pathways, as well as key molecules with roles in cell adhesion, all of which have essential functions in oocyte development. As for the CCs transcriptome, gene activity at 3 weeks and 9 weeks were found to be similar, suggesting relatively few changes in these vital somatic cells during early life. However, 20 genes were differentially expressed in CCs from 1-year-olds compared to 9 weeks of age, all showing down-regulation. Genes displaying altered expression in CCs of older females were involved in organ senescence, cell adhesion and gap junctions.

Limitations, reasons for caution: The study was conducted in mice. While murine follicle biology closely resembles that of humans, it is possible that some differences may exist, reducing the extent to which the results can be extrapolated to our species.

Wider implications of the findings: Whole transcriptome analysis of single oocytes revealed age-related changes in pathways relevant to oocyte viability. Supporting these pathways in-vivo or in-vitro might conceivably mitigate the impact of age on oocyte competence. Altered CCs gene activity suggests that the follicular microenvironment may also contribute to age-associated changes in oocyte competence.

Trial registration number: Not applicable.

P-400 AMH at advanced reproductive age can identify a subset of patients who have comparable IUI and IVF outcomes

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Study question: Does AMH levels can predict IUI and IVF outcomes among women of advanced reproductive age?

Summary answer: IUI and IVF have similar outcomes among women above 40 years old with higher AMH levels. Women with lower AMH should be referred to IVF.

What is known already: Advanced reproductive age (ARA) is associated with decreased probability of pregnancy after intra uterine insemination (IUI), in vitro fertilization (IVF) and with a reduction in the ovarian reserve. Anti-Müllerian hormone (AMH) is a good indicator of ovarian reserve. However, there is a considerable variation in ovarian reserve among women of ARA. AMH represent ovary biological age however it was suggested that chronological age is more important. Studies reported that women above the age of 40 and low AMH levels have poor outcome from IVF. However it is unclear whether high AMH at ARA is correlated with better pregnancy outcomes.

Study design, size, duration: Retrospective case series analysis of patients with ARA (35 years old and above) from a single center over 6 years (2009-2014).

Participants/materials, setting, methods: AMH and pregnancy outcomes were available for 384 patients. 281 patients underwent 1163 IUI cycles and 93 patients underwent 145 IVF cycles. Clinical pregnancy rates were compared among different age groups and between different AMH levels among the age groups. Male and mechanical factors were excluded.

Main results and the role of chance: Women over the age of 40 with a serum AMH level above 15 pmol/L had 10.2% chance of achieving clinical pregnancy after IUI and 14.3% after IVF ($p = 0.54$). In the same age group women with AMH level 8 to 15 pmol/L had 4.76% for clinical pregnancy by IUI and 13.6% by IVF ($p = 0.042$). Women over the age of 35 with AMH levels greater than 15 pmol/L had 17.36% chance for clinical pregnancy after IUI and 31.94% after IVF ($p = 0.006$). In the same age group women with AMH level 8 to 15 pmol/L had 8.48% for clinical pregnancy by IUI and 23.3% by IVF ($p = 0.001$).

Limitations, reasons for caution: Prospective studies are needed to confirm these results.

Wider implications of the findings: Pregnancy outcomes among women 40 years old and above with AMH level greater than 15 pmol/L are similar between IUI and IVF. Therefore, for women in this group it might reasonable to recommend IUI treatment as a first choice. However for other groups IVF should be first treatment choice.

Trial registration number: not applicable.

P-401 Evaluation the clinical and oocyte morphology parameters which may predict the fertilization failure in young poor responder patients

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Study question: Are there any predictive clinical and oocyte morphology parameters for the determination of the fertilization failure in young poor responder (POR) patients?

Summary answer: The number of oocyte–cumulus complex (COC) and Metaphase-II (MII) oocyte, and oocyte morphology may predict the fertilization failure in young POR patients.

What is known already: The ESHRE consensus criteria define a poor ovarian response (POR) as less than four oocytes retrieved. Advanced female age and severe sperm disorder were associated fertilization failure after intracytoplasmic sperm injection (ICSI). Also, oocyte quality is pivotal to determine the fertilization of the oocyte. Hence there is no study to evaluate the factors that affects fertilization after ICSI in young poor responder patients where advanced female age and severe male infertility are excluded.

Study design, size, duration: In this retrospective case-control study, we analyzed poor responder patients younger than 38 years old, including 87 patients with total fertilization failure (group 1) and 132 controls with normal fertilization (group 2) in 2-year period (2015-2016). Patients who have the POR criteria according to MII oocytes lower than 4 were included in the analysis.

Participants/materials, setting, methods: Mean age, body mass index (BMI), AMH levels, day-2 FSH and E2, duration of infertility, LH and E2 levels on hCG administration day, duration of stimulation, total dose of gonadotropins used, number of previous trials, COCs, and MII oocytes were examined and compared between the groups. Sperm concentration under 5×10^6 mL was not included to exclude severe male factor. Oocyte morphological evaluation were performed for each patient.

Main results and the role of chance: Characteristics of the groups were similar. Number of COC and MII oocytes, and E2 levels on hCG day were lower in fertilization failure group (Table 1). Smooth endoplasmic reticulum (SER) clusters were significantly higher in fertilization failure group (Table 2).

Table 1. Characteristics of the groups.

	Fertilization failure group	Controls with fertilization	P
Number of patients	85	132	
Age	33,2 ± 3,4	32,8 ± 3,4	0,32
BMI	24,6 ± 3,9	23,6 ± 4,4	0,08
Duration of infertility	4,8 ± 4,2	4,0 ± 3,0	0,13
D2 FSH	10,4 ± 4,1	9,4 ± 3,0	0,09
D2 E2	46,6 ± 32,1	51,6 ± 32,2	0,34

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Continued

	Fertilization failure group	Controls with fertilization	P
AMH	0,78 ± 0,9	0,99 ± 1,2	0,34
LH level on HCG day	4,6 ± 4,0	3,8 ± 3,1	0,15
E2 level on HCG day	**631 ± 582,6**	**798,9 ± 535,3**	**0,03**
No of COCs	**2,1 ± 2,1**	**3,5 ± 2,1**	**0,0001**
No of MII Oocytes	**1,2 ± 0,5**	**2,3 ± 0,7**	**0,0001**
Total dose of gonadotropins used (IU)	2027,9 ± 1573,4	1852,8 ± 1180,5	0,35
Duration of Stimulation (days)	7,3 ± 2,9	7,5 ± 2,1	0,58

Table 2.

	Fertilization failure group	Controls with fertilization	P
Dark cytoplasm or severe cytoplasmic granulation	111/407 % 27	33/187 % 17	0.16
Smooth endoplasmic reticulum clusters	**32/407 % 8**	**6/187 % 3**	**0.03**
Oval shape oocytes	12/407 %3	2/187 % 1	0.23
Large polar body shape	7/347 % 2	1/186 % 1	0.59
Increased perivitellin space	161/347 % 46	104/186 % 55	0.051

Limitations, reasons for caution: The study is limited by its retrospective nature. A higher sample size or a prospective randomized design might be used in the future studies to corroborate the current findings.

Wider implications of the findings: Our results showed that the number of COCs, MII oocytes and E2 levels on hCG day were lower in fertilization failure patients. In addition to the clinical parameters, the presence of the smooth endoplasmic reticulum clusters might be the useful parameters for prediction of fertilization failure in young POR patients.

Trial registration number: not applicable.

P-402 Inhibition of CXCL5-CXCR2 signaling improves the implantation of aging embryos for pregnancy

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Study question: Is defective cell signaling involved in the decline of embryo quality during aging?

Summary answer: Inhibition of CXCL5-CXCR2 signaling during culture of preimplantation embryos improves implantation of aging embryos, leading to successful pregnancies.

What is known already: Embryo quality declines with age due to dysfunction of mitochondria and intracellular organelles and increases in DNA damage induced by accumulation of oxidative stress and chronic inflammation. Such embryos have a low potential to implant and exhibit high proportion of miscarriage, even if the embryos showed good morphology. Despite many attempts, there is no established method to improve pregnancy rates in aging women.

Study design, size, duration: This laboratory-based observational study sampled surplus human blastocysts with same morphological grading from IVF patients in two different age groups: young: 25-27 years of age (n = 3) and aging: 39-45 years of age (n = 5), together with preimplantation embryos and serum samples from young (3-6 weeks of age) and aging (43-50 weeks of age) mice. We investigated differential cell signaling related to aging-associated decline of embryo quality.

Participants/materials, setting, methods: Human blastocysts were subjected to DNA microarray analyses to identify cell signaling factors associated with aging-induced decline in embryo implantation success. Mouse zygote cultures following blastocyst transfer were performed to assess the roles of candidate factors following treatment with candidate ligands, neutralizing antibodies to the ligand, and specific receptor antagonists. The expression of candidate factors and their cognate receptors were measured using real-time RT-qPCR. Serum levels of candidate factors were determined by ELISA.

Main results and the role of chance: In aging human blastocysts, 3,789 genes showed >5-fold increases as compared with young group. Among these genes, C-X-C motif chemokine 5 (CXCL5) showed 203-fold increases in aging embryos. In mouse embryos, the expression of CXCL5 was also increased during aging, whereas the expression of its cognate receptor, CXCR2 showed no change. Although IL8 and CXCL1 also bind CXCR2, their expressions were low and not increased by age. The expressions of both CXCL5 and CXCR2 were low in aging oviducts and uterus. However, CXCL5 expression was increased in aging lung, liver, spleen, and ovary, resulting in 1.7-fold increase in serum CXCL5 levels in aging mice, suggesting that CXCL5 could act on embryos via autocrine and paracrine manner in vivo. Although treatment of aging mouse two-cell stage embryos with CXCL5 neutralizing antibodies and CXCR2 antagonists up to the blastocyst stage did not affect embryo development judged by morphological evaluation, it significantly improved pregnancy success (control: 40.0 ± 14.1%, treatment: 82.1 ± 10.8%, $P < 0.01$). Conversely, treatment of young mouse zygotic stage embryos with CXCL5 peptide significantly decreased pregnancy success (control: 83.9 ± 4.5%, treatment: 34.4 ± 9.7%, $P < 0.01$), together with increases in aging markers (p21, p53, IL-6, and PAI-1) in CXCL5 treated blastocysts.

Limitations, reasons for caution: Due to difficulty to obtain sufficient number of human embryos, we investigated biological actions of CXCL5-CXCR2 signaling using mouse embryos and elucidated cellular and molecular changes. Clinical significance of these findings awaits further analyses.

Wider implications of the findings: Results presented here are consistent with chronic inflammation as one of the major causes of embryo aging and support a role of CXCL5-CXCR2 signaling in preimplantation embryos, providing new clues for pharmacological manipulation of aging embryos during culture and assessing embryo quality based on CXCL5 as an aging biomarker.

Trial registration number: Not applicable.

P-403 Clinical evaluation, birth rate and time to conception in women with unexplained recurrent miscarriages

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Study question: What is the live birth rate (LBR) and time to conception in a cohort of women with unexplained recurrent miscarriages?

Summary answer: Half of the women had a child one year after their investigation for unexplained recurrent miscarriage.

What is known already: Recurrent miscarriage (RM), defined as three or more consecutive miscarriages, occur in 1 % of all pregnancies. Age is independently the greatest risk factor for miscarriage. The risk increases from 9% at 20-24 years of age to 50% at 42 years. Previous studies have shown a live birth rate in unexplained RM of 50-80 % Median time to live birth in the subsequent

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pregnancy has been 113 weeks. No difference in LBR in subsequent pregnancy between primary, nullipara women, and secondary, women who have child/children before, their unexplained RM has been demonstrated.

Study design, size, duration: A retrospective cohort study of all women (N = 138) who underwent clinical evaluation for recurrent miscarriage at Södersjukhuset, Sweden between January 2012 and December 2014. All women had three or more consecutive miscarriages. Clinical examination were performed but also thyroid function, antiphospholipid syndrome, thrombophilia and chromosomal analyse were analyzed. Data, including time to pregnancy and pregnancy outcome, were collected until January 2017 from our own records and from five other hospitals in Stockholm.

Participants/materials, setting, methods: One hundred women (mean age 34.4 yr, SD 5.4) with unexplained RM were included in the study. 53 women (53%) were defined as primary recurrent miscarriage and 47% as secondary RM. 26 women (26%) received medical treatment. Four per cent of the women became pregnant with ART, the rest conceived naturally. Time to pregnancy was defined as the time between diagnoses of RM until the first day in the menstrual cycle in which conception took place.

Main results and the role of chance: In this study a pregnancy was successfully achieved by 87 women (87 %) and the cumulative live birth was 76 % (n = 76). Eleven women had two children during the study. The median time to pregnancy (TTP) resulting in a live birth was 19 weeks (0-113) and the median time to live birth 57 weeks (24-152). There was no significant difference in life birth rate if you received medical treatment for your unexplained RM or not (p = 0.144). The mean age was higher in the group of women (N = 13) who did not become pregnant (37.5yrs SD 5.6) compared to women who did (33.9yrs SD 5.2) (p = 0.009). No statistical significant difference in pregnancy rate, live birth rate, TTP or time to live birth, were found between women with primary or secondary recurrent miscarriage even though there were a significant age difference between the two groups; 32.9yrs and 36.1yrs (p = 0.001). The included women had a high premature rate of 10%. Time to pregnancy was significantly longer in women who had a premature birth; 44wks (0-98wks) compared to 13wks (0-113wks) (p = 0.002).

Limitations, reasons for caution: There is always a risk of information bias in a retrospective design that include detailed information about the women's ability to become pregnant during the study period. Another limitation is the limited access to information from patient's records.

Wider implications of the findings: Our results demonstrate a high live birth rate after unexplained RM and a short time to pregnancy and live birth. We should be able to counsel women that they have a high chance of child/children in a near future even if they have had unexplained RM.

Trial registration number: not applicable.

P-404 The impact of reproductive age on metabolism and gene expression indices of oocyte quality in germinal staged oocytes

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Study question: How does reproductive age alter oocyte energy metabolism and the molecular mechanism(s) regulating oocyte maturation and subsequent developmental competence or quality?

Summary answer: Pyruvate and glucose metabolism were significantly (P < 0.05) different in prepubertal vs. adult oocytes. Metabolic differences were associated with altered expression patterns of glycolytic pathway genes.

What is known already: The reproductive ageing process is associated with a deterioration of oocyte quality, which has been confirmed in both natural conception and assisted reproduction treatment. Several studies have linked the non-invasive and invasive measurement of embryo amino acid metabolism to embryo developmental potential and to be predictive of successful treatment. Furthermore, measurement of the embryo and metaphase II oocyte glucose, pyruvate and lactate metabolism have been proposed as determinants of embryo quality. The relevance of energy metabolism to the subsequent developmental competence of immature GV-staged oocytes has yet addressed.

Study design, size, duration: Prepubertal and adult GV sheep oocytes were used as a model to study the impact of reproductive ageing on metabolic and molecular markers of oocyte quality. Glucose, pyruvate, and lactate metabolism were quantified for 126 prepubertal and 144 adult GV oocytes. Gene expression was evaluated in 6 oocytes/group in relation to the functional assessment of oocyte metabolism using a quantitative real-time PCR array.

Participants/materials, setting, methods: Cumulus enclosed oocytes (CEOs) were harvested from abattoir-derived ovarian tissue from prepubertal and adult animals. CEOs were immediately stripped and the denuded oocytes were individually incubated in microdrops of defined media for 6 hours. Spent culture media were frozen for later carbohydrate metabolism analysis using an established enzyme-linked ultramicrofluorescent assays. For genetic study, individual GV oocytes were snap frozen in RNAGEM buffer before RNA extraction and SMART amplification for analysis by real-time PCR.

Main results and the role of chance: The data demonstrated that carbohydrate metabolism can be used to evaluate quality of GV-staged oocytes. Both glucose and pyruvate utilization showed significant differences between the 2 age groups studied (P = 0.014 and P < 0.0001, respectively) while no significant different was found in lactate production between ages (P = 0.889). Adult GV oocytes consumed more pyruvate and less glucose when compared to prepubertal GV oocytes. The data confirmed that oocytes utilize pyruvate rather than glucose as a major substrate for energy production. Molecular genetic analysis revealed that there were significant differences (P < 0.05) in expression of key genes including those involved in the glycolytic pathway. Increased mRNA expression of SLC2A3, SLC16A1, IGF1R, GSK3B, and PRDX2 was detected in prepubertal GV oocytes when compared to adult oocytes. This data served to both highlight the close link between molecular control mechanisms and the metabolic function of oocytes and shows how these relationships change with reproductive age.

Limitations, reasons for caution: Denudation of CEOs was necessary to facilitate measurement of glucose, pyruvate and lactate metabolism by individual oocytes as the multiple cells in the cumulus compartment would have a major impact on CEO metabolic assessment. Further, the oocyte denudation procedure per se might have impacted on oocyte metabolism and/or gene expression.

Wider implications of the findings: Assessment of energy metabolism is a non-invasive method of determining oocyte and embryo quality. Calibration of oocyte energy metabolism against other more invasive molecular genetic indices of oocyte health may be a useful means to fully characterize the developmental competence of oocytes.

Trial registration number: NA.

P-405 A prospective study comparing the simplified Walking Egg IVF culture system and ICSI in a selected group of patients

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Study question: What are the actual results when using the new simplified IVF procedure, the so-called Walking Egg IVF culture system?

Summary answer: Our results indicate an excellent fertilization rate and ongoing pregnancy rate when using the Walking Egg IVF culture system.

What is known already: The Walking Egg simplified IVF culture system (TWE) was originally developed for resource-poor countries in order to make IVF accessible and affordable for a larger part of the world population. In a previously published pilot clinical trial on a small series of patients it was shown that this method does not compromise fertilisation, embryo development and pregnancy outcome when compared to ICSI or regular IVF.

Study design, size, duration: From January until December 2016 we performed a prospective non-inferiority study comparing ICSI with TWE in a selected group of patients with a least 6 oocytes recovered at oocyte retrieval and an inseminating motile count of more than 0.3 million. Patients eligible for this study were couples requiring IVF as assessed by our standard protocol of infertility evaluation. ICSI was performed on 50 % of eggs; the remaining 50 % were inseminated in TWE.

Participants/materials, setting, methods: 170 ART cycles were started, in 163 an oocyte retrieval was performed. Half of the eggs were treated with ICSI or TWE. The best embryo (s) for transfer were chosen by an independent person who followed the Istanbul criteria. The main outcome result was the

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clinical pregnancy rate confirmed with a gestational sac and foetal heartbeat on ultrasonography at 8 weeks of gestation. Secondary outcome measures were the fertilisation rate (FR) and HCG positive cases.

Main results and the role of chance: Out of the 163 cycles no embryo transfer was performed in 18 cases (11 %) due to the absence of a good quality embryo (2 cases) or because of an increased risk for OHSS (16 cases) in which all embryos were cryopreserved. No fertilisation occurred in 8 (4.9 %) TWE cases and in 2 ICSI cases (1.2 %). An embryo transfer took place in all 165 cycles.

The fertilisation rate was 64 % for TWE, excluding the cases with no fertilisation the FR was 70.8 %. The FR of metaphase II oocytes by ICSI was 71.2 %.

In 56.5 % of cases (82/145) the transferred embryo(s) were the result of TWE, in 43.5 % the ICSI embryo(s) were transferred. Singe embryo transfer was performed in 60/82 (73.2 %) TWE cycles and in 44/63 (69.8 %) ICSI cycles. In the remaining cases a double embryo transfer took place.

HCG positive cases were noted in 46.3 % and 39.6 % of TWE and ICSI cases respectively. The CPR was 32.9 % (27/82) for TWE and 31.7 % (20/63) for ICSI, the difference was statistically not significant.

Limitations, reasons for caution: Our study results should be confirmed in other infertility centres. We have to consider that it was a selected group of patients with at least 6 eggs at oocyte retrieval. The value of TWE has to be confirmed in poor responder cases, and by using mild ovarian stimulation protocols.

Wider implications of the findings: According to our results the Walking Egg simplified culture system is equally effective compared to ICSI in a selected group of patients, even in moderate to severe male infertility cases. TWE can be used as a high quality but less expensive ART method when compared to regular IVF or ICSI.

Trial registration number: Not applicable.

P-406 Trends and reproductive outcomes of double gamete embryo donations: An analysis of a national data registry

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Study question: What are the trends and reproductive outcomes of fresh and frozen double gamete embryo donation cycles in UK?

Summary answer: Double gamete embryo donations have risen 13 fold with fresh donations resulting in higher live birth rate than frozen donations but with poorer reproductive outcomes.

What is known already: Reproductive options using double gamete donations are increasingly considered for single women, infertile couples needing both donor gametes and same sex couples. There is a shortage of egg and sperm donors in UK. Frozen embryos donated for the treatment of others, can be a useful resource but little is known about their utilisation in the UK. There is limited research on the reproductive outcomes of double gamete donations. Human Fertilisation Embryology authority (HFEA) regulates assisted conception treatments in UK and maintains an online anonymised data registry that is a helpful resource to analyse national data.

Study design, size, duration: This is a retrospective cohort study analysing all double gamete embryo donation cycles on the UK's anonymised national HFEA register between 1991 and 2012. There were 4486 double gamete embryo donation cycles of which 2058 (45.9%) were fresh and 2428 (54.1%) were frozen.

Participants/materials, setting, methods: Data was extracted from HFEA's registry for all women who underwent fresh or frozen double gamete donation cycles in UK over two decades. The reproductive outcome measures were live birth rate, miscarriage rate, multiple pregnancy, low birth weight and preterm birth in singleton pregnancies. Descriptive statistics and chi square statistic were used for statistical analysis with SPSS statistical software version 24. Statistical significance was $p<0.05$.

Main results and the role of chance: The fresh to frozen double gamete embryo donations ratio was 1:1 with a 13 fold increase seen over two decades (16-fold for fresh and 9-fold for frozen). The mean ($\pm$ SD) number of embryos transferred was similar in both groups (fresh 0.955 $\pm$ 0.83 vs frozen 0.958 $\pm$ 0.83). Most recipients (56.9%) were 40-50 years old with fresh embryo donations more likely in women aged >42 years whilst frozen embryo donations were more likely in women $\leq$ 42 years ($p<0.001$). Fresh embryo donations resulted in higher live birth rate (fresh 31.8%, frozen 19.1%; p 0.027) and lower miscarriage rate (fresh 0.0%, frozen 2.8%; p <0.001) than frozen embryo donations. However, fresh embryo donations were associated with a higher risk of low birth weight (fresh 11.4%, frozen 5.5%; p 0.002) and prematurity (fresh 32.3%, frozen 27.3%; p 0.01) in singleton pregnancies. Multiple pregnancies were also significantly higher amongst fresh embryo donations (fresh 11.3%, frozen 3.5%; p 0.033). Congenital abnormalities were higher among fresh than frozen transfers but this was not significant (fresh 1.7%, frozen 0.7%; p 0.431). Removal of donor anonymity in 2005 did not affect the number of fresh embryo donations (p 0.056) or frozen embryo donations (p 0.094).

Limitations, reasons for caution: Potential confounders such as smoking that affect live birth rate could not be accounted for because that information was not recorded on the HFEA database. It was not possible to report cumulative live birth rate per woman because of the anonymised nature of the data.

Wider implications of the findings: This study showcases the rising need for double gamete donation as a reproductive option. Acute shortage of gamete donors in UK may warrant looking at alternative strategies such as the use of embryo donation. Creating awareness and appropriate information provision may help donors and recipients to make an informed decision.

Trial registration number: Not applicable.

P-407 Role of latent female genital tuberculosis in unexplained infertility in the Indian subcontinent

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Study question: To assess the outcome of cases of unexplained female infertility or recurrent implantation failure following anti-tubercular treatment

Summary answer: Early Institution of anti-tubercular therapy restores fertility in a major proportion of cases.

What is known already: Infertility is the commonest symptom associated with genital tuberculosis and it seems to be an important under-diagnosed factor in infertility. Clinical presentation of this disease in the female reproductive tract is protean in nature and a vast majority of patients could be completely silent. There is no consensus in giving anti-tubercular treatment based on clinical suspicion, in absence of clear diagnostic criteria. With advent and acceptance of Tuberculosis-Polymerase Chain Reaction (TB-PCR), a large number of infertile patients are being detected with latent or sub-clinical infection.

Study design, size, duration: It was a longitudinal observational study conducted at an infertility centre in India over seven years (2010-2016). After detailed history and investigations, patients with unexplained infertility were included in the study. Those with other known causes of infertility were excluded. Enrolled cases were tested by multiplex polymerase chain reaction for confirming evidence of latent genital tuberculosis. The patients with positive TB-PCR received standard anti-tubercular therapy after reconfirming the test results.

Participants/materials, setting, methods: The participants in the study with positive TB-PCR test received standard nine months anti-tubercular treatment. The patients who completed the treatment were followed up for conception following treatment completion. The overall conception rate and conception rate within 6 months of completion of anti-tubercular treatment was studied.

Main results and the role of chance: A total of 757 patients were enrolled over the study duration. Of these, 576 (76.09%) were found to be positive for latent genital tuberculosis with multiplex TB-PCR test. After giving anti-tubercular therapy for 9 months, 224 (38.89%) of these 576 patients conceived. Of the 224 patients who conceived, 150 (66.96%) conceived within 6 months of completion of treatment.

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Limitations, reasons for caution: False positive results with TB-PCR do occur and the result has to be correlated clinically. Any doubt in the interpretation of result warrants a repeat test.

Wider implications of the findings: The results of this study can have a bearing on many patients of unexplained infertility who have been extensively investigated without a good outcome. A treatable infection, tuberculosis, if diagnosed and treated early can result in restoration of fertility and pregnancy with relatively less economic burden.

Trial registration number: not applicable.

P-408 Is it the ratio of exogenous LH and FSH administered for in vitro reproduction important for the treatment outcome?

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Study question: Is it the ratio of the exogenous LH to exogenous FSH administrated during ovarian stimulation for in vitro reproduction (IVF) important for improving the treatment outcome?

Summary answer: Exogenous LH/FSH ratio, independent of age and AMH, was positively correlated with the number of oocytes and embryos, that independently predicted the clinical pregnancy rate.

What is known already: It was previously suggested that the addition of exogenous LH to exogenous FSH during ovarian stimulation for IVF could improve the pregnancy rate, probably attenuating the increase of the late follicular progesterone level, which could impair the endometrial receptivity. The sweet spot ratio between exogenous FSH and LH it seemed to be 0.30-0.60. There are studies corelating the premature rise of woman serum progesterone during ovarian stimulation for IVF with this FSH/ LH ratio.

Study design, size, duration: We conducted a retrospective study in the department of reproductive medicine of a private hospital. 912 consecutive patients with all causes fertility (mean age 34,65 ± 4,29 years) underwent conventional IVF or ICSI between January 2013 – December 2016. We used the same culture system and a maximum of three embryos were transferred. Only one clinician established the ovarian stimulation protocol for each case in particular according with age, history, BMI, AMH and AFC.

Participants/materials, setting, methods: Reproductive outcome was evaluated by oocytes and embryos number and clinical pregnancies rate. Controlled ovarian hyperstimulation was performed using mixt protocols (human menopausal gonadotropin in association with recFollitropinum alpha or beta) The ratio between exogenous LH and FSH was calculated based on total dose of each medication. SPSS 13 was use for statistics.

Main results and the role of chance: We found that the oocytes and embryos number were negatively correlated with age, total FSH and total LH dose and positively correlated with AMH values. In turn LH/FSH ratios was positively correlated with oocytes ($r = 0.41$, $p < 0.0001$) and embryos number ($r = 0.22$, $p < 0.0001$). In multivariate regression model total LH and total FSH doses were independent negative predictors of oocytes and embryos numbers after adjustment for age and AMH. However, the LH/FSH ratio was positively and independently associated with oocytes (beta = 0.235, $p < 0.0001$) and embryos number (beta = 0.109, $p = 0.004$) after adjustment for age and AMH value. Although LH/FSH ratio was higher in patients who obtained pregnancy in comparison to those without pregnancy, after adjustment for covariates only age and oocytes/embryos number independently predicted clinical pregnancy.

Limitations, reasons for caution: Our results need to be evaluated in a prospective study including subgroups of patients according to age. We used in our study exogenous LH from human menopausal gonadotropin (HMG) which is completely different pharmacokinetic from recombinant LH, which it will be interesting to study separately.

Wider implications of the findings: Our data suggests that the clinician could influence the clinical outcome in IVF, by using a certained ratio of exogenous FSH/LH. This exogenous LH activity of HMG, probably determine the thecal conversion of progesterone to androgens precursors, avoiding late follicular progesterone rise that could impair endometrial receptivity.

Trial registration number: Not applicable.

P-409 Obstetric management of women who conceive following IVF: a systematic review of the more controversial aspects

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Study question: The aim of this systematic review is to formulate evidence based obstetric management strategy for women who conceive following IVF.

Summary answer: Women who conceive following assisted conception are at risk of obstetric complications should have high risk consultant-led individualised care during pregnancy to optimise pregnancy outcome.

What is known already: Pregnancy as a result of IVF or ICSI is associated with an increased incidence of several obstetrical and perinatal complications. It could be due to maternal and paternal characteristics, underlying medical conditions associated with subfertility and infertility, the use of fertility medications, sperm factors, laboratory conditions, culture medium, cryopreservation and thawing, prenatal genetic diagnosis, multiple gestations or a combination of these factors. Difference in obstetric management strategies is a significant factor contributing the outcome. This systematic review is aimed to provide evidence based obstetric management plan for controversial aspects.

Study design, size, duration: Evidence synthesis was done by performing a systematic literature search using Medline (1980-Aug 2016), Embase (1980-Aug 2016) and The Cochrane Library (Aug 2016) for relevant citations. Two reviewers independently reviewed the papers. Total, 394 citations were included in this study. Quality assessment analysis was performed using Newcastle-Ottawa scale.

Participants/materials, setting, methods: All studies comparing various obstetric interventions and surveillance strategies to reduce perinatal complications in IVF and ICSI pregnancies with a predefined control group for comparison were included. Primary outcome measure was neonatal morbidity and mortality. Secondary outcome measures were maternal morbidity including preeclampsia, risk of caesarean section, and development of gestational diabetes, placental insufficiency, and preterm delivery.

Main results and the role of chance: The risk of developing perinatal complications is significantly higher in the IVF conception. However we found paucity of evidence derived from interventional studies to reduce these risks. Based on current literature, obstetric management was subdivided into a) Pre pregnancy optimization, b) First trimester c) second trimester d) third trimester and e) postpartum. We reviewed 23 citations for calculating expected date of delivery, 42 citations to study role of induction of labour and 28 citations for growth scan. The risk of perinatal mortality was high with IVF (OR 1.87, CI 1.48-2.37). IVF conception was associated with two-fold increased risk of preterm birth and low birth weight in singleton pregnancies. The chances of induction of labour (OR 1.5, 95% CI 1.3-1.6) are high with IVF conception. In absence of consensus, it would be a good practice to establish the expected date of delivery based on embryo age and date of transfer. The risk of hypertensive disorders with donor egg is significantly high (OR 3.92; CI 3.21-4.78), therefore low dose aspirin could be used as primary prevention. In absence of evidence, use of serial growth scans and routine induction of labour at term should be weighed against benefits. Obstetric management should be personalised.

Limitations, reasons for caution: Evidence synthesis is mainly based upon prospective observational studies. Some heterogeneity between studies was observed. Only published data was included in the review.

Wider implications of the findings: IVF or ICSI conception should be treated as an independent risk factor for various pregnancy complications. Couples should be counseled carefully about the risks before treatment and antenatal visits. Appropriate surveillance strategies should be in place. IVF or ICSI pregnancies should be treated in standardised way to optimise perinatal outcome.

Trial registration number: na.

P-410 Influence of body mass index on the relationship between endometrial thickness and pregnancy outcome in frozen blastocyst embryo cycles

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Study question: Is there a positive correlation between endometrial thickness (ET) and body mass index (BMI), and does this impact pregnancy outcome?

Summary answer: BMI correlates significantly with ET. ET < 8 mm does not compromise pregnancy outcome in women with lower BMI, but may do so in those with higher BMI.

What is known already: Endometrial thickness (ET) has been shown to significantly affect clinical pregnancy and live birth rates in FET cycles, particularly those with ET < 8 mm (El Toukhy et al. Fertil Steril 2008). Rates of obesity are rising worldwide and it is known that success rates for all pregnancy outcomes in fresh IVF cycles are most favourable in cohorts with low and normal BMIs and progressively worsen as BMI increases (Provost et al. Fertil Steril 2016). However, there is limited data in the literature assessing whether BMI has an impact on ET and whether this impacts on pregnancy outcomes in blastocyst FET cycles.

Study design, size, duration: Retrospective analysis of data regarding FET cycles, prospectively entered into a computerised database over a 5 year period from 2012 until 2016 inclusive in our unit.

Participants/materials, setting, methods: All FET cycles with blastocyst transfer were included. Patient age, BMI, BMI category (underweight, normal weight, overweight, obese: WHO), use of hormone replacement therapy, ET prior to FET and number of embryos transferred were studied. Pregnancy outcomes included: positive pregnancy test rate, clinical pregnancy rate (ultrasound evidence of pregnancy:ESHRE) and livebirth rate per embryo transfer. We excluded women with a very difficult transfer. Data was analysed using one-way ANOVA, Chi-Squared and Pearson correlation.

Main results and the role of chance: During the 5 years, 560 FET cycles met the inclusion criteria. The mean age was 36.2 years (SD 3.0). 88.9% (n = 498) used HRT. 12.3% (n = 83) had two embryos transferred, the remainder had one. The mean BMI was 23.4 kg/m^2 (SD 3.1) and mean ET was 8.6 mm (SD 1.6). Overall, 54.1% (n = 303) had a positive pregnancy test, 36.3% (n = 203) had a clinical pregnancy and 26.4% (n = 148) had a live birth.

Mean ET increased with increasing BMI category (7.2 mm, 8.4 mm, 8.9 mm, 9.7 mm; p < 0.0001). Despite this, there were no differences between BMI groups in positive pregnancy test, clinical pregnancy or live birth rates.

There were significant positive correlations between increasing BMI and ET on positive pregnancy test, clinical pregnancy and live birth rates (r = 0.243 (p<0.0001); r=0.241 (p = 0.001); r=0.197 (p = 0.017)).

Pregnancy outcomes for ET<8 mm were compared with ET≥8 mm for BMI<25 kg/m^2 and ≥25 kg/m^2. There was a trend for a lower rate of positive pregnancy test in women with BMI≥25 kg/m^2 and ET<8 mm when compared with ET≥8 mm (50.0% vs. 60.6%;p = 0.19). However, this difference was lost in rates of clinical pregnancy (34.3% vs. 37.6%;p = 0.62) and live birth in this cohort (26.2% vs 26.6%;p = 1.00). In those with BMI<25 kg/m^2 there was no reduction in any oucome when ET was <8 mm.

Limitations, reasons for caution: Our clinic policy of optimising BMI prior to FET treatment means that very few patients were outside the extremes of BMI. Embryos did not undergo pre-implantation genetic screening. Ultrasound scanning may have been subject to inter-observer bias in measurement of ET.

Wider implications of the findings: Individual BMI should be taken into consideration when deciding on optimal ET for embryo transfer. ET < 8 mm may not jeopardise pregnancy outcome in women with BMI <25 kg/m^2. The development of a norm referenced test for BMI and ET may prove to be a helpful adjunct in clinical IVF setting.

Trial registration number: Not applicable.

P-411 A dynamic approach to estimating the chances of a live birth in couples with unexplained subfertility with and without treatment: a population-based record-linkage study

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Study question: How do the predicted chances of live birth change over time in couples with unexplained subfertility accounting for different fertility treatments?

Summary answer: Average chance of pregnancy (leading to live birth) within six months without treatment is 14% at three months post-fertility clinic registration (10% at twelve months).

What is known already: Couples who experience fertility problems but who have no absolute barrier to conception (unexplained infertility) can get pregnant naturally making it difficult for clinicians to decide when to commence active treatment and what that treatment should be. The use of assisted reproductive technology continues to increase and as healthcare costs rise it is important that fertility care is used appropriately. Our aim was to dynamically predict live birth whilst accounting for the commencement of fertility treatment in couples with unexplained subfertility.

Study design, size, duration: In this retrospective cohort study, a record-linked population based database containing fertility diagnosis, treatment and outcome information from 1,521 couples with unexplained subfertility was developed and analysed. Couples from the Grampian region of Scotland who were referred to a single fertility clinic in Aberdeen between 1998 and 2011 were included.

Participants/materials, setting, methods: Dynamic prediction using a landmarking approach was used to predict a pregnancy (leading to a live birth) within six months after registration at the fertility clinic. Prognosis was then updated monthly from three months post-registration up to 24 months. Predictors included female age, duration of infertility, and initiation of fertility treatment (clomifene, intra-uterine insemination (IUI) or in-vitro fertilisation (IVF)), the latter as a time-varying covariate.

Main results and the role of chance: Out of 1521 women with unexplained subfertility, 889 (58%) women did not need/receive any fertility treatment during follow-up. A total of 911 (60%) achieved a pregnancy leading to live birth over a maximum of 14 years, of which 640 (70%) were treatment independent pregnancies. In couples with a previous pregnancy, couples treated with superovulation and IUI had a higher chance of a live birth over 6 months (Hazard ratio (95% CI) =2.51 (1.47 to 4.30); for IVF HR=1.53 (0.82 to 2.85)) compared to those who were untreated. In couples with no previous pregnancy, those treated with IVF had almost twice the chance of a live birth compared with couples receiving expectant management (HR=1.93 (1.26 to 2.95). Empirical clomifene with treatment had no beneficial effect. Three months after registration at the fertility clinic, a 30 year-old with two years of primary subfertility has a 20% chance of becoming pregnant over the next 6 months (leading to live birth) without treatment. Her probability would increase to 41% if she started IVF at three months (37% with superovulation and IUI, 29% with clomifene). Twelve months after registration these chances decrease to 15%, 30%, 26% and 21% respectively.

Limitations, reasons for caution: This study was conducted using single centre data and may not be generalizable to other centres. We were unable to adjust for potential predictors including ethnicity and antral follicle count.

Wider implications of the findings: In women with secondary infertility our results show that IUI treatment is effective. For those with primary infertility, IVF can result in higher chances of success over the next 6 months compared to expectant management.

Trial registration number: not applicable.

P-412 Evaluation of 15,788 in vitro fertilization (IVF) cycles for predictive value of anti-Müllerian hormone (AMH) levels on clinically usable embryos and ongoing pregnancy

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Study question: We examined a large multi-centre data set to investigate the value of AMH levels in predicting clinically usable embryos and ongoing pregnancy.

Summary answer: The relationship between AMH levels and clinically usable embryos or ongoing pregnancy rates is not linear and depends on the AMH level ranges.

What is known already: AMH is a useful marker of quantitative aspects of ovarian reserve and response to ovarian stimulation; however, no large-scale studies have investigated the relationship between AMH levels and metrics related to oocyte quality such as number of clinically usable embryos or ongoing pregnancies. Therefore, we analyzed a large multi-centre data set to investigate AMH's predictive value for these outcomes.

Study design, size, duration: We performed a retrospective cohort study on 15,788 patients undergoing IVF at 13 centres in the United States (2010-2016). We included each patient's first IVF retrieval and excluded cycles using cleavage stage embryos or preimplantation genetic screening (PGS). Ongoing pregnancy analysis (defined by fetal heartbeat upon discharge to obstetrician) included fresh single or double embryo transfer cycles. Clinically usable embryos included transferred and cryopreserved embryos in each retrieval cycle.

Participants/materials, setting, methods: To investigate the relationship between AMH levels and usable embryos, we performed Poisson regression using piecewise-cubic splines to capture non-linear relationships. We performed logistic regression using piecewise-cubic splines on AMH levels and ongoing pregnancy. Each model controlled for age groups (<35, 35-37, 38-40, 41-42, >42 years) and the model of ongoing pregnancy controlled for number of embryos transferred. Because of sample size, analysis of AMH levels exceeding 5 ng/mL only included patients under 41 years.

Main results and the role of chance: When we controlled for patient age, we found that AMH levels were correlated with both clinically usable embryos and ongoing pregnancy in a non-linear manner ($p<0.0001$). While patient age was also a significant predictor of both outcomes, there was no interaction between age and AMH levels.

As AMH levels increase, the number of usable embryos predicted increases at varying rates: for an AMH level increase from 0 to 1 ng/mL, the predicted number of usable embryos increases by ~0.4; for an AMH level increase from 1 to 5 ng/mL, the predicted number increases by ~1; and for an AMH level increase from 5 to 10 ng/mL, the predicted number increases by ~0.2.

In contrast, the relationship between AMH levels and the likelihood of ongoing pregnancy exhibited a different non-linear curve. While we also observed that the likelihood of pregnancy rose with increasing AMH levels, the relationship was mainly limited to AMH levels between 0.5-3 ng/mL: likelihood of pregnancy increases by 6-7% as AMH levels increase from 0.5 to 3 ng/mL. Interestingly, for AMH levels >3 ng/mL, predictions were observed to plateau, with no additional increase in the likelihood of pregnancy, despite the number of useable embryos predicted being higher.

Limitations, reasons for caution: Analysis was performed across multiple centers and included measurements from multiple laboratories. Our study excluded PGS cycles, which are becoming more routine in the United States. The impact of this and other protocol and assay differences on the predictive value of AMH will need to be further tracked.

Wider implications of the findings: Our results show that AMH has value in helping to set patient expectations for IVF outcomes. Although patients with high AMH are predicted to have higher numbers of clinically usable embryos, this outcome does not necessarily translate into higher ongoing pregnancy rates for AMH levels above 3 ng/mL.

Trial registration number: Not applicable.

POSTER VIEWING SESSION
IMPLANTATION AND EARLY PREGNANCY

P-413 HLA-E was involved in the inhibition of NK cell cytotoxicity toward JEG-3 by progesterone

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Study question: To investigate if HLA-E, one of the MHC-Ib molecules plays a role in the regulation of NK cell cytotoxicity toward trophoblasts by pogesterone.

Summary answer: Progesterone inhibited NK cell cytotoxicity toward trophoblast cell line JEG-3, while the silence of HLA-E expression diminished the inhibitory effect of progesterone.

What is known already: Progesterone was known to play essential roles in maintaining local immune tolerance at the maternal-fetal interface during pregnancy, while the underlying mechanisms are largely unknown. HLA-E, one of the MHC-Ib molecules expressed in trophoblsts, was suggested to be contributed to the establishment of immune tolerance at maternal-fetal interface. In our preliminary study, progesterone was found to inhibit the NK cell cyotoxicity toward trophoblast cell JEG-3. We performed this study to determine if HLA-E plays a role in the regulation of NK cell cytotoxicity toward JEG-3 by progesterone.

Study design, size, duration: In this control study, the JEG-3 cells were divided into 5 groups: the blank control, progesterone treatment, transfection of lentivirus carrying siRNA targeting HLA-E before progesterone treatment, transfection of lentivirus carrying siRNA targeting HLA-E and transfection of the negative control siRNA. Independent experiments were repeated three times with triplicates for each treatment.

Participants/materials, setting, methods: The JEG-3 cells were purchased from Cell Resource Center, IBMS, CAMS/PUMC (China). The expression silence of HLA-E in JEG-3 cells was induced through transfection with the lentivirus carrying siRNA targeting HLA-E. JEG-3 cells were treated as described above and collected as target cells 48 h after different treatments. The NK cells were obtained from peripheral blood mononuclear cells of non-pregnant healthy women. The cyotoxicity of NK cells toward JEG-3 was evaluated using Lactate Dehydrogenase (LDH) assay.

Main results and the role of chance: In this study, we found that progesterone decreased NK cell cytotoxicity toward JEG- 3. The NK cell cyotoxicity against JEG-3 transfected with lentivirus carrying siRNA targeting HLA-E was highly enhanced compared to the controls. Further, Silence of HLA-E expression before progesterone treatment diminished the inhibitory effect of progersterone on NK cell cytotoxicity toward JEG-3. Our data suggested that HLA-E was involved in the inhibition of NK cell cytotoxicity toward JEG-3 by progesterone.

Limitations, reasons for caution: Here JEG-3, an extravillous trophoblast cell model was used as target cells to investigate the regulation of NK cell cytotoxicity. Further studies are needed to determine if HLA-E plays a role in the regulation of NK cell cytotoxicity toward trophoblasts from early gestational placenta.

Wider implications of the findings: HLA-E, although not fully understood, contributes to the immunologic tolerance at maternal-fetal interface. We found that HLA-E was involved in the inhibition of NK cell cytotoxicity toward JEG-3 trophoblast cells by progesterone, which may provide a new clue to investigate the mechanisms of immunologic tolerance at maternal-fetal interface.

Trial registration number: This study was supported by grants from the National Natural Science Foundation of China (81200453), and Science & Technology Department of Sichuan Province (2014SZ0001).

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P-414 A prospective study on reproductive outcome following oral antibiotic treatment for chronic endometritis in infertile women with a history of repeated implantation failure

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Study question: Is antibiotic treatment effective for histopathologic cure of chronic endometritis (CE) and improvement of reproductive outcome in infertile women suffering from repeated implantation failure (RIF)?

Summary answer: Oral antibiotic treatment is a safe and effective therapeutic option to improve the live birth rate in infertile women with RIF/CE.

What is known already: CE is an oligosymptomatic local inflammatory disease characterized by plasmacyte infiltration within the endometrial stromal areas and found in substantial population of infertile women with a history of RIF (14% - 31%) as well as unknown etiology (28%) and recurrent pregnancy loss (9% - 13%). A retrospective study showed that antibiotic treatment significantly improves the reproductive outcome in the subsequent embryo transfer (ET) cycle in women suffering from RIF, but the impact of CE on embryo implantation remains controversial.

Study design, size, duration: Prospective longitudinal study. From November 2011 to July 2014, endometrial biopsy was performed in 421 infertile women with RIF. CE was histopathologically diagnosed using immunohistochemistry. The infertile women with RIF/CE desiring treatment were given a 14-day consecutive administration of oral antibiotic agents. The histopathologic cure of CE was evaluated in the second/third-look endometrial biopsy obtained during the following menstrual cycle. Their reproductive outcome in the subsequent three fresh/cryopreserved-thawed ET cycles was followed-up until March 2016.

Participants/materials, setting, methods: The sections of the paraformaldehyde-fixed paraffin-embedded endometrial biopsy samples were immunostained with a monoclonal antibody against CD138, a specific marker for plasmacytes. Under a light microscope, the immunoreactive cells were enumerated in at least 20 non-overlapping high-power fields within the stromal areas. The endometrial stromal plasmacyte density index (ESPDI) was calculated as the sum of the CD138+ cell counts divided by the number of the high-power fields evaluated. CE was diagnosed as ESPDI $\geq$ 0.25.

Main results and the role of chance: CE was identified in 142 out of 421 (33.7%) infertile women with a history of RIF. Oral doxycycline was finally administered to 118 women with RIF/CE (83.1%). The histopathologic cure rate of CE in the second-look endometrial biopsy was 92.3%. Meanwhile, nine women resistant to doxycycline in the RIF/CE group were further treated with a combination of oral metronidazole and ciprofloxacin hydrochloride. The overall cure rate following two-step oral antibiotic treatment strategy was 99.1%. The cumulative clinical pregnancy rate up to three ET cycles (p = 0.032, RR 1.34, 95%CI 1.02-1.76) was significantly higher in the cured RIF/CE group (45.7%) than in the RIF/non-CE group (34.1%). The miscarriage rate in the first ET cycle (p = 0.96, RR 0.97, 95%CI 0.34-2.79) and cumulative three ET cycles (p = 0.73, RR 0.87, 95%CI 0.38-1.94) was similar between the cured RIF/CE group (4.3% and 6.9%, respectively) and RIF/non-CE group (4.4% and 8.0%, respectively). The live birth rate in the first ET cycle (p = 0.031, RR 1.48, 95%CI 1.03-2.12) and cumulative three ET cycles (p = 0.037, RR 1.39, 95%CI 1.02-1.90) was significantly higher in the cured RIF/CE group (32.8% and 38.8%, respectively) than in the RIF/non-CE group (22.1% and 27.9%, respectively).

Limitations, reasons for caution: The limitation of this study is that the design is not a randomized controlled trial. A potential bias is that compared with the RIF/non-CE group, the RIF/CE group underwent more endometrial biopsy, which potentially affected the reproductive outcome in the RIF/CE group via its endometrial scratching effect.

Wider implications of the findings: In this prospective study, we found CE in about one-third of infertile women with a history of RIF. The histopathologic cure rate and live birth rate in the fresh/cryopreserved-thawed ET cycles following oral antibiotic treatment are encouraging in patients with RIF/CE. Randomized controlled trials are required to confirm these results.

Trial registration number: UMIN-CTR 000006536

P-415 The effect of endometrial scratch on implantation and pregnancy rate for patients undergoing natural-cycle frozen-thawed embryo transfer: a randomized study

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Study question: Can endometrial scratch (ES) improve the implantation and pregnancy rate for patients undergoing natural-cycle frozen-thawed embryo transfer (FET)?

Summary answer: Endometrial scratch does not have any beneficial effect on an unselected group of women undergoing FET in natural cycles.

What is known already: A recent Cochrane review included 14 RCTs concluded a favourable effect of ES with an increased clinical pregnancy rate based on polled results from 13 RCTs including 1972 women and an increase in live birth or ongoing pregnancy rate from 9 RCTs including 1496 women (Nastri et al. 2015). Whilst the studies included in the Cochrane review were based on fresh ET cycles, there have been 3 studies examining the impact of ES in FET cycles (Dunne and Taylor 2014, Aflatoonian et al. 2016, Kanazawa et al. 2016) but they were mostly HRT-FET and each of them presented different results.

Study design, size, duration: This is a prospective double-blind randomized control study conducted among patients who were scheduled for FET cycles using non-donor oocytes in the Assisted Reproductive Unit, the tertiary referral centre affiliated with the Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, during the period of March 2013 to April 2016.

Participants/materials, setting, methods: Women who were suitable for natural-cycle FET were recruited. Patients who had uterine anomaly or pathology such as endometrial polyp, endometriomas >4 cm and hydrosalpinx were excluded. A total of 299 patients were randomized to receive endometrial scratch (n = 115) or endocervical manipulation as control (n = 114), and 196 patients had embryo transfers (93 patients in each group). Endometrial scratch or endocervical manipulation would be performed at the mid-luteal phase of preceding menstrual cycle before FET.

Main results and the role of chance: Our study showed no significant difference in the implantation (ES group 36.5% versus control group 32%, P = 0.458; RR 1.143, 95% CI 0.802-1.627) and pregnancy rate (ES group 48.4% versus control group 40.3%, P = 0.462; RR 1.125, 95% CI 0.822-1.540), as well as the clinical implantation (ES group 41.9% versus control group 37.6%, P = 0.549; RR 1.114, 95% CI 0.782-1.588) and ongoing pregnancy rates implantation (ES group 34.4% versus control group 32.3%, P = 0.756; RR 1.067, 95% CI 0.710-1.602) between the two groups.

Limitations, reasons for caution: Our study included patients with variable numbers of previous IVF or ET attempts, while it is recognised that ES is more likely to have beneficial effect in women with 2 or more ET failures, subgroup analysis of our cases would inevitably lacks sufficient power to draw any conclusion.

Wider implications of the findings: This is the first RCT to evaluate the impact of ES on clinical outcomes in women undergoing natural-cycle FET. And we have found that ES did not have any beneficial effect or adverse impact, with implantation and pregnancy rates very similar between those who did and did not have ES.

Trial registration number: ChiCTR-TRC-12002389

P-416 Frozen-thawed embryo transfer cycles have a lower incidence of ectopic pregnancy compared with fresh embryo transfer cycles

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Study question: To compare the incidence of ectopic pregnancy in women undergoing frozen-thawed embryo transfer cycles and fresh embryo transfer cycles.

Summary answer: The risk of ectopic pregnancy is lower in frozen-thawed embryo transfer cycles than fresh embryo transfer cycles, and blastocyst transfer could further decrease the rate.

What is known already: Infertility has been considered to be an important risk factor of ectopic pregnancy and undesirable pregnancy outcomes. In IVF-ET, the embryos are directly transferred into the uterine cavity, but a significant

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number of ectopic localizations of gravidity occurred. A recent review describes that the incidence of ectopic pregnancy occurring after IVF ranges from 2.1% to 8.6% of all clinical pregnancies.

Study design, size, duration: A retrospective cohort study on the incidence of ectopic pregnancy in fresh and frozen-thawed embryo transfer cycles from January 1st, 2010 to January 1st, 2015.69,756 in-vitro fertilization- embryo transfer (IVF-ET) cycles were analyzed

Participants/materials, setting, methods: Infertile women underwent frozen transfer cycles or fresh transfer cycles

Main results and the role of chance: The clinical pregnancy rate per embryo transfer was lower in fresh embryo transfer cycles compared with frozen-thawed embryo transfer cycles (40.8% vs. 43.1%, p<0.001). Frozen-thawed embryo transfer is associated with a lower incidence of ectopic pregnancy per clinical pregnancy, compared with fresh embryo transfers (OR=0.306; 95%CI 0.244-0.386). Female age and body mass index (BMI) have no influence on ectopic pregnancy. In the frozen-thawed embryo transfer cycles, blastocyst transfer shows a lower incidence of ectopic pregnancy (0.8% vs. 1.8%, p = 0.02) compared with day3 cleavage embryo transfer.

Limitations, reasons for caution: descriptive, only in one reproductive medical center

Wider implications of the findings: agreement with literature

Trial registration number: 2014-1-4091

P-417 Function and hormonal regulation of the serum and glucocorticoid inducible kinase 1 in the human trophoblast-derived HTR-8/SV neo cells

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Study question: Whether E2/P4 stimulation has influences on SGK1 expression and cellular function in the human trophoblast-derived HTR-8/SV neo cells and what is the related molecular mechanism.

Summary answer: It indicates that SGK1 may be partly responsible for the effect of hormone on cell proliferation, differentiation, metastasis, and vessel remodeling in HTR-8/SV neo cells.

What is known already: Hormone including estrogens (E2) and progesterone (P4) were reported to profoundly affect trophoblast cellular changes in the human first trimester placentation. The serum- and glucocorticoid-inducible kinase 1 (SGK1) is also known to exerts embryonic function in cell differentiation, maturation, and vascular remodeling during the embryo development.

Study design, size, duration: We used in vitro human trophoblast-derived HTR-8/SV neo cells culture system. All experiments were performed three times individually. The study was approved by the Ethics Committee of Women's hospital,Zhejiang University.

Participants/materials, setting, methods: Cell viability, differentiation, metastasis, vessel remodeling ability and apoptosis were detected in HTR-8/SV neo cells after treated with E2/P4. Dual luciferase reporter gene assay and siRNA-mediated SGK1 knockdown were performed. We also evaluated the role of SGK1 in the E2/P4 stimulation in the mixture of HTR-8/SV neo cells and decidual cells as well as chick embryo.The expression of SGK1, VEGF, TGF-β, MMP2, MMP9, phosphorylation of AKT and STAT3 were demonstrated by qRT-PCR and/or estern blot.

Main results and the role of chance: The results suggested the combination of E2(10 nM)+ P4(100 nM) resulted in an elevated cell viability, differentiation, metastasis, and vessel remodeling ability compared with the other groups. Simultaneously, the treatment of E2(10 nM)+ P4(100 nM) protect HTR-8/SV neo cells from apoptosis. Consistent with this result, the treatment of E2(10 nM)+ P4(100 nM) also promoted the expression of SGK1, VEGF, and TGF-β demonstrated by qRT-PCR and western blot. Next, we performed Dual luciferase reporter gene assay and siRNA-mediated SGK1 knockdown to explore the effect of E2/P4 on SGK1 transcriptional activity. Our findings indicated that combination of E2(10 nM)+P4(100 nM) significantly promotes the proliferation, invasion, migration, and vessel remodeling of HTR-8/SV neo cells via upregulating SGK1. E2/P4 treatment caused a rapid increase in the level of MMP2, MMP9, and phosphorylation of AKT and STAT3, and inhibition of SGK1 resulted in a decreased response for the treatment of E2/P4 demonstrated by western blot. Moreover, we evaluated the role of SGK1 in the E2/P4 stimulation in the mixture of HTR-8/SV neo cells and decidual cells as well as chick embryo. The data revealed that the promoted invasion, migration (in the mixture of HTR-8/SV neo and decidual cells), and angiogenic remodeling ability (in chick embryo) induced by E2/P4 was mediated by SGK1.

Limitations, reasons for caution: The present study is only in vitro experiment. In our further study, we will build an spontaneous abortion animal model to demonstrate the fundamental role of SGK1 in embryo implantation and placentation during early pregnancy and its' related disease in vivo.

Wider implications of the findings: Our study revealed the importance of SGK-1 on cell proliferation, differentiation, metastasis, and vessel remodeling in the human trophoblast-derived HTR-8/SV neo cells, which may provide us a better understanding of the complexity of molecular dynamics in the key processes of implantation and placentation during early pregnancy.

Trial registration number: N/A.

P-418 Impact of Galectin-1 on Trophoblast stem cell differentiation and invasion during embryo implantation

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Study question: What's the role of Galectin-1 in Trophoblast stem cell differentiation and invasion during embryo implantation?

Summary answer: Co-culture with endometrial epithelium cells or its secretion can elevate the expression of Gal-1 in TSCs, and promote the differentiation and invasion of TSCs.

What is known already: Trophoblast stem cells differentiation in an orderly manner, which plays an important role in the process of embryo implantation, placentation and maintenance of early pregnancy. At the maternal-fetal interface, the dialogue is crucial between trophoblast cells and endometrial epithelial cells. Galectins constitute a growing family of lectins with carbohydrate recognition domains (CRDs) sharing a consensus sequence of 130 amino acids. Gal-1 exerts biological effects including cell adhesion, metastasis, cell growth regulation and apoptosis in various tissues and cells by recognition of glycan ligands. Previous studies shows differentially expression in pathological and normal placenta.

Study design, size, duration: In the present study, we used the co-culture model to simulate the normal physiological process, aiming to identify galectin-1 expression in the trophoblast stem cells during the implantation, and to see whether the expression of galectin-1 is affected by the endometrial epithelial cells. Moreover, to better understand the pathophysiologic role of Gal-1 during implantation, we investigated the effect of gal-1 on differentiation and invasion in TSCs during implantation.

Participants/materials, setting, methods: In this study we used the trophoblast-endometrium co-culture model. We induced the differentiation of trophoblast stem cells in differentiation medium. qRT-PCR was used to detect the mRNA level of each cell type markers, fusion markers and Gal-1 during differentiation of TSC cells. Wound healing and transwell invasion assay were used to detect the migration and invasion ability in each group. Recombination Gal-1 was used to stimulate TSCs in differentiation, wound healing and transwell invasion assay.

Main results and the role of chance: The differentiation status of cultured cells was indicated by the relative mRNA levels of TSC marker genes (Esrrb, Eomes), TGC marker genes (Plf, Ctsq), SpT marker (Tpbpa), and fusion marker (E-cadherin). To explore whether secreted factors derived from endometrial epithelial cells stimulate the invasion capacity of TSCs, we set up a Wound healing assay and a chemotactic Matrigel invasion assay. Co-culture with IK cells or IK cells secretion can elevate the expression of Gal-1 in TSCs, and promote the differentiation and invasion of TSCs. Specifically, we demonstrated that

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recombinant Gal-1 can also promote the differentiation and invasion of TSCs. This finding suggests that during implantation, some of IK cells secretion increase the expression of Gal-1 in TSC, which in turn induces trophoblast differentiation and invasion in vitro.

Limitations, reasons for caution: he experiments were mainly carried out in vitro. Animal models may be further applied in the following research.

Wider implications of the findings: Our research reflects the physiological response of TSCs to endometrial epithelium cells at the implantation and very early placentation stage. The present study provides new experimental evidence for a pivotal role for Gal-1 in promoting TSCs differentiation and invasion during implantation and suggests a potential therapeutic approach for recurrent miscarriage.

Trial registration number: Our study is not a cliical trial.

P-419 Crosslinked hyaluronan gel can improve clinical pregnancy rate of IVF/ICSI patients with moderate to severe intrauterine adhesion

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Study question: Severe intrauterine adhesion (IUA) may lead to frequent implantation failure. Will crosslinked hyaluronan gel application after adhesiolysis improve embryo implantation outcome in patients with severe IUA?

Summary answer: Data in current study show that multiple application of hyaluronan (HA) gel reduced intrauterine adhesion, improved endometrial receptivity, and increased pregnancy rate after embryo implantation.

What is known already: Severe IUA is characterized by extended scar adhesion between endometrial mucous and fibrosis (non-functional scar tissue), and frequently results in infertility and implantation failure. Although adhesiolysis is performed to eliminate adhesions and create environment beneficial to embryo implantation, reoccurrence of adhesion often develops after surgery and the quality and receptivity of endometrium may not be enhanced for effective embryo implantation. HA materials have been reported to prevent adhesion and improve quality of endometrium due to its ability to facilitate angiogenesis and modulation of inflammatory reactions. HA enriched embryo transfer medium improved implantation after IVF procedures and increased clinical pregnancy rate.

Study design, size, duration: In this consecutive case study, 48 patients diagnosed as moderate to severe IUA according to the standard of American Fertility Society (Score $\geq$ 5) due to previous different intrauterine procedures and experienced multiple embryo implantation failure of IVF were enrolled from May to September of 2016 at our Center. This study was approved by the hospital ethics committee. All patients received written informed consent. Quality frozen embryos for each patient were available during this procedure.

Participants/materials, setting, methods: Adhesions were separated using hysteroscopic scissors until normal uterine anatomy was achieved; then an IUD and 3 ml of crosslinked HA gel (MateRegen® gel) were implanted into the uterine cavity. HA gel was installed again into uterine cavity when IUD was removed one month later and then one week thereafter. Endometrium samples were collected for histology examination. Embryo transfer was performed according to standard methods. Endometrium thickness was measured and the clinical pregnancy rate was monitored.

Main results and the role of chance: Using HA gel effectively reduced reoccurrence of intrauterine adhesion. The AFS score dropped from 8.5 preoperatively to 1.2 postoperatively. Observed under hysteroscopy, endometrial mucous appears much normal with rich blood supply. Histological evaluation under microscopy revealed that the number of mucous glands per scope (x10) increased from 15 prior to HA gel application to 29 afterward. The average thickness of endometrium at implantation was 7.64 mm for patients received HA gel, in contrast to the 7.40 mm without using HA gel (historical data). The clinical pregnancy rate reached 29% with multiple application of HA gel, in contrast to the 18% without using HA gel (historical data). All of those data indicated that application of HA gel improved the quality and receptivity of the endometrium, achieved a favorable microenvironment for embryo implantation, and eventually enhanced the clinical pregnancy rate. Many women with severe IUA develop thin endometrium even after surgical intervention. The minimum optimal thickness of endometrium for implantation is 7 mm. Hormonal therapy and growth factors have been employed to stimulate endometrial regeneration to prepare implantation. HA gel might play multiple roles including adhesion prevention, inflammation modulation, and angiogenic regeneration; altogether HA gel contributed to the enhanced clinical pregnancy rate in current study.

Limitations, reasons for caution: This is a consecutive case study. The results from this study were compared with the historical data in the authors' institute and literatures. Therefore, a randomized multiple center study with control group is desired in order to achieve a comprehensive conclusion.

Wider implications of the findings: When women with moderate to severe IUA are treated to receive IVF, comprehensive approaches are needed. Preventing adhesion, regenerating endometrium, and improving quality and receptivity of endometrium should be focused. Crosslinked HA gel is able to stay inside uterine for up to 2 weeks and could achieve those therapeutic purposes.

Trial registration number: N/A.

P-420 Unusual twinning resulting in chimerism: a systematic review on monochorionic dizygotic twins

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Study question: What are the possible causes and (clinical) consequences of a monochorionic dizygotic (MCDZ) pregnancy?

Summary answer: Assisted reproductive technology is responsible for the origin of most MCDZ pregnancies. Chimerism is demonstrable in 90.3% of the twins, leading to various diagnostic difficulties.

What is known already: Traditionally it is understood that dizygotic twins always have a dichorionic placenta. Recently, several studies have reported dizygotic twins with an unusual placentation: MCDZ twins. Vascular anastomoses in the monochorionic placenta can connect the two different fetal circulations, resulting in blood chimerism. This means that in one organism two blood cell lines exist, from two genetically different zygotes. This is a well-known phenomenon in cattle, where chimerism is recognized as the origin of the *freemartin* (an infertile female calve lacking the Müllerian duct derivatives). In human, little attention has been devoted to monochorionic placentation in dizygotic twins and clinical consequences.

Study design, size, duration: We performed a systematic review of the literature, in accordance with the PRISMA guidelines. The search was conducted using PubMed, Embase.com and ISI/Web of Science, with use of the following terms (including synonyms and closely related words): "dizygotic twins and monochorionic", "twins and chimerism" and "freemartinism".

We considered all case reports and case series of MCDZ twins. We selected only papers in which a specific description of the placenta was available.

Participants/materials, setting, methods: By this literature search 1877 records were identified. After removal of the duplicates, 1219 records were assessed against our criteria, based on their titles and abstracts. We identified 31 unique cases of MCDZ twin pregnancies, reported in 27 articles.

From all included cases the following data were extracted: baseline data, method of conception, pregnancy data, birth outcomes, testing for chimerism and physical examination of the twins. Relative frequencies were calculated and expressed in percentages.

Main results and the role of chance: Assisted reproductive technology (ART) was responsible for the origin of the MCDZ pregnancy in 82.1% of the cases, especially double embryo transfer in IVF/ICSI cycles seems a risk factor.

Most (83.9%) of the reported MCDZ twin pregnancies lead to a live birth of twins. Pregnancy loss under 24 weeks occurred in 6.5%. Twin-to-twin transfusion syndrome was reported in 16.1% of the pregnancies, in two cases followed by laser therapy.

In all 31 cases an ultrasound in early pregnancy had reported absence of the lambda sign, confirming a monochorionic diamniotic placenta. However, when

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sex-discordance was noted on a following ultrasound, 7.4% of these pregnancies were nonetheless assumed to be dichorionic.

Blood chimerism was demonstrable in 90.3% of the twins, leading to various diagnostic difficulties, pre- and postnatally. One case describes erroneous sex differentiation due to chimerism, this lead to a gonadectomy because of suspected gonadal dysgenesis in a completely normal healthy girl.

In 15.4% of the sex-discordant MCDZ twins a genital anomaly is reported in one of the twins, including one girl with aplasia of the Müllerian derivatives – possibly resembling the freemartinism in animals.

Limitations, reasons for caution: An association of MCDZ twinning and ART is confirmed in this review, whether there is a causal effect remains uncertain. Moreover, it is possible that placental transfusion between sex-discordant twins results in genital anomalies. A publication bias, especially regarding ART pregnancies and clinical consequences of MCDZ twinning, must be considered.

Wider implications of the findings: Most MCDZ twins are discovered by accident, it can be argued that it is more common than has been assumed. Awareness is important, with subsequently correct medical strategy in pregnancy measures and diagnostic testing. A challenging task for research is to identify the causes and long-term consequences of twin chimerism.

Trial registration number: Not applicable.

P-421 Endometrial and embryo exosomes for embryo-endometrial cross talk at implantation

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Study question: embryonic stem cells would selective incorporate several biologically active components (including glycosphingolipids, GSLs) in exosomes, and their role as preventing the embryo from being attacked by the maternal immune system

Summary answer: GSLs were significant expressed in exosomes derived from ESC, indicating the GSLs have potential modulatory role on embryo implantation and decidual programming of human pregnancy

What is known already: Establishment of pregnancy requires synchronized growth between the endometrium and the blastocyst. Functional interaction between these occurs both during the pre-implantation phase of embryo implantation and during placentation. Pregnancy is a unique event in which a fetus, despite being genetically and immunologically different from the mother, develops in the uterus. Successful pregnancy implies avoidance of rejection by the maternal immune system. Exosomes released from the endometrium and the embryo are present in uterine fluid.

Study design, size, duration: In this in vitro study, we examined whether GSLs could be transfected from exosomes derived from ESC to human immune cells. To demonstrate the immunomodulatory capacity of exosomes derived from ESC. To test macrophage M1/M2 polarization, indicating the role of exosomes in embryo implantation and pregnancy.

Participants/materials, setting, methods: Human villus trophoblast cells were isolated from the abortus tissue from healthy women undergoing pregnancy termination of a pregnancy at 6- to 12-wk gestation, after informed consent. Embryonic stem cells (ESC) were from ESC cell lines. GSLs transfected from exosomes derived from ESC to monocytes. Macrophage M1/M2 polarization was tested by flow cytometry with CD68/CD80/CD163 markers. Transmission electron microscopy (TEM) images of isolated exosomes were performed.

Main results and the role of chance: GSLs were successfully transfected from exosomes derived from ESC to monocytes. Pretreatment with exosomes derived from ESC significantly induced macrophage M2 polarization. Moreover, isolated exosomes from villus trophoblast cells were confirmed by transmission electron microscopy images. Pretreatment with exosomes derived from villus trophoblast cells of a normal pregnancy significantly induced macrophage M2 polarization compared with anembryonic pregnancy.

Limitations, reasons for caution: Functional interaction between these occurs both during the pre-implantation phase of embryo implantation and during placentation

Wider implications of the findings: exosomes derived from ESC and villus trophoblast cells of a normal pregnancy significantly induced macrophage M2 polarization. Our findings represent a new concept regarding the immunomodulatory capacity of exosomes from embryos and villi, suggesting that exosomes have potential modulatory role on embryo implantation and decidual programming of human pregnancy.

Trial registration number: Non.

P-422 A randomized double-blinded controlled trial of human chorionic gonadotropin as luteal phase support in natural cycle frozen embryo transfer

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Study question: Does the use of human chorionic gonadotropin (hCG) as luteal phase support in natural cycle frozen embryo transfer (FET) increase the ongoing pregnancy rate?

Summary answer: The use of hCG in natural cycle FET did not improve the ongoing pregnancy rate.

What is known already: The use of luteal phase support in stimulated cycles was associated with higher live-birth rate and the results were similar using hCG or progesterone.

Study design, size, duration: This is a randomised double-blinded controlled trial of 450 women recruited between July 2012 and October 2015.

Participants/materials, setting, methods: Women with regular cycles undergoing natural cycle FET were recruited. Serial serum hormonal concentrations were used to time natural ovulation and at most two day 2 cleavage embryos were replaced. They were randomised into one of the two groups: (1) the treatment group receiving 1500 IU hCG on the day of FET and 6 days after FET and (2) the control group receiving normal saline on these two days.

Main results and the role of chance: The ongoing pregnancy, implantation and miscarriage rates were comparable between the two groups. In the treatment group, there were significantly more cycles with top quality embryos transferred and a significantly higher serum oestradiol level but a comparable serum progesterone level 6 days after FET. No significant differences were observed in serum oestradiol and progesterone levels 6 days after FET between pregnant and non-pregnant women. In the multivariate logistic regression, the number of embryos transferred is the only significant factor predictive of the ongoing pregnancy rate of natural cycle FET.

Limitations, reasons for caution: This study only included FET with cleavage stage embryos and hCG was used as luteal phase support, but not vaginal progesterone.

Wider implications of the findings: The findings in this study did not support the use of hCG as luteal phase support in natural cycle FET.

Trial registration number: NCT01931384.

P-423 Detection for chromosomal aberrations in early miscarriages following assisted reproductive technology (ART) treatment Using array-CGH

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Study question: Whether array-CGH (Array comparative genomic hybridization) is a better diagnosis tool for chromosomal aberrations detection in product of conception (POC) of first trimester miscarriages following ART treatment?

Summary answer: ART treatment does not increase the risk of chromosomal aberrations in those who miscarry. Array-CGH could detect additional genetic abnormalities compared with conventional karyotyping.

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What is known already: Of these first trimester miscarriages, about 50% are due to the presence of large-scale chromosome abnormalities.Maternal cell contamination (MCC) and suboptimal quality of chromosome preparations are adjunctive limiting factors to karyotype analysis.Array CGH is a powerful molecular cytogenetic technique for quickly scanning through an entire genome for imbalances.Till now there are no well-designed studies for miscarriage POC sample detection in ART patient.

Study design, size, duration: 102 patients with first-trimester miscarriages were investigated by array-CGH using a 100-Kb resolution platform. Patients be collected who refer to reproductive center of Renji Hospital, Shanghai from Aug 2015 to Feb 2016 because of clinical abortion with a subsequent dilation and evacuation performed.

Participants/materials, setting, methods: Patients grouped by type of conception as follows: conventional IVF (in vitro fertilization) (n = 35), ICSI (intracytoplasmic sperm injection) (n = 31), and control (natural conception or intrauterine insemination [IUI]) (n = 36); All couples have normal karyotypes. The results were visualized with Genomic Workbench Standard Edition 5.0.14 (Agilent), annotated against build NCBI 37 (human genome assembly UCSC hg19, Feb.2009).

Main results and the role of chance: There is no statistic difference in the abnormality rate between three study groups. Trisomy constitutes most of the abnormalities, followed by micro duplications/deletions, monosomy, et al. The abnormality rate is significantly higher in mild stimuli group. The frequency of normal embryonic karyotype increase as the number of miscarriages increases. 20 copy number variants (CNVs) were identified. 9 CNVs were submicroscopic genomic gains and 11 were losses. Nineteen CNVs were defined as common CNVs. Only one CNV was classified as unique. Some CNVs shows more than once. The common CNV in the 15q11.2 region resulted in being the most frequent CNVs,followed by common CNVs in the14q11.2, Xq22.2, 1p36.13, 14q11.1, and 15q22.31 regions.

Limitations, reasons for caution: Array CGH cannot detect balanced chromosome abnormalities. We still cannot apply it to POC of multiple pregnancies. Sometimes, the findings fall into "variant of unknown significance (VOUS)", then the clinical significance of the observed change is uncertain. And it is more expensive than traditional cytogenetic.

Wider implications of the findings: The contribution of CNVs to miscarriages seems to be complex. Genes involved in these CNVs may be interesting for the study of early human development and pregnancy effects. Future studies should record a complete CNV burden and characteristics for a more comprehensive assessment of their role in miscarriage.

Trial registration number: N/A.

P-424 Endometrial-embryo crosstalk via extracellular vesicles pathway

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Study question: To study whether endometrial extracellular vesicles (EVs) are involved in embryo-endometrial cross-talk.

Summary answer: EVs are presented in the mouse ULF and could transfer microRNAs into embryos and exert functions.

What is known already: EVs are membrane-bound vesicles released by a variety of cells into the extracellular microenvironment. They can transfer proteins, small RNAs and mRNAs via the extracellular environment to cells at distant sites to promote inter-cellular communication.

Study design, size, duration: Mouse model were applied in this study. The experiments were repeated for at least five times and at least three mice were included in each examination group.

Participants/materials, setting, methods: ULF-EVs were isolated from mouse ULF by sedimentation. The morphology, size, marker expression and microRNA expression of the EVs were characterized. Besides, the roles of EVs in embryo implantation were analyzed by in-vitro and in-vivo models respectively.

Main results and the role of chance: The ULF-EVs have a mean diameter of 59.6 ± 20.3 nm and expressed the exosome enriched proteins CD63, HSP70 and TSG101. CD63 was also detected in the mice uterine epithelium at different pregnancy stages suggesting that epithelium is a source of ULF-EVs. Transmission electronic microscopy(EM) discovered EV-shaped vesicles in the uterine cavity and glandular cavity of the endometrium. MicroRNA let-7a expression was higher in dormant stage mouse embryo, endometrial epithelial cells and ULF-EVs than the respective samples from receptive stage or estradiol-activated stage. Moreover, there was a positive association of let-7a expression between the blastocysts and ULF-EVs, suggesting that let-7a could be transferred from endometrial epithelial cells to the embryos by EVs. Scanning EM showed EV-shaped vesicles were attached to the surface of the embryo. Confocal microscopy showed internalization of labelled EVs to the blastocysts. Coculture of let-7a enriched EVs with blastocysts inhibited the expression of c-Myc, a let-7a target gene, and decreased DNA synthesis in the embryos. Taken together, these results demonstrated that the EVs could be internalized into the mouse embryo and exerted function. Finally, we showed that exosome inhibitor GW4869 suppressed EVs generation and lead to reduction in attachment rate and implantation rate in in vitro and in vivo.

Limitations, reasons for caution: Our observations are based only on mouse and in-vitro model. Different situations may apply in humans.

Wider implications of the findings: As EVs appear to have a crucial role in the course of reproduction, they are excellent research candidates for a better understanding of the underlying molecular activities associate with the developing of endometrial receptivity and their potential role as markers of endometrial receptivity.

Trial registration number: NA.

P-425 Low dose bisphenol compounds affect development and reproductive ability of offspring mice by prenatal exposure

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Study question: Whether bisphenol compounds (BPA, BPF and BPS) have similar effect on developmental and reproductive capacity of F1 mice when exposed to bisphenol compound prenatally.

Summary answer: BPA affects development and fertility of offspring mice with higher potency than BPF and BPS, which suggests that BPF and BPS are safer than BPA.

What is known already: Growing evidence suggest that BPA affects humans and rodent endocrine and reproductive system. It also affect the behavior or development of children when they are exposed to BPA perinatally. With the restricted usage of BPA in some countries, chemicals structurally similar to BPA (e.g. BPF and BPS) are used as without thorough investigation on their effect on human reproduction.

Study design, size, duration: Adult female ICR mice were fed with corn oil (vehicle), diethylstilbestrol (DES) 5 μg/kg/day, BPA or BPF or BPF 500 μg/kg/day for 30 days, and then mated with untreated male. Treatment was continued until delivery (N = 3-5 for each group). The reproductive ability of F1 offspring was studied.

Participants/materials, setting, methods: Body weight of F1 offspring was measured from postnatal day 21 (PD21) to 42 (PD42). Onset of puberty of female F1 offspring was determined on the day of vaginal opening. Length of estrous cycle of female F1 offspring was studied. The number of implantation site on pregnancy day 7 was investigated in three mating groups: (1) treated female mated with untreated male, (2) treated female intercross with male, (3) untreated female mated with treated male.

Main results and the role of chance: Vehicle and bisphenol groups produced similar litter size. While no offspring was found in DES group. The body weight of BPA/BPF/BPS treated female and male F1 offspring was much lower than the vehicle group on PD21, and the body weight of the F1 offspring exposed to BPA was the lowest among all bisphenol treatment groups. However, the body weight of female F1 offspring in all groups was comparable on PD42; however, the male offspring from the BPA and BPS groups were lighter than vehicle group. The date of puberty onset of female offspring from the BPA group was delayed and the estrous cycle length was longer when compared to vehicle group. In the BPA group, the number of implantation site on PD7 of treated female offspring mated with untreated male mice was significantly higher than the vehicle group; while the implantation site of treated female offspring mated with treated male was fewer than the vehicle group. However, untreated female mice mated with treated male

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offspring have similar number of implantation site to the vehicle group. In BPF and BPS treated F1 offspring, the number of implantations site in three mating groups was similar to vehicle group.

Limitations, reasons for caution: In this study, limited number of pregnant mice were exposed to bisphenol compounds. Only observation of affected reproductive ability was investigated. Mechanism is still unclear. And the effect of these bisphenol compounds in other endocrine systems need further investigation.

Wider implications of the findings: Since the toxicity of BPA to human beings is wildly recognized. BPA substitutes have weaker potency than BPA in development and reproductive ability of mice. Our preliminary data suggested these substitutes have less detrimental effects on human reproductive function.

Trial registration number: Nil.

P-426 Double endometrial scratching = double benefit for pregnancy rate?

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Study question: Can a double endometrial scratching in two consecutive menstrual cycles in the luteal phasesversus one prior to embryo transfer improve the pregnancy rate in patients withv recurrent implantation failure (RIF)?

Summary answer: RIF patients do benefit from double endometrial scratching and reveal a statistically significant higher pregnancy rate compared to RIF patients with a single scratch.

What is known already: Pregnancy rates in RIF patients remain unsatisfactorily low although the embryo culture methods in ART programs have improved. The main problem of RIF patients seems to be caused by an insufficient endometrial receptivity. Even when a triple lining and sufficient growth of the endometrium detected by ultrasound controls during the stimulation phase and a minimum of one top quality embryo was transferred a pregnancy does only appear unsatisfactory low percentages. Up to date the identification of the correct individual window of implantation remains expensive.

Study design, size, duration: Retrospective case-control study of 14 RIF patients with double endometrial scratching in consecutive cycles before embryo transfer versus 42 RIF patients with only one scratching in the luteal phase before the embryo transfer was performed, university setting, 2.5 years (April 2014-November 2016).

Participants/materials, setting, methods: The study was approved by the ethical committee of the university of Düsseldorf and patients gave a written consent. RIF patients had a minimum of 2 previous embryo transfers (maximum 10 transfers). Control RIF patients conducted scratching only once. Statistical analysis: Student´s T-Test and odd´s ratio. Age of patients, number of previous embryo transfers, day of transfer, number of good quality embryos did not vary.

Main results and the role of chance: Consecutive double endometrial scratching before the embryo transfer improves the pregnancy rate in RIF patients with an odd of 3.8 (95% confidence interval 1.0633 to 13.2787). Double scratching can easily be performed in 2 consecutive menstrual cycles in the luteal phases and is not cost-intensive for the patients.

Limitations, reasons for caution: Retrospective case-control study.

Wider implications of the findings: This new approach raises more questions about the endometrial signature and a possible memory of endometrial and immune cells within the endometrium. Furthermore it questions the current approach of a single endometrial scratching in patients with RIF to improve the implantation and live birth rates.

Trial registration number: Medical Center of Düsseldorf study number 4394 R.

P-427 Addition of prednisolone and low molecular weight heparin (LMWH) in patients with repeated implantation failures (RIF): a cohort study

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Study question: Can the co-administration of prednisolone and LMWH be an effective treatment for RIF patients undergoing ART?

Summary answer: The co-administration of prednisolone and LMWH does not seem an effective treatment, in terms of improved clinical pregnancy rates, in RIF patients undergoing ART.

What is known already: Both LMWH and prednisolone have been tested as separate adjuncts for the improvement of the clinical outcomes in RIF patients undergoing IVF/ICSI, with negative results. The rationale for using both concurrently was based on their promoting role in the implantation and placentation processes, where they promote a better tolerance of the immune system toward the embryo and a reduction of the local inflammatory reaction to the transfer procedure. In a previous pilot study, we observed a trend favoring the co administration in terms of improved pregnancy outcomes that could not reach statistical significance.

Study design, size, duration: This is a three-center retrospective cohort study conducted at three Universities. A total of 115 patients were recruited from February 2012 to July 2016. Definition of RIF was based on the failure of implantation in at least two IVF/ICSI attempts, where two high-grade quality embryos were transferred. Sample size calculation was based on the pilot study, ending up with 57 participants in each group to provide a significance of 0.05 and a power of 0.8.

Participants/materials, setting, methods: All patients underwent the same COH protocol that they had undergone in the previous cycle. Our primary analysis was performed to provide a direct comparison between groups. In order to reveal potential independent predictors that contributed to the change of the primary outcomes, we planned to perform logistic regression analysis, if differences were observed. The primary outcome measures were clinical pregnancy and miscarriage rates. Secondary outcome parameters included embryological and ICSI cycle characteristics.

Main results and the role of chance: Patients and basic cycle characteristics were comparable between groups, in terms of age, smoking habits, duration and type of subfertility, basal FSH and AFC values, parity, total dose of gonadotrophins and number of previous RIF cycles, oocytes retrieved and embryos transferred (all p values>0.05). Biochemical and clinical pregnancy rates were similar for both groups [23/57 (40.4%) vs. 14/58 (24.1%), and 17/57 (29.8%) vs. 11/58 (19%), p = 0.063, and 0.175, respectively], although the first parameter did not marginally reach statistical significance. Similarly, miscarriage rates were comparable between groups (35.7% vs. 34.8%), as well as live birth rates [15/57 (26.3%) vs. 9/58 (15.5%), p = 0.154].

Limitations, reasons for caution: The lack of blinding and randomization are linked with unknown confounders and selection bias. Another limitation is the reduced cohort size, together with the addition of other arms, comparing LMWH and prednisolone, separately.

Wider implications of the findings: The differences observed of 16% and 10% in clinical pregnancy and live birth rates using the co-administration of both drugs did not manage to reach statistical significance, so that we cannot recommend it for routine use in this category of subfertile patients. A full-scaled RCT would definitely be more accurate.

Trial registration number: N/A.

P-428 Gene expression profiling of human peri-implantation endometria before and after hysteroscopic polypectomy in subfertile patients with endometrial polyps

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Study question: Does hysteroscopic polypectomy affect endometrial gene expression in subfertile women?

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Summary answer: No consistent effect was found in the gene expression in the paired endometrial samples prior to and after polypectomy.

What is known already: Endometrial polyps may negatively impact endometrial receptivity through various mechanisms, and surgical removal of the polyps may improve fertility outcome in subfertile patients. Some studies showed that hysteroscopic polypectomy reduces nuclear factor-kappa B expression, increases concentrations of insulin-like growth factor binding protein-1, tumour necrosis factor α and osteopontin in the endometrium. No published study has examined the effect of hysteroscopic polypectomy on global gene expression, and we aimed at studying this.

Study design, size, duration: This was a prospective observational study in a university-affiliated hospital. We recruited 9 subfertile women who were found to have endometrial polyp on saline infusion sonohysterogram and scheduled for hysteroscopic polypectomy before in-vitro fertilisation.

Participants/materials, setting, methods: For each patient, endometrial biopsies were taken before and after hysteroscopic polypectomy 7 days after luteinizing hormone surge (LH+7). We compared the gene expression profiles of the paired endometria from 3 women using Affymetrix Gene 2.0 ST Array. Quantitative-PCR on TaqMan assays was used to confirm the microarray result. Furthermore, 8 selected receptivity genes were used to compare the gene expression profiles with natural (LH+7, n = 8) and stimulated (hCG+7, n = 8, excessive responders) endometrial samples.

Main results and the role of chance: Microarray analysis demonstrated that no genes were commonly differentially expressed in all 3 paired samples. The expression of 16 genes (hERα, hERβ, OLFM1, OLFM2, IL15, CXCL14, GPX3, CCL4, UPKIB, PAEP, MMP26, SLC1A1, MUC15, MUC16, TM4SF4 and TFPI2) that were differentially expressed (≥2-fold) in 2 of the 3 paired samples were confirmed with quantitative-PCR in all 9 paired samples. No consensus change on 8 receptive genes (OLFM1, IL15, CXCL14, GPX3, PAEP, MMP26, SLC1A1 and MUC16) expression was found after polypectomy when compared to endometrial samples taken on LH+7 or hCG+7.

Limitations, reasons for caution: The main limitation of the present study was the small sample size.

Wider implications of the findings: This was the first study in the literature evaluating the effect of hysteroscopic polypectomy on endometrial receptivity by microarray evaluation of global gene expression. Polypectomy may modulate other endometrial environment conducive to embryo implantation. Further study with larger sample size is needed.

Trial registration number: Not applicable.

P-429 Is the peripheral blood transcriptome profile 9 days after blastocyst transfer in pregnancy destined to miscarriage different from pregnancy which subsequently progressed to live birth?

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Study question: Are there any differences in the peripheral blood transcriptome profiles 9 days after single blastocyst transfer between pregnancies which subsequently progressed to live birth or miscarriage?

Summary answer: There were significant differences in peripheral blood transcriptomic profile 9 days after single blastocyst transfer between pregnancies which subsequently progressed to live birth or miscarriage.

What is known already: In women who received IVF treatment, serum human chorionic gonadotrophin (hCG) measurement 14 days after oocyte retrieval or 9 days after blastocyst transfer can establish if pregnancy has occurred. The majority of pregnancies progress to live birth but some end up in miscarriage. Women who later miscarried appeared to have lower serum hCG level 9 days after blastocyst transfer compared with those which progressed to live birth. However, there is significant overlap in hCG levels between the two groups for the hCG measurement to be of significant discriminatory value.

Study design, size, duration: This is a prospective, cross-sectional, observational study. 100 women who underwent IVF treatment had blood sample taken 9 days after blastocyst transfer for hCG measurement and a portion of the blood sample stored for later transcriptome study. Six samples from women whose pregnancy progressed to live birth and 6 women whose pregnancy later miscarried were randomly chosen for transcriptome study.

Participants/materials, setting, methods: Total RNA were extracted from peripheral blood, and purified for RNA sequencing. Differential gene expression and transcriptome patterns were studied by using DESeq2 package.

Main results and the role of chance: The two groups had comparable hCG levels 9 days after blastocyst transfer but there were 143 significantly differential expressed genes in the miscarriage group. These deferentially expressed genes were mainly enriched in the immune related pathways.

Limitations, reasons for caution: Quantitative PCR study is in progress to validate the differentially expressed genes.

Wider implications of the findings: Peripheral blood transcriptome study 9 days after blastocyst may reflect the success or failure of the implantation process. The panel of differentially expressed genes may be used to predict the outcome of pregnancy.

Trial registration number: None.

P-430 Inner cell mass splitting in 8-shaped blastocysts does not increase monozygotic twinning and negative effects on newborns in PGD/PGS patients

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Study question: Does inner cell mass (ICM) splitting in 8-shaped blastocysts increase monozygotic twinning and negative effects on newborns compared to fully hatched blastocysts in PGD/PGS patients?

Summary answer: ICM splitting in 8-shaped blastocysts does not increase monozygotic twins and negative effects on newborns compared to fully hatched blastocysts in PGD/PGS patients.

What is known already: The use of assisted reproductive technology (ART) has been reported to increase the incidence of monozygotic twinning (MZT), especially after blastocyst transfer. It has been reported that 8-shaped hatching of blastocyst increased the ICM splitting in mouse, which may provide insights into the increased risk of human MZT after blastocyst transfer. In PGD/PGS patients based on blastocyst biopsy, 8-shaped hatching of blastocyst is frequently seen because zona openings were made on day 3 embryos, and ICM may be trapped and splitted in a small zona opening.

Study design, size, duration: Patient selection standard: 1) PGD/PGS based on blastocyst biopsy; 2) frozen cycle and single blastocyst transfer. Seventy 8-shaped blastocysts with ICM splitting and 571 fully hatched blastocysts (control group) were selected from Mar-1-2013 to Dec-31-2015. This clinical outcomes and neonatal parameters were retrospectively analyzed. tocyst biopsy, 8-shaped hatching of blastocyst is frequently seen because zona openings were made on day 3 embryos, and ICM may be trapped and splitted in a small zona opening.

Participants/materials, setting, methods: Patients underwent controlled ovarian stimulation in CITIC-Xiangya Reproductive and Genetic Hospital. Oocytes were collected 35-36 hours after hCG trigger and fertilized by ICSI. A 20μm hole was made on zona of day 3 embryo by laser. Blastocysts were biopsied and vitrified on day 5 or 6. The biopsied TE cells were analyzed by SNP array, FISH or PCR. A tested blastocyst was thawed and transferred to each patient in the frozen cycle.

Main results and the role of chance: Seventy 8-shaped blastocysts with ICM splitting and 571 fully hatched blastocysts were thawed and transferred to patients by single blastocyst transfer. No significant difference was found in respect of clinical pregnancy/implantation (61.4% vs. 58.8%), MZT pregnancy (2.1% vs. 2.3%), miscarriage (20.9% vs. 16.5%) and live birth rate (47.9% vs. 48.0%) between the two groups. Totally, 34 and 276 infants were born in the two groups, respectively. No significant difference was found in respect of MZT birth (0% vs. 0.73%), gender ratio (percentage of boys, 52.9% vs. 62.3%), mean

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gestational age (wk)(38.8 ± 1.7 vs. 38.9 ± 1.7), preterm birth (8.8% vs. 7.7%), mean birth weight (kg)(3.38 ± 0.50 vs. 3.42 ± 0.53), low birth weight infant (5.9% vs. 2.9%), birth defect (5.9% vs. 1.4%) between the two groups.

Limitations, reasons for caution: The results of this study should be regarded with caution because of its limitations, mainly the retrospective design, confounding factors, and small sample size.

Wider implications of the findings: This study does not support the hypothesis that ICM splitting by a small zona opening is a potential mechanism of MZT in human.

Trial registration number: No. 31171379 approved by the Ethical Committee of CITIC-Xiangya.

P-431 Patients with RPL history undergoing PGS have a lower rate of miscarriage

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Study question: Should PGS be offered to patients with a history of recurrent pregnancy loss (RPL)?

Summary answer: The study demonstrates that PGS results in similar to lower miscarriage rates than non-RPL patients and therefore could be beneficial for RPL patients.

What is known already: Patients with a history of recurrent pregnancy loss (RPL) have traditionally been offered expectant management (EM) as standard of care. Aneuploidy is prevalent and leads to clinical miscarriage (CM). In an effort to reduce CM we are suggesting PGS as an alternative strategy, at least to older patients to improve live birth rates.

Study design, size, duration: This retrospective cohort study included a total of 430 cycles of PGS in patients with a history of RPL resulting in pregnancy (+sac) from 30 fertility clinics. The cycles were attempted between 2011 and Dec 2015. Centers reporting substantial number of procedure pregnancy follow up were included in the study.

Participants/materials, setting, methods: Blastocyst biopsies from cycles performed at multiple fertility clinics were referred to the same reference laboratory for analysis. Genetic analysis was performed by array Comparative Genome Hybridization (aCGH) or NGS. CM Rates were compared to 2014 SART data for all patients who underwent ART (frozen cycles) not limited to those with history of RPL, controlling per clinic and age.

Main results and the role of chance: A total of 430 patients who had a history of RPL reported a positive pregnancy test (PPT). The overall PPT rate in our dataset was similar to that of the national ART average. The study looked at CM among patients of different age groups. For PGS-RPL patients under 38, an average of about 12.45% embryos were lost post-implantation, either as empty sacs or clinical losses after FHB was detected. This rate was higher for patients up to 38 years in SART data (17.70%, $p<0.05$). In contrast, patients in the age group of 38-40 showed a miscarriage rate of only 9% while SART miscarriage rate was almost 23% ($p<0.05$). Similarly for the age group of 41-42 within the PGS-RPL group, miscarriage rate was about 9% while it was almost 27% in SART data ($p<0.05$). Finally for patients older than 42;PGS-RPL patients fared better with miscarriage rates of about 22% while SART data showed a miscarriage rate of about 33%. Embryo loss per center was measured and it ranged from as low as 5% (1/22) to as high as 13.5% (5/37). The miscarriage rate for individual centers was significantly lower for RPL-PGS group versus the regular ART group from SART ($p<0.05$).

Limitations, reasons for caution: Dataset does not include details of luteal support, biopsy methods, size of the embryo biopsy, and freezing methods. Comparison of the PGS-RPL group was not done with non-PGS RPL group due to unavailability of such data.

Wider implications of the findings: The results indicate an advantage for RPL patients, which should miscarry more than non-RPL patients. PGS-RPL patients of all ages had a clear advantage with significantly lower miscarriage rates compared to all ART patients. These results advocate for judicious use of PGS for RPL patients of all ages.

Trial registration number: Not applicable.

P-432 PSG1 protein stimulates natural killer cell proliferation via the NKP44- and DAP12-associated signaling pathways

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Study question: We hypothesize that pregnancy-specific glycoproteins (PSGs) could regulate immune cell functions during trophoblast invasion via interactions with the natural killer (NK) cell receptor-mediated signaling pathways.

Summary answer: The PSG1-mediated NK cell proliferation was significantly reduced following the knockdown of natural cytotoxicity triggering receptor 2 (NCR2/NKP44) or DNAX activation protein 12 (DAP12).

What is known already: Human PSGs are secreted carcinoembryonic antigen (CEA)-related cell adhesion molecule-related members of the immunoglobulin superfamily. PSG family proteins are among the most abundant trophoblast-derived proteins in human maternal blood in late pregnancy, and select PSG proteins have been shown to regulate the proliferation and migration of trophoblast, endothelial, and immune cells. In addition, dysregulation of PSG expression has been associated with gestational pathology and cancer-related angiogenesis. Furthermore, PSG proteins could mediate these events by interacting with the Smad- and integrin αIIbβ3-associated signaling pathways.

Study design, size, duration: To investigate how PSGs regulate the proliferation and migration of immune cells during trophoblast invasion, we analyzed the effects of a recombinant PSG1 protein on the proliferation of human NK92-MI cells with or without knockdown of the NKP44 or DAP12 protein.

Participants/materials, setting, methods: Cultured human NK92-MI cells were treated with different concentrations of a recombinant PSG1 protein following transfection with a control vector or a shRNA knockdown vector (NKP44-shRNA-pGPU6 or DAP12-shRNA-pGPU6). The expression of NKP44 and DAP12 proteins in cells were analyzed by Western blotting analysis; whereas cell proliferation was quantified by the MTS assay.

Main results and the role of chance: Recombinant PSG1 protein significantly stimulated the proliferation of human NK92-MI cells dose-dependently. Western blotting analysis showed that the transfection of NKP44 or DAP12 shRNA vector significantly decreases the levels of endogenous NKP44 or DAP12 protein, respectively ($p < .05$). Importantly, knockdown of NKP44 or DAP12 protein in NK92-MI cells significantly reduced the PSG1-mediated cell proliferation ($p < .05$); whereas the transfection with an empty vector had no effects on cell proliferation. These data indicated that PSGs might directly or indirectly interact with NCR-associated signaling pathways to affect the functions of NK or trophoblast cells during embryo implantation.

Limitations, reasons for caution: Because the receptor(s) for PSG proteins remain to be identified, it is not clear how PSG1 mechanically affects NK cell proliferation via the NKP44/DAP12 signaling pathway.

Wider implications of the findings: Our observation provides the first evidence that PSGs may regulate immune cell proliferation and migration by

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signaling in conjunction with the NK cell receptor pathways. This action may represent an important regulatory step in the process of trophoblast invasion and maternal tolerance of the semi-allogenic fetus during pregnancy.

Trial registration number: Not applicable.

P-433 Freeze-all policy is not correlated with improved IVF outcomes in poor responders

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Study question: Does the freeze-all strategy improve in vitro fertilization (IVF) outcomes in poor responders?

Summary answer: Although the freeze-all policy has been related to better IVF outcomes in normal and high responders, poor ovarian responders don´t benefit from the freeze-all strategy.

What is known already: Although fresh embryo transfer is still the routine method in IVF cycles, elective frozen–thawed embryo transfer – the freeze-all policy – has emerged as an alternative for selected IVF treatments. Many questions have been arisen concerning the adverse effects of controlled ovarian stimulation (COS) on the endometrium and endometrial receptivity. Thus, the implementation of the freeze-all policy would avoid these possible deleterious effects of COS and embryo transfer could be performed in a more physiologic uterine environment. However, it is still uncertain whether this strategy might be beneficial for all patients undertaking IVF treatments, especially poor ovarian responders.

Study design, size, duration: This was a retrospective cohort study conducted between January 2012 and December 2016. A total of 433 poor responder patients (following Bologna criteria) fulfilled the inclusion/exclusion criteria and were included in the study. Accepting an alpha risk of 0.05 and beta risk of 0.2 in a two-sided test, it was necessary 149 patients per group to obtain statistically significant results. There were 277 patients in the fresh group and 156 in the freeze-all group.

Participants/materials, setting, methods: The patients were submitted to COS with gonadotropin-releasing hormone (GnRH) antagonist protocol, and cleavage stage embryo transfer. Data were described as the mean ± standard deviation or percentages. The statistical analysis was performed using Student's t test, the chi-square test, and logistic regression models. A *P* value of <0.05 was considered statistically significant. The main outcome measure was ongoing pregnancy rate. The secondary outcomes were implantation and clinical pregnancy rates.

Main results and the role of chance: The patient's mean age in the freeze-all group was 39.5 ± 3.6 and 39.7 ± 3.8 in the fresh group (P = 0.54). The mean number of embryos transferred (nET) was 1.53 ± 0.6 and 1.60 ± 0.6 (P = 0.12) in the freeze-all and fresh groups respectively. The ongoing pregnancy rates did not significantly differ between freeze-all and fresh group (9.5% versus 10.1%, Odds Ratio [OR] 0.95, 95% CI 0.49-1.83; *P* = 0.87) and also the clinical pregnancy rates (14.1% versus 13.7%, OR 1.03, 95% CI 0.59-1.82; *P* = 0.91). The implantation rates were 9.6% and 10.3% (*P* = 0.77) in the freeze-all and fresh groups respectively. In the logistic regression analysis (including age, AFC, number of retrieved oocytes, number of mature oocytes, nET, and fresh versus freeze-all strategy), the age (*P* < 0.001) and the nET (*P* = 0.039) were the only independent variables associated with ongoing pregnancy rates.

Limitations, reasons for caution: This was a retrospective study, which rendered it subject to bias.

Wider implications of the findings: The results of this study emphasize the importance to evaluate adequately the patients that really benefit from the freeze-all strategy, concerning the improved IVF outcomes. Although this strategy may benefit some patients, there are no benefits in implementing the freeze-all strategy in poor ovarian responders.

Trial registration number: -.

P-434 Implantation rate following frozen embryo transfer in leukaemia inhibitory factor supplemented medium

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Study question: The aim of the study was to determine whether leukemia inhibitory factor (LIF) enriched transfer medium improves implantation and pregnancy rates in frozen embryo transfer (FET) cycles.

Summary answer: Supplementing transfer medium with 10 ng/ml LIF does not appear to improve implantation and pregnancy rates following FET.

What is known already: Leukemia inhibitory factor plays a central role in the control of implantation. LIF regulates multiple processes prior to and during implantation such as endometrial transformation into receptive state, embryo-endometrial interaction, stromal decidualization, tropfoblast invasion, blastocyst growth and development and uterine leukocyte infiltration. Abberant LIF production is linked to implantation failure.

Study design, size, duration: Prospective controlled study. Results are presented for 1542 IVF cycles with FET between April, 2014 and September, 2015.

Participants/materials, setting, methods: 1712 blastocysts with a grade of BB or higher were cryopreserved on day 5 or day 6 by vitrification technique. Warming was performed at least two hours prior the FET. 798 blastocysts (737 cycles) were then cultured and transferred in global HP medium containing 10 ng/ml LIF. The rest 914 blastocysts (812 cycles) cultured and transferred in global HP medium served as controls. The volume of medium injected with the embryos did't exceed 20mkl.

Main results and the role of chance: The mean female age and number of embryos per transfer were comparable between two groups. Clinical pregnancy was confirmed by the sonographic observation of a gestational sac. Implantation and clinical pregnancy rates were statistically analyzed using Chi-square test.

Results are summarized in Table 1.

Table 1. Group characteristics and cycle outcomes.

	Global HP	Global HP + 10 ng/ml LIF	Chi-square test
Mean age	35.2 ± 2.4	34.6 ± 2.8	-
#blastocysts transferred	914	798	-
#ET cycles	812	737	-
#blastocyst transferred/ET cycles	1,1	1,1	-
implantaion rate(%)	39,6%	39,2%	NS (p > 0.05)
Clinical pregnancy rate%	42,5%	41,8%	NS (p>0.05)

Limitations, reasons for caution: Due to data absence at the time of this abstract submission, we didn't compare ongoing pregnancy rates.

Wider implications of the findings: There is strong evidence that leukemia inhibitory factor (LIF) is related to embryo development and implantation. In our study LIF in concentration of 10 ng/ml did not improve the implantation rate. Futher studies with use of other LIF concentrations are required in order to detect its positive effect.

Trial registration number: NA.

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P-435 Technique with intrauterine fiberscope and curettage of the endometrium (IFCE) improves the pregnancy rate for infertile patients with repeated embryo implantation failures-a randomized controlled trial

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Study question: Does our technique with intrauterine fiberscope and curettage of the endometrium (IFCE) improve the reproductive outcome for infertile patients with repeated embryo implantation failures?

Summary answer: The clinical pregnancy rate for infertile patients with repeated embryo implantation failures was improved by IFCE.

What is known already: Although two randomized controlled trials (RCTs) showed that the use of intrauterine fiberscope did not improve live birth rate, some reports have shown that local injury by endometrial biopsy increases the pregnancy rate in infertile patients with repeated embryo implantation failures. Although we have developed a new technique with IFCE, the clinical efficacy is unclear.

Study design, size, duration: A randomized controlled trial was conducted [IFCE group (n = 448) and non-IFCE group (n = 448)] from December 2013 to August 2016. The patients were randomized using computer-generated randomization. All infertile patients with repeated embryo implantation failures (mean age, 37.9 years) provided informed consents at our clinic. The study was approved by the Institutional Review Board. The primary outcome was the clinical pregnancy rate.

Participants/materials, setting, methods: IFCE was performed in the follicular phase. After observation using intrauterine fiberscope, an original thin metal curette was inserted through the cervical os and advanced gradually into the uterine cavity until resistance was felt. Furthermore, after a single scratch was made in the uterine cavity, the curette was removed to confirm endometrial sampling. If found, endometrial polyps were removed prior to embryo transfer. All patients were given antibiotics. $P < 0.05$ was considered as statistically significant.

Main results and the role of chance: The mean clinical pregnancy rate was 41.74 % in the IFCE group and 6.03 % in the non-IFCE group. Multiple logistic regression analysis indicated that the clinical pregnancy rates were influenced by the conduction of IFCE ($P < 0.001$, odds ratio 11.17, 95% confidence interval: 7.27–17.16), regardless of the patient's age ($P = 0.09$) and BMI ($P = 0.95$). Therefore, regardless of the patient's age and BMI, the clinical pregnancy rate was significantly higher in the IFCE group than in the non-IFCE group. There was no difference in adverse events in both groups.

Limitations, reasons for caution: The sample size in our RCT was sufficient. However, the reproducibility of our study should be investigated by other studies in the near future.

Wider implications of the findings: Our RCT has shown that our new technique with IFCE improves the clinical pregnancy rate for infertile patients with repeated embryo implantation failures. Therefore, our new technique with IFCE will help for the improvement of reproductive outcomes in infertile patients.

Trial registration number: UMIN000025679.

P-436 Pregnancy outcomes of women with a congenital unicornuate uterus after in vitro fertilization-embryo transfer

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Study question: Are the pregnancy outcomes of women with a unicornuate uterus worse than those of women with a normal uterus?

Summary answer: A unicornuate uterus was associated with an increased risk of early pregnancy loss, premature delivery,perinatal mortality, low birth weight (LBW) and low live birth rate

What is known already: Although normal pregnancies can occur in some patients with a clinically asymptomatic unicornuate uterus, this condition has been related to miscarriage, premature delivery, low birth weight, perinatal mortality and other complications.

Study design, size, duration: A retrospective, single-center cohort study was conducted of 238 women with a unicornuate uterus and 818 women with a normal uterus who experienced a clinical pregnancy after in vitro fertilization-embryo transfer (IVF-ET) treatment.

Participants/materials, setting, methods: This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). Two hundred and thirty-eight patients with a unicornuate uterus were chosen as the study group (183 with singleton and 55 with twin pregnancies), and 818 patients with a normal uterus were selected as the control group (567 with singleton and 251 with twin pregnancies). The outcome measures included pregnancy and obstetric outcomes, which were compared between the unicornuate and control groups.

Main results and the role of chance: A unicornuate uterus was associated with an increased risk of early pregnancy loss [adjusted odds ratio (aOR) 1.88, 95% CI 1.25-2.83; p = 0.002], premature delivery (aOR 2.11, 95% CI 1.45-3.07; p < 0.001), perinatal mortality (aOR 3.35, 95% CI 1.89-5.94; p < 0.001), LBW (aOR 1.43, 95% CI 1.04-1.79; p = 0.005) and very low birth weight (VLBW, aOR 2.58, 95% CI 1.53-4.34; P < 0.001). Additionally, significantly lower rates of term delivery (aOR 0.43, 95% CI 0.31-0.58; p <0.001) and live birth (aOR 0.27, 95% CI 0.13-0.57; p = 0.001) were observed in women with a unicornuate uterus compared to those of the controls.

Limitations, reasons for caution: Our center was only a reproductive center; thus, all pregnancy outcomes were obtained via telephone calls or faxes. Therefore, some detailed obstetric complications were not studied here. Additionally, this was a retrospective study, and the control group was not screened randomly, which may cause selection and confounding biases.

Wider implications of the findings: Our findings can be used to counsel women whose pregnancies are complicated by a unicornuate anomaly and help guide appropriate antenatal treatment and surveillance.

Trial registration number: None.

P-437 Glycodelin-A interacts with L-selectin on CD16-CD56bright natural killer cells to induce endothelial cell angiogenesis and trophoblast invasion

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Study question: How does glycodelin-A (GdA) regulate the action of $CD16^{-}CD56^{bright}$ natural killer (NK) cells on trophoblast and endothelial cell functions?

Summary answer: GdA binds to L-selectin of the $CD16^{-}CD56^{bright}$ NK and induces secretion of angiogenic factors, which in turn regulate endothelial cell angiogenesis and trophoblast invasion.

What is known already: Glycodelin-A (GdA) is an abundant glycoprotein in the decidua. The carbohydrate chains of GdA interact with sialylated glycan receptors such as selectin and siglec of resident decidual leukocytes and invading trophoblasts, modulating their functions for a successful pregnancy. Aberrant expression of GdA in serum, endometrial tissue and uterine flushing is associated with unexplained infertility, pregnancy loss, and preeclampsia. $CD16^{-}CD56^{+}$ natural killer (NK) cells are the most abundant leukocyte population in the decidua. These cells regulate trophoblast invasion during spiral arteries remodeling, and mediate the homeostasis and functions of the endothelial cells.

Study design, size, duration: Human $CD16^{-}56^{bright}$ NK cells were isolated from female peripheral blood by immuno-magnetic beads and fluorescence

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activated cell sorting. They were cultured with GdA at a physiological dose of 5 μg/mL. The phenotypes, the secretion of angiogenesis-related soluble proteins and the biological activities of the resulting NK cells were studied.

Participants/materials, setting, methods: GdA was purified from human amniotic fluid by affinity chromatography. Secretion of angiogenesis-related soluble proteins were determined by cytokine array and flow cytometry. The effect of the identified angiogenic proteins were studied by corresponding blocking antibodies. Endothelial cell angiogenesis was determined by tube formation assay. Cell invasion and migration were determined by trans-well invasion/migration assay. GdA binding on the NK cells and the expression of decidual NK cell markers were analyzed by flow cytometry.

Main results and the role of chance: GdA treatment increased secretion of vascular endothelial growth factor (VEGF) and insulin-like growth factor-binding protein 1 (IGFBP-1) from the $CD16^{-}CD56^{bright}$ NK cells, the suggested immature precursors of tissue-specific NK cells, but not the $CD16^{+}CD56^{dim}$ NK cells. Serum-free conditioned medium derived from GdA-treated $CD16^{-}CD56^{bright}$ NK cells induced tube formation of of HUVEC endothelial cells in vitro, and this effect was nullified by anti-VEGF antibody. Similarly, data suggested that IGFBP-1 mediated the stimulatory activities of the conditioned medium on invasion of primary extravillous trophoblast isolated from 1st trimester termination of pregnancy and trophoblastic cell line JEG-3. Fluorescently labeled GdA has a significantly stronger binding to $CD16^{-}56^{bright}$ NK cell than the $CD16^{+}CD56^{dim}$ NK cell. The sialic acid-dependent glycan receptor L-selectin was expressed in former but not the latter NK cells. The binding was sialic acid dependent, and desialylated GdA failed to bind to both NK cell subsets. Anti-L-selectin function blocking antibody (CD62L) treatment prevented the binding of fluorescently labeled GdA to the $CD16^{-}56^{bright}$ NK cells, and abolished the actions of GdA on NK cells in terms of VEGF and IGFBP-1 secretion. GdA enhanced the expression of decidual NK cell marker CD9 in the $CD16^{-}CD56^{bright}$ NK cells.

Limitations, reasons for caution: Most of the conclusions were based on human peripheral blood $CD16^{-}56^{bright}$ NK cells. Confirmation of these results using human decidual natural killer cells is required. on.

Wider implications of the findings: This is the first study on the role of GdA in $CD16^{-}56^{bright}$ NK cell functions. Further study on the glycosylation of GdA and its biological role will provide better insight of its molecular nature, and thus offer opportunity for development of new preventive intervention and treatment approach for pregnancy complications.

Trial registration number: Nil.

P-438 Collagen mixed with epidermal growth factor promotes the endometrial HOXA10 expression as well as endometrial growth in patients with thin endometrium

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Study question: Does collagen mixed with epidermal growth factor (EGF) increase the expression of endometrial *HOXA-10* in the mid-luteal phase of infertile patients with thin endometrium, compared with EGF alone?

Summary answer: EGF-loaded collagen stimulates the endometrial *HOXA10* expression in the mid-luteal phase and improves the endometrial growth effect of EGF in infertile patients with thin endometrium.

What is known already: Treatment of poorly developed thin endometrium is still challenging. Several growth factors and cytokines including EGF and granulocyte colony-stimulating factor (G-CSF) have been shown to stimulate the endometrial growth in patients with thin endometrium. In 2016, we have presented that collagen enables sustained release of EGF and improves the treatment effect of EGF on thin endometrium of infertile women. However, study on the effect of EGF-loaded collagen on endometrial receptivity markers including *HOXA10* in patients with thin endometrium has not been reported.

Study design, size, duration: We analyzed the results of a total of 87 consecutive infertile women with thin endometrium < 6 mm who underwent intrauterine treatment with either EGF-loaded collagen solution (study group, n = 50) or EGF alone solution (control group, n = 37) between March 2014 and November 2016 for this retrospective study. Endometrial tissue in the mid-luteal phase was obtained in 15 patients included in the study group and 12 patients included in the control group in the same period.

Participants/materials, setting, methods: In the study group, slow intrauterine infusion of 0.8% collagen gel (Collabarrier, Dalimtissen. Co. Ltd., Seoul, Korea) in which 200 ng/ml EGF was loaded was performed every 2-3 days during the follicular phase of 4 to 5 menstrual cycles. In the control group, EGF alone solution with same concentration was used during same period. Endometrial biopsy was performed in the mid-luteal phase of last treatment cycle. Endometrial *HOXA10* mRNA was analyzed by realtime RT-PCR.

Main results and the role of chance: Study and control groups were similar with respect to patient's characteristics. The treatment duration and number were also comparable between the two groups. The peak endometrial thickness was significantly higher in the study group of 7.51 ± 0.68 mm compared with 6.86 ± 0.66 mm in the control group ($p < 0.001$) and resistance index (RI) of subendometrial artery (SEA) was also significantly lower in the study group ($p < 0.001$). Proportion of patients with blood flow of subendometrial artery (SEA) after treatment was also significantly higher in the study group ($p = 0.021$). Relative amount of HOXA-10 mRNA in the endometrium was significantly higher in the study group of 15.85 ± 5.76 compared with 3.21 ± 2.70 in the control group ($p < 0.001$)

Limitations, reasons for caution: This study may have a limitation due to a small number of sample available. Inaddition, it is difficult to know whether the positive effect of EGF-loaded collagen on endometrial *HOXA-10* expression is caused by the collagen itself or by the sustained release of EGF.

Wider implications of the findings: This new treatment regimen may not only promote endometrial growth but also enhance embryo implantation by increasing endometrial *HOXA-10* expression in infertile patients with thin endometrium.

Trial registration number: No.

P-439 Clinical signification of low-grade blastocyst among sibling embryos from the same cohort

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Study question: How do we select and transfer the low-grade stage blastocysts which make up about 40% of all blastocysts in ART treatment.

Summary answer: Each low-grade blastocyst has moderate hatch potential. The pregnancy rate of the other embryos after successful first transfer is moderate except for women over 40.

What is known already: We can obtain some morphological types of blastocyst with oocyte retrieval. Usually, the best blastocysts are selected from the same cohort and are transferred in order. In Japan we are not permitted to perform PGS. According to the Gardner classification, expanded blastocysts (G3 to G5) have a higher pregnancy rate than low-grade blastocysts (G1 and G2). Of the blastocysts we come across, 17.4% are G1 and 24.9% were G2. We often have to think about what to do with surplus low-grade blastocysts after an unsuccessful result of the first embryos transfer from the same cohort.

Study design, size, duration: This is retrospective, single center study (conducted January 2015 to December 2016).

Participants/materials, setting, methods: This study included 329 vitrified blastocyst in the G1 stage and 670 in the G2 stage, and all blastocysts were vitrified at day 5. Pregnancy rate was detected after thawed single embryo transfer. For experimental analysis, we examined the hatch potential of 63 low-grade blastocysts. Back ground of the groups are no significantly difference.

Main results and the role of chance: The hatching rate of vitrified G1 blastocysts in vitro was 31.3% (10/32), and that of vitrified G2 blastocysts was 88.5% (23/26), p<0.001. The ongoing pregnancy rates of G1 and G2 blastocysts were significantly different (25.1% (231/919) and 12.7% (55/432), respectively, p<0.001). Blastocysts were divided into two groups according to the situation. Group A was the low-grade blastocysts that deserved the second

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grade ranking of their cohort that remained after successful single higher-grade blastocyst transfer. Group B was also the low-grade blastocysts that deserved second-grade ranking of their cohort that remained after failed single higher-grade blastocyst transfer. The ongoing pregnancy rates of G2 blastocysts in Group A and Group B were 33.6% (47/140) and 23.4% (43/184), respectively, p<0.05. The ongoing pregnancy rates of G1 blastocysts in Group A and Group B were 18.6% (13/70) and 6.0% (6/100), respectively, p<0.01. The ongoing pregnancy rates of G2 blastocysts from patients 39 and under and G2 blastocysts from patients 40 and over were 30.7% (206/670) and 9.8% (25/255), respectively, p<0.01. The ongoing pregnancy rates of G1 blastocysts from patients 39 and under and G1 blastocysts from patients 40 and over were 15.2% (50/329) and 4.5% (5/110), respectively, p<0.01.

Limitations, reasons for caution: There is the opinion that G1 and G2 grade blastocysts should be cultured and undergo PGS for analyzing euploid embryos. However, in Japan PGS is not permitted to any ART treatment. In this study, we evaluated the pregnancy rates from the morphological blastocyst status.

Wider implications of the findings: We need to carefully consider whether we should transfer the remaining low-grade blastocysts or try to do another retrieval according to the pregnancy rates of those low-grade blastocysts. Except for G1 low-grade blastocysts from women 40 and over it might be beneficial to transfer low-grade blastocysts.

Trial registration number: self-funded

P-440 Subchorionic hematomas are associated with hormone replacement treatment, but do not have any negative impact on pregnancy and miscarriage rates

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Study question: Are subchorionic hematomas (SCH) associated with hormone replacement treatment (HRT) and do they influence ongoing pregnancies?

Summary answer: It was found that SCH was associated with HRT, but no negative influence on pregnancy and miscarriage rates was detected.

What is known already: SCH in pregnancies with first-trimester vaginal bleeding is the most common sonographic abnormality in the presence of a live embryo. First-trimester bleeding is notably higher in pregnancies following IVF/ICSI as compared to natural births. However, several reports have shown that pregnancies derived from IVF/ICSI are not associated with an increased risk of spontaneous abortion. On the other hand, other reports have shown that the incidence of preterm delivery (<37 gestational week) after fresh embryo transfers (ET) is higher in patients with first trimester bleeding as compared to patients without first trimester bleeding.

Study design, size, duration: This retrospective study of 4,835 pregnancies was conducted between January 2011 and December 2015, including 684 fresh ET and 4,835 frozen- thawed ET (ovulatory cycles: n = 476, and HR cycles: n = 4,359), all of which achieved the development of a gestational sac (GS) following single embryo transfer. Identical twin pregnancy was excluded from this study. The presence of SCH was defined by transvaginal ultrasound, which was observed by15 gestational weeks.

Participants/materials, setting, methods: The occurrence of SCH after fresh ET was compared with the occurrence of SCH after frozen-thawed ET. Among frozen-thawed ET cycles, the risk of SCH was compared between ovulatory cycles and HRT cycles. Furthermore, the fetal heart beat (FHB) and the birth rates were compared between patients with SCH and without SCH among HRT cycles.

Main results and the role of chance: The occurrence of SCH after frozen-thawed ET was significantly higher as compared to SCH after fresh ET (16.3% vs. 8.2%; p<0.001). Among frozen-thawed ET cycles, the incidence of SCH with HRT was significantly higher than that without HRT (17.1% vs. 8.8%; p<0.001). Among the HRT cycles, the positive FHB rate was significantly higher in patients with SCH as compared to patients without SCH (95.7% vs. 86.9%; p<0.001). Similarly, birth rates in HRT cycles were significantly higher in patients with SCH than in patients without SCH (84.4% vs. 74.0%; p<0.001). Regarding pre-term birth rates in HRT cycles, there was no significant difference between patients with SCH and patients without SCH (7.6% vs 8.5%; p = 0.49).

Limitations, reasons for caution: Other factors, such as high blood pressure, prior obstetrical complications and family history, which may be associated with SCH, are under investigation following this study.

Wider implications of the findings: A higher incidence of SCH with HRT may indicate excessive hormonal levels in the endometrium. Although no negative impact on pregnancy and miscarriage rates was detected in patients with SCH, careful treatment for SCH is necessary to ensure successful live birth.

Trial registration number: Not applicable.

P-441 CD147 regulates human extravillous trophoblast invasion via Wnt/β-Catenin Signaling Pathway

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Study question: What are the underlying mechanisms that CD147 regulates human extravillous trophoblast invasion?

Summary answer: CD147 on the plasma membrane and extracellular vesicles (EVs) of human extravillous trophoblast enhances trophoblast invasion through the activation of Wnt/β-Catenin signaling pathway.

What is known already: CD147 (basigin or EMMPRIN) is a transmembrane glycoprotein exists as protein complexes on the plasma membrane of human trophoblasts. It regulates trophoblast invasion by modulating matrix metalloproteinases (MMPs) and enzymes of the urokinase plasminogen activator (uPA) system. Secretory CD147 is mainly generated by microvesicle shedding. Reduced placental CD147 expression is associated with pre-eclampsia, but the mechanism of actions remains unclear. A key feature in preeclampsia is the defect in trophoblast invasion during early placentation process.

Study design, size, duration: The expression of CD147 on human extravillous trophoblasts and trophoblast-derived EVs was studied. Their roles and mechanism on trophoblast functions were assessed.

Participants/materials, setting, methods: Trophoblast invasion was evaluated by transwell invasion assay. The spent medium from placental explants, isolated trophoblasts or trophoblastic cell-line JEG-3 was collected for EV isolation by differential centrifugation. EVs uptake and trafficking were assessed by flow cytometry and live cell confocal imaging. CD147 expression on EVs was determined by immune-electron microscopy. CD147 suppression on trophoblasts was achieved by siRNA knockdown or functional blocking antibody. The involvement of Wnt pathway was confirmed by pharmacological activators/inhibitors.

Main results and the role of chance: CD147 was demonstrated to be expressed on the trophoblast plasma membrane or trophoblast-derived EVs in form of protein complex. Inhibition of CD147 suppressed trophoblast invasion and β-catenin activation. The involvement of Wnt pathway in the effects of CD147 was further supported by the studies using Wnt activator or inhibitor. We further demonstrated that the isolated trophoblast-derived EVs could be uptake by other trophoblasts in a time-dependent manner which acted as a cargo for CD147 trafficking.

Limitations, reasons for caution: Most of the above findings are based on *in vitro* model. Their possible association with pathological placental development during early pregnancy needs to be confirmed.

Wider implications of the findings: The outcome of this project gives a better understanding on the role of CD147 in regulating early placentation. Our findings also indicate the possible use of trophoblasts-derived CD147, including both cell surface CD147 and secretory CD147, as test for early prediction of trophoblast functions.

Trial registration number: Not applicable.

P-442 Exposure to Mancozeb impairs embryo implantation via disrupting embryo- epithelium interaction in vitro trophoblast/endometrial cells co-culture model

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Study question: Will Mancozeb affect implantation using spheroid-endometrium co-culture model? What is the underlying mechanism that Mancozeb affect endometrium receptivity?

Summary answer: Mancozeb decreased spheroid attachment onto endometrial epithelial cells in vitro and modulated steroid hormone receptor expression and in turns embryo implantation.

What is known already: Mancozeb, is a manganese/zinc containing fungicide belonging to the ethylene bisdithiocarbamate (EBDC) family. Mancozeb has already been classified as a potential endocrine disrupting chemical (EDC), which is reported to modulate androgen and thyroid hormone receptor pathways in recent years. It is widely used in agriculture to protect the fruits, crops and vegetables from fungal diseases. Mancozeb has short half-life (1~3 days) and very low acute mammalian toxicity. However, the role of Mancozeb on human reproductive process especially embryo implantation remains largely unknown.

Study design, size, duration: We used the JEG-3 and endometrial adenocarcinoma Ishikawa cells lines to mimic embryo implantation. Methotrexate (5 μM) was used as a positive control. JEG-3 and/or Ishikawa cells were treated with different concentrations of Mancozeb (0.01 μg/ml, 0.1 μg/ml, 1 μg/ml and 3 μg/ml) for 24 or 48 hours.

Participants/materials, setting, methods: Total RNA and protein were extracted from Ishikawa and JEG-3 cells. The attachment rate of JEG-3 spheroids and the expression of adhesion molecules (N-catenin and E-cadherin) were measured. The expression of Wnt/β-catenin signaling molecules and steroid receptors (ER, PR and AR) transcripts and proteins in Ishikawa cells were measured by qPCR and Western blotting, respectively. The function role of ERb receptor on implantation was confirmed by inhibitor.

Main results and the role of chance: Mancozeb did not suppress cell proliferation at concentration less than 10 μg/ml. Mancozeb (>1 μg/ml) could decrease the attachment rates when both JEG-3 and Ishikawa cells were treated. The decrease in attachment rate was more prominent by treating the Ishikawa than JEG-3 cells only. Mancozeb affected the expression of N-cadherin in JEG-3 but not in Ishikawa cells, and down-regulated ERβ transcript and protein levels in Ishikawa cells. No change was observed for the expression of ERα, PR and AR. To confirm the role of ERβ in embryo implantation, we used highly selective ERβ antagonist PHTPP (10 μM) on Ishikawa cells and found PHTPP could reduce the attachment rate in spheroids attachment assay which indicate the association of ERβ in implantation. However, in Wnt/β-catenin signaling pathway, no significant change was observed under environmental conditions.

Limitations, reasons for caution: As Mancozeb has a relative short half-life in the cells and environment, its metabolite ethylene thiourea (ETU) should be evaluated. Present study was laboratory study, data from in vivo studies and concentration of Mancozeb and its metabolite in patient with fertility problem should be investigated.

Wider implications of the findings: This study indicates that fungicide Mancozeb could affect implantation process through steroid hormone receptor. Countries with high usage on Mancozeb should be investigated in women with high serum Mancozeb level seeking for infertility treatment since exposure to Mancozeb happens via food chain easily.

Trial registration number: N/A.

P-443 Endometrial receptivity assessment using transcriptomic approach 'Win-test' for personalized embryo transfer

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Study question: The aim of this study was to optimize pregnancy outcome using personalized care management by combining embryo replacement according to the endometrial receptivity evaluation in patients with repeated implantation failures.

Summary answer: Individual evaluation of endometrial receptivity allows a personalized patient care management and improves pregnancy outcome for patients with repeated implantation failures (RIFs)

What is known already: Many approaches for human endometrial receptivity including microarray has been previously reported. However, efficiency of the tests according to the clinical results is still debated. The aim of this study is to evaluate the endometrial receptivity (ER) in RIFs patients who have had at least 3 to 7 implantation failures using Window implantation test (Win Test). The principal of concept which consisting for screening for 13 specific genes of implantationwindow.

Study design, size, duration: An endometrial biopsy is performed during the implantation windows. According to the Win-test result, the embryo transfer strategy was established: transfer at the blastocyst stage at the specific day during the implantation windows where endometrium has been diagnosed as 'receptive'; or transfer at day 2 or day 3 post-fertilization where endometrium is considered as 'partially receptive'. Clinical pregnancy was defined by visualization of a gestational sac with a positive foetal heartbeat.

Participants/materials, setting, methods: The Win Test consists to measuring the expression level of 13 genes predictive of endometrial receptivityin endometrial biopsies. RNAs were extracted and determination of the mRNA expression levels of our biomarkers was performed by qRT-PCR. Then data were converted into a score by using algorithms.

Main results and the role of chance: Analyses of endometrial receptivity status (n = 799) in a large cohort of infertile patients revealed that a strong inter-patient variability i) of the moment of the opening of the implantation window, as well as ii) of the duration of this implantation. Then, as soon as the cycle day where endometrium was said receptive, a personalized embryo transfer can to be perform in the respect of the synchronization of the fœto-maternal dialogue. Using this strategy, the biochemical and clinical pregnancy rate in this group of patients (n = 151) with previous RIFs were 38.3 and 32.7 %, respectively.

Limitations, reasons for caution: Personalized embryo transfer has been performed in 151 RIFs patients only. The benefits of this innovative strategy will be analyzed in PGS patients.

Wider implications of the findings: A personalized embryo transfer according to the endometrial receptivity status diagnosed by transcriptome approach improves pregnancy outcome

Trial registration number: Not applicable.

P-444 Comparative evaluation of Notch signaling pathway in the endometrium of women with PCOS, endometriosis, RIF and healthy fertile controls during luteal phase

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Study question: Does Notch signaling pathway alter in women with polycystic ovary syndrome (PCOS), endometriosis and Repeated Implantation Failure (RIF) compared to healthy fertile women?

Summary answer: Notch signaling components show different expression in PCOS, endometriosis and RIF patients in comparison with normal women.

What is known already: Prior evidence have suggested Notch signaling pathway plays important role in endometrial remodeling, decidualization, and implantation

Study design, size, duration: In this case–control study, human endometrial biopsies were taken from ten PCOS patients, ten endometriosis, 10 RIF patients and ten healthy fertile women in the secretory phase.

Participants/materials, setting, methods: Total mRNA were extracted from endometrial tissues of PCOS, endometriosis and RIF patients (on day 3-5 after ovulation, n = 10) and healthy fertile individuals (on day 3-5 after ovulation, n = 10) during luteal phase, then the expression of Notch1, -2, -3, Jagged1, -2 and survivin as a downstream target of Notch signaling pathway were measured by QRT-PCR.

Main results and the role of chance: All the endometrial samples expressed Notch1, Notch2, Notch3, Jagged1,2 and survivin. There were significant differences ($p<0.05$) for Notch1,Jagged1,2 and surviving between free-disease and disease groups.

Limitations, reasons for caution: The main limitation of this study is a low number of human endometrial samples.

Wider implications of the findings: This study provides new insights into the role of Notch signaling pathway in endometrial receptivity.

Our data also indicate that Notch signaling may be promising molecular pathway for the assessment of implantation capacity in several female reproductive diseases.

Trial registration number: N/A.

P-445 Cumulative live birth rate after preimplantation genetic screening in repeated implantation failure: is there still a place for replacing genetically uncreened embryos?

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Study question: What is the cumulative ongoing and live birth rate after preimplantation genetic screening (PGS) with trophoectoderm biopsy and array comparative genomic hybridization (aCGH) analysis in repeated implantation failure (RIF)?

Summary answer: Per patient cumulative ongoing pregnancy and live birth rate after PGS was 76.3%. Euploidy rate was 52.4%.

What is known already: Not much neither known nor published.

A uniform definition is still lacking and, even though embryo aneuploidy is by far the most important factor in RIF, PGS benefit is still questioned.

Unfortunately, the few RCT that we are aware of assessing PGS in RIF, performed D3 biopsy and FISH analysis and a recent non-randomized trial performing trophoectoderm biopsy and comprehensive chromosome screening (CCS), focused on implantation and pregnancy rates.

We consider that RIF studies using PGS should refer to trophoectoderm biopsy and CCS and that success rates, also in RIF, should be reported as cumulative ongoing / live birth rates.

Study design, size, duration: Retrospective study carried out in a University-affiliated fertility clinic (January 2015 - June 2016).

N = 38 couples with RIF: absence of implantation after consecutive embryo transfer (ET) of at least 4 D3 embryos or 2 blastocysts of good quality, as recently defined. Cases with abnormal karyotype, trombophilia, autoimmune disorders, uterine abnormalities, hydrosalpinx or severe male factor were excluded. Women aged >38 were excluded as at those ages, >50% of embryos have been reported to be aneuploid.

Participants/materials, setting, methods: 38 couples with RIF underwent one PGS cycle. Trophoectoderm biopsy was performed when embryos reached the blastocyst stage (D5, D6, D7). Blastocysts were subsequently vitrified. CCS was done with aCGH analysis.

Frozen ET was scheduled either on natural (16%) or artificial cycle (84%). ET was always done under ultrasound guidance.

Main results and the role of chance: Mean woman's age was 35.5 ± 2.4 and mean number of previously transferred embryos was 6 ± 2.7.

With a mean number of 15.2 ± 5.3 inseminated oocytes per patient, fertilization rate was 79.4% and blastocyst rate was 53.1%. Mean number of biopsied blastocysts per patient was 6.4 ± 3.5. Observed euploidy rate was 52.4%. All cases reached ET.

Interestingly, 25% of D3 good quality embryos did not reach the blastocyst stage and 51% of those that reached it were found to be aneuploid.

A total of 50 frozen ET were performed (12 patients with unsuccessful 1st ET underwent a 2nd ET, still no 3rd ET). Mean number of transferred embryos was 1.4 ± 0.5 (single ET was performed in 58% of cases, in 56% of them >1 euploid embryo was available). Clinical pregnancy rate per ET was 68% (34/50) with a miscarriage rate of 14.7% (5/34). Out of 50 ET, 11 are currently ongoing pregnancies (>20 weeks, 22%) and 18 have resulted in a live birth (36%). Globally, 6 multiple pregnancies have been observed.

Implantation rate was 56.3%. Per patient cumulative ongoing / live birth rate was 76.3%. Out of the 9 patients that haven't achieved an ongoing pregnancy / live birth, 6 still have frozen euploid embryos.

Limitations, reasons for caution: Our main limitations are the retrospective nature of the study and the small sample size.

Wider implications of the findings: According to our results, PGS should be offered in RIF patients. Furthermore, shouldn't embryo euploidy be a prerequisite to be considered in a future RIF definition?.

Other factors should be identified in RIF patients that don't succeed after ET of euploid blastocysts and mosaicism cannot be ruled out.

Trial registration number: NCT03001648.

P-446 The absence of ovulation negatively impacts endometrial receptivity: results from an experimental model of anovulation

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Study question: Does anovulation induced by continuous light exposure adversely affect the expression of endometrial receptivity markers in rats?

Summary answer: Induced anovulation in rodents reduces expression of LIF and PROKR1, crucial endometrial receptivity molecules, even though anovulatory rats show exuberant development of the uterine structure.

What is known already: Infertile patients due to ovulatory disorders have sub-optimal pregnancy rates when they undergo assisted reproductive technology as compared to normal ovulatory patients. Yet, they present a wealth of clinical and laboratory features in common with women displaying recurrent implantation failures. The complex endocrine-metabolic milieu of the anovulatory patient constitutes an obstacle in the evaluation of the specific effect of anovulation on endometrial function. Whether anovulation itself is the main cause of endometrial receptivity disturbance in this subset of patients remains to be further investigated.

Study design, size, duration: 14 rats displaying anovulation induced by continuous light exposure were compared to 13 ovulatory rats, exposed to natural light 12/12 hours, concerning biomolecular and histological parameters of endometrial receptivity and function. The average experimental period of each included animal was 2 months. The analysis and comparisons between animals' uteri was done after precise establishment of the estrus cycle phases through serial vaginal cytology. A single experienced operator performed all cytological and histologic evaluations.

Participants/materials, setting, methods: 27 adult females Rattus norvegicus albinus (Wistar) were sorted into 2 groups: experimental group, n = 14, under continuous illumination and anovulatory cycles; and control group, n = 13, under natural 12/12 light/dark alternation and ovulatory cycles. The uterine samples were assessed as to the concentration of LIF and PROKR1 by sandwich ELISA. Further, transversal uterine sections stained in hematoxylin and eosin were studied for endometrial thickness, myometrial thickness, glandular surface and vascular surface.

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Main results and the role of chance: Rats in both groups presented similar weight, number of vaginal cytological smears and uterine mass. Animals in the experimental group were significantly older than those in the control group (84 days vs. 74 days, P = 0.01). In addition, anovulatory rats showed exuberant uterine structural development with hypertrophy of the epithelial, muscular, glandular and vascular elements, which were easily noted by histological analysis. Specifically, anovulatory rats displayed greater endometrial thickness (712 μm vs. 399 μm, P < .0001), myometrial thickness (228 μm vs. 151 μm, P < .0001) and vascular surface (60,354 μm^2 vs. 43,066 μm^2, P = 0.029). There was a tendency to statistical signification (P = 0.096) in the glandular surface comparison toward the anovulatory group. It is noteworthy that anovulation was associated with a decline of expression of endometrial receptivity markers LIF (385,921 ng/ml vs. 517,567 ng/ml, P = 0.009) and PROKR1 (1,643 pg/ml vs. 3,337 pg/ml, P < .0001), meaning that structural development itself doesn´t mean, necessarily, a favorable uterine environment for embryo implantation in terms of molecular expression pattern.

Limitations, reasons for caution: As an experimental study in rodents, results can´t be directly extrapolated to humans. Anovulatory rats were 11% older, which could have partially influenced results. The low reproducibility of histological measurements needs to be noticed, even though its effect was minimized by single operator evaluations.

Wider implications of the findings: The lower activity of fetal-maternal LIF-PROKR1 signaling pathway could have an influence in the lower implantation rates among anovulatory patients. This study raises new questions concerning the role of LIF and PROKR1 in the diagnosis of endometrial factor infertility, and also as whether they could be used as targeted therapies.

Trial registration number: Not applicable.

P-448 A randomized controlled clinical trial: the treatment of tamoxifen with patients of thin endometrium undergoing frozen-thawed embryo transfer

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Study question: Endometrial thickness <7 mm is always a criterion for fresh IVF cycle cancellation and all embryos will be cryopreserved. However, improving endometrial growth in such patients is very difficult.

Summary answer: Tamoxifen compared with letrozole drugs in patients of thin endometrium undergoing frozen-thawed embryo transfer (FET) can increase endometrial thickness and improve implantation rate.

What is known already: Up to now, ther are no standard treatment guidelines for thin endometrium. Tamoxifen, Some research supported that tamoxifen (TAM) compared with clomiphene for ovulation induction can significantly improve the endometrial thickness,but there are few reports about the use of tamoxifen with patiens having thin endometrium in frozen-thawed embryo transfer.

Study design, size, duration: A total of 133 women with thin endometrium scheduled for FET (January 2014–June 2016) were enrolled in an open-label randomized clinical trial to ovarian stimulation with letrozole per oral (n = 72,2.5 mg/day from Day3-7) or tamoxifen per oral (n = 61,40 mg/day from Day 3-7)

Participants/materials, setting, methods: We studied subfertile couples undergoing FET. Further inclusion criteria were female age ≤40 years, number of embryos transferred ≤2 and at least one of good quality, failure of grownth of endometrium in hormone replacement therapy. The primary end-point was clinical pregnancy.

Main results and the role of chance: The serum estradiol level of LE group both on HCG and transfer day [(1193.80 ± 629.64)ng/L vs(2776.30 ± 157.34) ng/L;(1195.90 ± 820.30)ng/L vs (2129.40 ± 1208.71) ng/L,p = 0.000] were statistically lower, serum luteinizing hormone level were statistically higher than TAM group [(20.48 ± 15.50)IU/L vs (10.59 ± 8.34)IU/L,p < 0.05]; implantation rate of LE group were statistically lower than TAM (39.32% vs 45.83%, p = 0.001). The endometrial thickness and serum E2 and P levels in TAM cycles were significantly higher compared with those in hormone replacement therapy cycle [(8.49 ± 1.36)mm vs (6.43 ± 0.96) mm, p = 0.018].

Limitations, reasons for caution: Since the sample size is small, this conclusion needs confirmation with large one.

Wider implications of the findings: Tam compared with LE for patients of thin endometrium undergoing FET can increase endometrial thickness and improve implantation rate, thus providing a new solution.

Trial registration number: ChiCTR-INR-16007965

P-449 Placental chorionic villi of the first trimester human placenta: a lectin study

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Study question: How does the glycocode of the human chorionic villi changes during the first trimester of pregnancy?

Summary answer: A significant modification in the distribution of carbohydrate epitopes bearing fucose and sialic acid could be revealed during the first trimester of pregnancy

What is known already: Although several studies have been published on sugar distribution in human placenta, they are mainly limited to terminal placentae and also lead to conflicting results. A few studies addressed the glycosylation status of different placental cells with particular reference to macrophage-like cells called Hofbauer cells in terminal placentae.

Study design, size, duration: We approached the study of cellular glycosylation in human placenta by using chorionic villi explants obtained by microdissection. A total of 24 placentae were obtained from women undergoing voluntary abortion and signing an informed consensus according to the guidelines of the University of Siena Ethical Committee.

Participants/materials, setting, methods: Isolated chorionic villi explants from first trimester human placentae (weeks 7, 9, 10, 12) were fixed and embedded in paraffin for histochemical analysis using biotinylated Concanavalin A (ConA), *Maackia amurensis* (MAA), *Sambucus nigra* (SNA) and *Lotus tetragonolobus* (LTL) lectins. Alternatively, samples were solubilized and analyzed by two dimensional electrophoresis followed by lectin blotting with the same probes.

Main results and the role of chance: The two methodological approaches used in this study allowed us to provide general information on the expression of selected carbohydrate epitopes in the whole tissue and on their specific localization in the cellular compartments. Glycoconjugates bearing fucose and sialic acid terminal epitopes are almost absent in seven weeks placenta explants while they became expressed in weeks 9 to 12 with a specific localization on endothelial and Hofbauer cells with MAA reactivity extending to the throfoblast. On the other side ConA reactivity was consistently found on throphoblast cells, their glycocalix and the stroma. By two dimensional electrophoresis followed by lectin blotting we could reveal non only exposed epitope carbohydrates but also processing and/or stored glycoproteins. As expected ConA labeling revealed several dozens of glycoproteins bearing N-linked oligosaccharides at all different stages of pregnancy. On the other side MAA, SNA and LTL lectins are restricted to few glycoproteins with a tendency to a reduction in the periods under analysis (weeks 7 to 12). Careful controls were performed and a significant role for chance may be excluded.

Limitations, reasons for caution: This has to be considered a pilot study. It has to be completed with a more specific analysis of the tentatively identified glycoproteins with specific antibodies and/or with protein identification by mass spectrometry

Wider implications of the findings: These preliminary results suggest that changes in endothelial cells may allow selective migration of Hofbauer cells from the blood flow in the first trimester of pregnancy and a more general role in stromal cells maturation and differentiation.

Trial registration number: None.

P-450 Construction of gel based 3D endometrial co –culture systems: Can human mesenchymal stem cells be an alternative?

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Study question: Which is more effective for construction of gel based 3D endometrial co–culture systems, endometrial cells or human mesenchymal stem cells?

Summary answer: Mesenchymal stem cells obtained from human umbilical cord can also be used for the same purpose and in a more efficient manner in some ways.

What is known already: Implantation is a complex process consisting of three phases: apposition, attachment-adherence and penetration. The rate of implantation failure in first trial of spontaneous pregnancies is 25-40%. It is very difficult to mimic implantation in vitro since the process takes place in a 3D environment. 3D co-cultures are more convenient for blastocyst behavior, during invasion. The perfect 3D culture has not been revealed successfully yet. One of the methods for 3D cultures is use of endometrial cells in a gelatinous matrix. On the other hand mesenchymal stem cells were also demonstrated to have positive effects on the developing embryo.

Study design, size, duration: This study reveals the comparative effects of two different gel-like extracellular matrix based 3D endometrial co-culture systems, one constructed with mouse endometrial stromal and epithelial cell, the other with human mesenchymal stem cells obtained from human umbilical cord matrix. The comparative analysis was held at hours 48 and 72 by IF, WB and qRTPCR and by confocal and multiphoton microscopy for Invasion depth.

Participants/materials, setting, methods: Mouse blastocysts were cultured in three groups as: i. routine IVF drop culture group, ii. the group that holds mouse blastocysts ongel decorated with endometrial epithelial cells and endometrial stromal cells, iii. the group that holds mouse blastocysts on immnunocompetent human mesenchymal cell decorated gel based 3D co-culture. The groups were compared in terms of structure, function and invasion by e-cadherin, LIF, MMP9 and progesterone receptor Caspase-9 and live/dead cell assay were also performed.

Main results and the role of chance: Here we propose two very effective gel-based 3D in-vitro implantation models supported by either mesenchymal stem cells or endometrial cells, compared with each other and compared with a routine ivf culture. Both of them can be used to see the in-vitro development and survival of the blastocyst together with the newly differentiating trophoblasts. The comparative analysis was made between groups and also in the group itself from 48 to 72 hours. From 48 to 72 hours e-cadherin decreased in all groups with a high amount of decrease in endometrial cell decorated group. At the end of 72 hours endometrial co-culture was found to be very effective for expression of progesterone receptors, while human MSC supported balstocysts for production of LIF efficiently. Invasion parameter MMP9 expression was high with both cultures, while routine group has also increased MMP9 interestingly. Endometrial co-cultures supported the blastocyst to invade horizontally under the implantation area as depicted by the increase of dead cells, and human MSC also supported the blastocysts for invasion, implicating that human MSC can be used in vitro for generation of 3D endometrial co-cultures, with a further impact for effective in vivo use.

Limitations, reasons for caution: This study is based on use of mouse endometrial epithelial and stromal cells, though human MSCs are also used. The limitation is the inter-species implantation differences between of mouse and human. Human blastocysts were intentionally not used for such trial, since there is a major ethical concern

Wider implications of the findings: 3D culture for embryos still stands for an unsolved problem. Though the comparative methods proposed here are based on mouse blastocyst implantation, they provide information about the effects of human mesenchymal stem cells on implantation models, which can be alternatively tried for human embryos on 3D cultures before embryo transfer.

Trial registration number: This is not a clinical trial.

P-451 A Retrospective Comparative Study of Lymphocyte Immunotherapy With and Without Aspirin: Pregnancy Outcomes of Patients With Unexplained Recurrent Miscarriage

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Study question: To evaluate the efficacy and safety of lymphocyte immunotherapy (LIT) with and without aspirin for patients with unexplained recurrent spontaneous abortion (URSA)

Summary answer: LIT combined with aspirin improved the pregnancy outcomes of patients with URSA. The combination therapy was also relatively safe.

What is known already: Lymphocyte immunotherapy (LIT), a possible treatment for URSA, in which the prospective mother is tolerized by subcutaneous injections with paternal or third party lymphocytes. The aim is to induce female immunosuppression and thereby reduce the abortion rate.Another possible reason for URSA may be excessive coagulation in the mother. There is evidence that the placental vasculature of women with URSA bears microthrombi and infarctions.Daily low-dose aspirin inhibits platelet aggregation and can thereby prevent thrombotic disorders such as coronary artery disease.It is also logical to hypothesize that combining the two approaches might yield better pregnancy outcomes in women with URSA.

Study design, size, duration: This retrospective cohort study included all consecutive patients with URSA who were treated with LIT alone or with aspirin in 2009–2014 in a tertiary care hospital. The rates of successful pregnancy (defined as a pregnancy that continues past 20 weeks' gestation) and live birth were determined. The factors that influenced these pregnancy outcomes were identified by multiple logistical regression analysis. The partum maternal complications were recorded. The offspring were followed up for 1–6 years.

Participants/materials, setting, methods: The cohort consisted of all consecutive patients with URSA who were treated with LIT with or without aspirin in the Peking University Third Hospital between January 2009 and December 2014. Patients were excluded if they had not become pregnant within 1 year after the end of LIT or their medical records were not complete.Aspirin, 100 mg was administered daily starting at the beginning of LIT and continuing until 20 weeks' gestation.

Main results and the role of chance: Of the 61 patients in the LIT-alone group, 36 had a successful pregnancy (30 term deliveries, four preterm deliveries, and two induced labors due to fetal malformations). Of the 109 patients in the combination-therapy group, 82 had a successful pregnancy (73 term deliveries, five preterm deliveries, two induced labors due to fetal malformation and two induced labors due to fatal cord accidents). Thus, the pregnancy success rates of the LIT-alone and combination-therapy groups were 59% and 75.2%, respectively. This difference was statistically significant ($P < 0.05$). To calculate the live birth rate, the six patients who underwent induced labor because of fetal malformation or fatal cord accidents were not counted. Thus, 34 (55.7%) patients in the LIT-alone group and 78 (71.6%) patients in the combination-therapy group gave birth to a live infant. This difference was statistically significant ($P < 0.05$). Multiple logistical regression analysis indicated that aspirin acted as an independent factor that protected gestation. Neither LIT nor aspirin associated with excessive maternal and fetal complications.The present study compares for the first time the pregnancy outcome of patients with URSA who underwent LIT with or without aspirin.

Limitations, reasons for caution: The limitation of our study is a retrospective analysis, and RCTs of the two therapies are needed.

Wider implications of the findings: Women with URSA were treated with LIT, it yielded significantly higher rates of successful pregnancy and live birth when it was combined with aspirin.The low rates of complications in our cohort also suggest that LIT combined with aspirin is relatively safe. Hence, this method deserves more clinical recognition and application

Trial registration number: Not applicable.

P-452 Intrauterine infusion of human autologous peripheral blood mononuclear cells in patients with repeated failed attempts of optimum endometrial development in FET cycle: A Pilot study

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Study question: Does autologous peripheral blood mononuclear cells (PBMC) help in optimum endometrial development in patients with several failed FET cycles due to inadequate endometrial thickness and vascularity?

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Summary answer: Intrauterine infusion of PBMC resulted improvement of endometrial development in 27 amongst 37 patients studied, with 5 pregnancies following 15 transfer results declared so far.

What is known already: Autologous PBMC is one of the most convenient source for stem cells generation. This process is non-invasive, cost effective and causes minimal discomfort to the patient. PBMC has been shown as a source of pluripotent stem cells for regeneration of blood cells, nerve cells, epithelial cells, myofibroblast, muscle cells, bone cells and endometrial cells. There are studies depicting the role of PBMC with or without hCG priming for increasing the invasive potential of embryos as a therapeutic modality in recurrent implantation failure. There is a case report of regeneration of endometrium using PBMC in case of Asherman syndrome.

Study design, size, duration: Prospective study, with sample size of 37 patients, from August 2016 and continuing till date of submission of this abstract. The study was designed to evaluate possible improvement in endometrial thickness and vascularity following intrauterine autologous PBMC compared to the mean of their previous records in failed IVF attempts (min. 3 and max. 10) due to inadequate endometrial development. Mononuclear cells were isolated from patient's peripheral blood by density gradient centrifugation using commercially available lymphoprep.

Participants/materials, setting, methods: Setting: Institute of Reproductive Medicine, Kolkata, India

Patients received oestradiol valerate 6.0 mg daily from day2 till endometrial thickness reached ≥6.5 mm with significant increase in blood flow, when progesterone was administered. End diastolic velocity(EDV) was considered as the main marker of endometrial vascularity. On day5, isolated mononuclear cells suspended in 500 μL normal saline were infused into uterine cavity and embryo transfer was performed around day25. Inadequate endometrial response (thickness/vascularity) resulted in cancelation of embryo transfer.

Main results and the role of chance: The patients were categorized in 4 groups with regard to their response to therapy;- a) Significant increase in thickness (6.2 ± 0.8 vs 7.3 ± 0.9, P = 0.003) and vascularity (8.55 ± 1.79 vs 10.05 ± 1.66, P = 0.04) in 12 patients (44.44%) b) significant increase in vascularity (7.68 ± 1.62 vs 9.24 ± 1.66, P = 0.02) but not in thickness (6.8 ± 0.7 vs 7.2 ± 0.7, NS) in 2 patients (7.4%) c) significant increase in thickness (6.7 ± 1.2 vs 7.9 ± 1.3, P = 0.02) but not vascularity (7.84 ± 1.32 vs 8.68 ± 1.47, NS) in 13 patients (48.14%) d) no significant increase either in thickness (6.5 ± 0.8 vs 7.2 ± 1.1, NS) or in vascularity (7.45 ± 1.13 vs 8.22 ± 1.31, NS) in the rest 10 patients.

5 pregnancies were achieved out of 15 embryo transfer results declared so far. 4 pregnancies occurred when both thickness and vascularity improved and in one, than vascularity rather thickness showed improvement. No pregnancies were recorded in other two groups.

The probability of events happening due to chance is low although effect of catheter insertion and invoking cytokine production accelerating the endometrial development in presence of estrogen supplementation cannot be ruled out.

It is still premature to ascertain the increase in endometrial thickness and vascularity due to PBMC because some other factor like catheter insertion might have resulted in identical endometrial changes.

Limitations, reasons for caution: Number of patients studied till now is not adequate to make a valid conclusion. But results achieved already appear promising. However, there were patients whose cycles were cancelled implying that some unknown patient or endometrial characteristics may exist which may influence in achieving optimum endometrial preparation for successful blastocyst implantation.

Wider implications of the findings: Till date other than surrogacy, there is no proven treatment of repeated thin endometrium in FET cycles.

It is cost effective and minimally invasive procedure and can result in pregnancy without taking recourse to surrogacy.

Trial registration number: Not applicable.

P-453 Thin endometrium transcriptome analysis reveals a potential mechanism of implantation failure

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Study question: To investigate the molecular mechanisms how thin endometrium is involved in implantation failure.

Summary answer: Implantation failure in the thin endometrium appears to be associated with aberrantly activated inflammatory environment and aberrantly decreased response to oxidative stress.

What is known already: Women with thin endometria have lower pregnancy rates, largely due to implantation failure. We recently found high blood impedance in the uterine radial artery in patients with a thin endometrium, and that vitamin E, l-arginine, and sildenafil citrate treatments, which increase blood flow of the uterine radial artery, helped to thicken the endometrium. This suggests that low blood flow to the endometrium reduces its thickness. However, little information is available regarding the molecular mechanisms how a thin endometrium caused by low blood flow of the uterine radical artery is involved in implantation failure.

Study design, size, duration: A total of six women with a history of infertility were recruited into the study. The patients were classified into two groups: normal-thickness endometrium group and thin endometrium group. The cutoff value of endometrial thickness between normal and thin endometrium was defined as 8 mm based on our previous studies.

Participants/materials, setting, methods: Thin and normal endometrial tissue was obtained from a total of six women during the midluteal phase of the menstrual cycle. The transcriptomes were analyzed with a microarray containing 54,120 probes supporting 18,599 genes. Differentially expressed genes were classified according to Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Main results and the role of chance: The hierarchical dendrogram clearly separated the thin and control endometria in the mRNA expression profiles. We identified 318 upregulated genes and 322 downregulated genes in the thin endometrium compared to the control endometrium. The upregulated genes in the thin endometrium were related to immunity processes such as 'response to external stimulus", 'defense response", 'leukocyte mediated immunity", 'immune response", 'immune effector process" and 'regulation of immune system process". GO and KEGG pathway analyses indicated that the thin endometrium possessed aberrantly activated immunity and natural killer cell cytotoxicity accompanied with increased inflammatory cytokines such as interferon gamma (IFN-γ). The downregulated genes in the thin endometrium were related to metabolic processes such as 'small molecule catabolic process", 'single-organism catabolic process", 'organic acid catabolic process" and 'carboxylic acid catabolic process". The GO terms include carnitine palmitoyltransferase 1 (CPT1), 3-Hydroxy-3-Methylglutaryl-CoA synthase 2 (HMGCS2) and 3-oxoacid CoA-transferase 1 (OXCT1), which are known to play important roles in generating energy in cells and tissues. Two KEGG pathways that have a strong association with the downregulated genes are 'butanoate metabolism" and 'metabolic pathways". These pathways include HMGCS2, OXCT1, xanthine dehydrogenase (XDH), isocitrate dehydrogenase 1 (IDH1) and carbonyl reductase 3 (CBR3).

Limitations, reasons for caution: A higher sample size design could be used in future studies to corroborate the current findings.

Wider implications of the findings: This study revealed that thin endometrium possesses an aberrant Th1-pro-inflammatory/Th2-anti-inflammatory balance and increased cytotoxic condition, and that a protective response to oxidative stress is impaired in the thin endometrium. These aberrant molecular mechanisms in thin endometrium may be associated with implantation failure.

Trial registration number: None.

P-454 The effect of hydrosalpinx volume various pretreatments on clinical pregnant rate of FET Cycle

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Study question: To assess the extent of hydrosalpinx which affect outcomes of frozen embryo transfer(FET) and the effect of difference pretreatments of hydrosalpinx prior to FET cycle.

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Summary answer: Patients with hydrosalpinx more than 3 cm have lower clinical conception rate. The effect of the four different pretreatments of hydrosalpinx has no significant differences.

What is known already: The hydrosalpinx secreted by epithelial cells in dilated fallopian lumens are toxic to embryos. They interfere with embryonic implantation, conception, and increase abortion rate. However, whether all the hydrosalpinx need to be deal with and what is the optimal pretreatment of hydrosalpinx have not been conclusive.

Study design, size, duration: A retrospective analysis of 716 patients was performed in this study. The data of the extent of hydrosalpinx and the effect of difference pretreatment groups(including Hydrosalpinx clamps, ligation, salpingectomy and Fallopian tube embolization) were collected from FET cycles which were carried out from January 2013 to April 2016 at CITIC Xiangya Reproductive and Genetic hospital.

Participants/materials, setting, methods: Infertile females with hydrosalpinx were divided into exposed group (pretreated before FET cycle, n = 557) and control group (untreated before FET cycle, n = 159). Patients in exposed group were further divided into four subgroups by difference pretreatments, including hydrosalpinx clamps, ligation, salpingectomy and fallopian tube embolization. Clinical pregnant rate in exposed group was compared to control group. We also compared the clinical outcomes among different pretreatments subgroups in exposed group.

Main results and the role of chance: All participants who met the following criteria were included: (i) both ovaries present; (ii) basal FSH<10 IU/l, estradiol<80 pg/ml; (iii) normal uterine cavity; (iv) frozen embryos better than 6II; and (v) no current or past diseases affecting the administration of gonadotrophins. There is no significant differences between exposed group and control group in general conditions, including age of female patient, duration of infertility, as well as the level of BMI, FSH, LH and E_2. In the first FET cycle, there was no significant difference in clinical pregnancy rate between exposed group (60.68%, 338/557) and comparison group (57.23%, 91/159).However, for cumulative clinical pregnancy rate, the exposed group was much higher than comparison group (68.4% vs. 58.5%, p<0.05).In exposed group, the was no significant difference in clinical pregnancy rate among the four different pretreatments subgroups (P > 0.05)In comparison group, the clinical pregnant rate of female patient with hydrosalpinx more than 3 cm was significantly lower than patient with hydrosalpinx less than 3 cm (p<0.05).But in exposed group, there was no significant difference of clinical pregnant rate between the patient with hydrosalpinx more than 3 cm and less than 3 cm (p>0.05).

Limitations, reasons for caution: A new treatment scheme was put forward and the effect of four difference pretreatment groups was compared in this study. However, the small sample size of exposed women might lack sufficient power for the investigation of differences in pretreatment groups.

Wider implications of the findings: The present study is the first indicating the correlation of pretreatments with the volume of hydrosalpinx.The female patient with the volume of hydrosalpinx more than 3 cm should receive any one kind of pretreatment prior to IVF.

Trial registration number: NA.

P-455 Differential microRNA profile in the endometrial fluid (EF) in natural cycles, HRT, and controlled ovarian stimulation (COS) triggered with hCG (COS+hCG) or GnRHa (COS+GnRHa)

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Study question: What is the miRNAs profile in the EF in the same patients undergoing consecutively a natural cycle, a HRT cycle, a COS+hCG and finally COS+GnRH-a cycle?

Summary answer: We identify significant differences in miRNAs patterns among natural cycle, HRT, COS+GnRH-and COS+hCG throughout the luteal phase in the same group of patient.

What is known already: It has been demonstrated that aspiration of EF in the same transfer cycle does not affect pregnancy rate and is being used for the search of minimally invasive biomarkers of endometrial function. We have already demonstrated that miRNAs are produced in the glandular epithelial endometrium and secreted into the EF. Specifically, we have identified 20 differentially expressed miRNAs throughout the natural menstrual cycle (Development, 2015). But the impact of hormonal replacement therapy (HRT) or controlled ovarian stimulation triggered with hCG (COS+hCG), or GnRHa (COS+GnRHa) on EF miRNA signature in the same patient has not been yet explored.

Study design, size, duration: EF aspirates (n = 114) were obtained from healthy ovum donors volunteers (n = 47), aged 18-34 with regular menstrual cycle, normal karyotype, and BMI between 19-29 Kg/m2. Each patient underwent consecutively a natural cycle, a HRT cycle, then a COS cycle in which final oocyte maturation was induced by hCG (COS+hCG), and finally a COS cycle in which GnRH-a was used for final maturation trigger (COS+ GnRHa).

Participants/materials, setting, methods: EF were collected in natural cycle at days LH+0 (n = 9), LH+3 (9), LH+5 (7), and LH+7 (2). HRT at P+0 (9), P+1 (8), P+3 (8) and P+5 (6). COS+hCG at hCG+0 (10), hCG+3 (3), hCG+5 (3), hCG+7 (4). Finally, COS+GnRHa at GnRHa+0 (10), GnRHa+3 (9), GnRHa+5 (9), GnRHa+7 (8). Total RNA was sequenced using Illumina HISeq2000/100 and bioinformatically analyzed. The gene expression profiles from the Endometrial Receptivity Analysis (ERA) were used for target analysis.

Main results and the role of chance: A comparative analysis revealed significant differences (p<0.05) in miRNAs secreted in EF between natural cycle and HRT/COS cycles. In the non-receptive phase (LH+0; P+0; hCG+0 and GnRH-a+0), natural cycles differed from COS cycles in 24 miRNAs, and HRT and COS cycles exhibit 27 differential expressed miRNAs. In the pre-receptive phase (LH+3/LH+5; P+1/P+3; hCG+3/hCG+5 and GnRHa+3/GnRHa+5), HRT and COS+GnRH-a displayed 10 miRNAs differentially expressed. The biggest differences were found during the receptive phase (LH+7; P+5; hCG+7 and GnRHa+7) where 26 miRNAs were differentially expressed between HRT and COS+GnRH-a, 21 miRNAs in natural vs. COS+GnRH-a, etc,. Thereby, HRT and COS+GnRHa displayed the most dissimilar profiles along luteal phase, while HRT and natural cycles the most similar. MiRNAs obtained from the comparisons of pre-receptive vs. receptive phase were considered as biomarkers candidates. In detail, hsa-miR-708-5p, hsa-miR-30d-5p, hsa-miR-4446-3p, hsa-miR-873-3p, hsa-miR-30b-3p as candidates in natural cycles, hsa-miR-1299, hsa-miR-7974, miR-409-5p) in COS+GnRH-a, in HRT (miR-324-5p, miR-223-3p), and in COS+hCG (hsa-miR-203a-3p). Functional analysis evidenced 41 ERA genes target. Annotation of these genes in KEGG, showed 56 pathways, which could be involved in endometrial receptivity such as FoxO signaling, Wnt signaling, focal adhesion, and cell adhesion molecules.

Studing funding: Grant for Fertility Innovation 2013. Merck Serono; IGENOMIX S.L

Limitations, reasons for caution: Aspiration of EF is considered minimally invasive. However, the introduction of a catheter into the uterine cavity carries a slightly risk of infection. Another critical aspect is the small amount of sample recovered, for this reason, maximum care must be taken to minimize contamination with blood and endometrial tissue.

Wider implications of the findings: This NGS-based study demonstrates for the first time the differential miRNA profile in EF in natural cycle versus different hormonal treatments. It offers the basis to understand how miRNAs are involved the human endometrial biology. This work identifies differential expressed miRNAs that could serve as new diagnostic for endometrial receptivity.

Trial registration number: It was consider as a biomedical study (Non clinical Trial with drug or medical device). FV and CS contributed equally.

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P-456 Clinical pregnancy outcome after double dose intrauterine instillation of granulocyte colony-stimulating factor(G-CSF) in the unresponsive thin endometrium in frozen embryo transfer

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Study question: Does double dose intrauterine instillation of G-CSF in patients with unresponsive thin endometrium improve clinical pregnancy outcome following frozen embryo transfer.

Summary answer: Double dose intrauterine G-CSF instillation results in higher clinical pregnancy rates in patients with unresponsive thin endometrium undergoing frozen embryo transfer.

What is known already: G-CSF, a biologic response modifier has the beneficial effect on clinical pregnancy outcome in Assisted Reproductive Technology (ART). However, some studies failed to demonstrate such benefit. Possible reasons for this, as concluded in a recent meta-analysis are the use of the relatively low dose of G-CSF and one-time administration. They also concluded that cohort studies are required to investigate the appropriate route and dose of G-CSF administration.

Study design, size, duration: A prospective observational cohort study over 6 months, carried out on 200 patients undergoing FET, aged between 23-40 years. All patients had two cancelled cycles due to thin endometrium (<5 mm on day 12/13 of cycle) after receiving conventional treatment with oral and topical oestrogen. 3 patients were lost to follow up.197 patients underwent 300 microgram intrauterine G-CSF instillation (97 patients received second dose). Primary and secondary outcome measures were endometrial thickness and clinical pregnancy.

Participants/materials, setting, methods: All patients received oral and topical oestrogen from day 2/3 of cycle, endometrial thickness (ET) was reassessed on day12/13 by transvaginal ultrasonography. Subjects having ET 4-5 mm received intrauterine instillation of 300 microgram G-CSF. After 72 hours patients having an ET increase of 1.5 mm or more underwent FET; others received 2nd dose of G-CSF after another 72 hours. Those with 1.5 mm ET increase from day 12/13, underwent FET, others having cycle cancelled.

Main results and the role of chance: Out of 197 subjects, 100 (50.76%) had ET increase more than 1.5 mm after 72 hours of the 1st dose of G-CSF (mean 6.17 mm ± SD 0.81), significantly higher than value after conventional treatment (p value<0.001).Out of 97, receiving the 2nd dose of G-CSF, 70 (72.16%) had minimum ET increase of 1.5 mm after another 72 hours (mean 6.5 mm+SD 0.74), significantly higher than value after conventional therapy(p value<0.001). 27 out of 197(13.70%) had their cycles canceled.

Out of 100 patients undergoing FET after 1st dose of G-CSF 49(49%) had clinical pregnancy documented by transvaginal ultrasonography. Rest 97 received the 2nd dose of G-CSF of which 70 underwent FET. Out of this 97 receiving the second dose,23(23.71%) had the clinical pregnancy. Overall 197 patients with unresponsive thin endometrium receiving either 1 or 2 doses of G-CSF 72((36.55%)attained clinical pregnancy.

Limitations, reasons for caution: The absence of control, the safety of 2nd dose of G-CSF instillation.

Wider implications of the findings: This study reaffirms utility of G-CSF instillation in unresponsive thin endometrium in ART. It opens the feasibility of using 2nd dose of G-CSF to enhance clinical pregnancy outcome in such cases. Furthermore, endometrial thickness of less than 5.5 mm may be considered for G-CSF administration to proceed to clinical pregnancy.

Trial registration number: Not applicable.

P-457 Higher incidence of colonization with Gardnerella vaginalis and gram-negative anaerobes in patients with recurrent miscarriage and elevated peripheral natural killer cells

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Study question: Are there associations between the vaginal microbiota and peripheral/uterine natural killer cells (NK cells) in patients with recurrent miscarriage (RM).

Summary answer: Patients with RM and elevated peripheral NK cells suffer more often from colonization by Gardnerella vaginalis and gram-negative anaerobes.

What is known already: Screening of vaginal microbiota in RM is not included in international guidelines. However, there are subgroups of RM patients with a history of gestational disorders like preterm rupture of the membranes or late miscarriage that are associated with vaginal infections. So far it is unknown whether changes in the vaginal microbiota are also reflected by the composition of immune cells in the peripheral blood. NK cells are major players during early pregnancy and therefore predisposed as a target of scientific interest when associations between vaginal microbiota and immunologic effects are focused.

Study design, size, duration: Between November 2011 and March 2016, n = 243 couples with ≥ 3 consecutive RM were included in this study.

Participants/materials, setting, methods: Vaginal swabs were taken and send to the Department of Infectious Diseases, Medical Microbiology of the University of Heidelberg for analysis by microscopy and culture. Further, a cervical swab was taken in n = 187 patients and the presence of Chlamydia trachomatis was evaluated by a molecular assay. In addition, peripheral blood levels of CD45+CD3-CD56+CD16+ peripheral NK cells (determined by four-color fluorescence flow cytometry) and CD56+ uterine NK cells (uterine biopsy, determined by immunohistochemistry) were evaluated.

Main results and the role of chance: The prevalence of *Gardnerella vaginalis* colonization in RM patients was 19.0%, *gram-negative anaerobes* 20.5%, *Candida* species 7.9%, group B *Streptococcus* 11.0% and *Enterobacteriaceae* 14.8%. Commensal *lactobacilli* were absent in 14.5% of the women. *Chlamydia trachomatis* was detected in n = 1 case (0.53%). The prevalence of *Gardnerella vaginalis* in RM patients with elevated peripheral NK cells (> 280/ µl) was significantly higher compared to patients with normal peripheral NK cells (29.0 vs. 14.9 %, Odds ratio (OR) 2.32, 95% Confidence interval (CI) 1.19 to 4.52, p = 0.012). In addition, in these subgroups, a higher prevalence of *gram-negative anaerobes* was detected (29.0 vs. 17.2 %, OR 1.96, 95% CI 1.02 to 3.76, p = 0.041). No difference of the vaginal microbiota was detected between the subgroups of RM patients with normal vs. elevated uterine NK cells.

Limitations, reasons for caution: The lack of a control group hints comparisons to healthy individuales.

Wider implications of the findings: RM patients with elevated peripheral NK cells suffer more often from colonization by *Gardnerella vaginalis* and *gram-negative anaerobes*. This might indicate an association between the vaginal microbiota, local inflammation, changes in immune parameters and recurrent miscarriage.

Trial registration number: n.a.

P-458 The effects of oxygen, pH and temperature in mammalian reproduction and their importance in human reproduction

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Study question: What are the modulators and effects of oxygen, pH and temperature in mammalian reproduction?

Summary answer: Modulators of biophysical parameters in the female reproductive tract are multifactorial; fine control is crucial in optimal fertilisation, implantation and development of the early conceptus.

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What is known already: Despite advances in IVF techniques and ovarian stimulation regimens, implantation rates per embryo transfer still remain low, and pregnancy rates have not risen accordingly. IVF laboratories strive to ensure that the process of handling gametes *in-vitro* closely mimics the *in-vivo* environment. However, ironically, a limiting step to improving outcomes is the lack of knowledge and data on the actual *in vivo* oxygen concentration, pH and temperature within the reproductive tract, which are likely to be dynamic rather than constant.

Study design, size, duration: A systematic review.

Participants/materials, setting, methods: A systematic literature search was performed using electronic databases including Medline, Embase, Cochrane Library and Pubmed to identify original and review articles addressing the biophysical parameters (pO2, pH and temperature) in the female reproductive tract. The search included all listed studies published between 1946 to November 2015. Search terms included 'oxygen', 'pH', 'hydrogen ion concentration', 'acid base' and others terms. We also used special features and truncations to identify synonyms and broaden the search.

Main results and the role of chance: Our search generated 18, 685 records and 60 articles were included. pO2 (oxygen tension) within the female reproductive tract shows cyclical variation and minute to minute oscillations which may be influenced by uterine contractility, hormones, the autonomic system, cardiac pulsatility, and myometrial and smooth muscle integrity. Fine balanced control of pO2 and avoidance of overwhelming oxidative stress is crucial for embryogenesis and implantation. The pH in the female reproductive tract is graduated, with lowest pH in the vagina (pH 4.42) increasing towards the Fallopian tubes (pH 7.94), reflecting the variation in site-specific microbiome and acid-base buffering at the tissue/cellular level. The temperature variation in humans is cyclical by day and month. In humans, it is biphasic, increasing in the luteal phase; with the caudal region of the oviduct 1-2 degrees cooler than the cranial portion. Temperature variation is influenced by hormones, density of pelvic/uterine vascular beds and effectiveness of heat exchange locally, crucial for sperm motility and embryo development. We have identified significant deficiencies and inconsistencies in the methods used to assess these biophysical factors within the reproductive tract. We have suggested technological solutions including the development of methods and models for real time, *in-vivo* recordings of biophysical parameters.

Limitations, reasons for caution: Due to the heterogeneity of studies, a valid meta-analysis of data was not possible within this systematic review.

Wider implications of the findings: The notion of 'back to nature' in assisted conception was suggested 20 years ago. However, the strategy for embryo culture to mimic nature has largely been ignored. Our inability to assess the *in-vivo* reproductive tract environment in real time is contributory; addressing this will enable precise and personalised fertility treatment.

Trial registration number: N/A.

P-459 High prevalence of chronic endometritis in women diagnosed with hydrosalpinx during in vitro fertilization treatment

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Study question: Should women diagnosed with hydrosalpinx during in vitro fertilization (IVF) treatment be screened for concurrent chronic endometritis (CE)?

Summary answer: Women diagnosed with hydrosalpinx during IVF treatment have a high prevalence (32%) of immunohistochemically confirmed CE, and should thus be screened for concurrent CE.

What is known already: Hydrosalpinges represent a chronic inflammatory condition that adversely affects implantation and pregnancy rates in IVF cycles, by altering endometrial receptivity. When diagnosed during IVF treatment, hydrosalpinges are surgically treated before embryo transfer. CE is defined as a persistent inflammation of the endometrial lining, and is best diagnosed with immunohistochemical identification of plasma cells in the endometrial stroma. Studies suggest CE negatively impacts fertility outcomes by altering endometrial receptivity, and its prevalence is higher in patients with infertility, recurrent implantation failure and recurrent pregnancy loss. However, it remains unclear whether there is a relationship between hydrosalpinges and CE.

Study design, size, duration: A pilot prospective observational study at the IVF center and the department of Reproductive medicine of Angers University Hospital, between June 2015 and September 2016.

Participants/materials, setting, methods: Patients aged 18 to 42 years, undergoing IVF for various indications, in whom a hydrosalpinx was diagnosed before or during cycles, were included. A laparoscopy was scheduled for the removal of hydrosalpinges and an endometrial biopsy performed to rule out CE. The diagnosis of hydrosalpinx was confirmed by histological examination while CE was diagnosed using immunohistochemistry stains for CD138 (≥5 plasma cells/field). Women with CE were treated with Doxycycline 100 mg bid for 14 days.

Main results and the role of chance: 25 patients were included. Mean age was 34.2 ± 4.4 years. The causes of infertility were: Tubal factor (36% [9/25]), endometriosis (28% [7/25]), poor ovarian reserve (16% [4/25]), anovulation due to polycystic ovarian syndrome (20% [5/25]). Hydrosalpinx was bilateral in 56% (14/25) and unilateral in 44% (11/25) of cases. 48% (12/25) of patients had a unilateral salpingectomy, 44% (11/25) had a bilateral salpingectomy and 8% (2/25) had bilateral tubal ligation with filshie clips. The diagnosis of hydrosalpinx was histologically confirmed in 92% (23/25) of women. All patients had an endometrial biopsy, and the prevalence of immunohistochemically confirmed CE was 32% (8/25). Median follow-up after surgery was 10.9 ± 5.9 months. Three patients were lost to follow-up. Following IVF and embryo transfer in 22 patients, eight clinical pregnancies (fetal heartbeat at 7 weeks) (36%), one ectopic (4.5%) and one biochemical pregnancy (4.5%) were recorded. Out of the eight patients treated for CE, two were lost to follow-up, three had an endometrial biopsy after treatment which confirmed the absence of CE, and one clinical pregnancy was achieved (17%).

Limitations, reasons for caution: Our pilot study was limited by the small number of participants, and was not powered enough to compare pregnancy rates between women without CE and the ones treated. Moreover, randomized controlled trials are needed to assess the impact of CE treatment on pregnancy rates and outcomes in IVF cycles.

Wider implications of the findings: Our study shows a high prevalence of CE in patients diagnosed with hydrosalpinx during IVF treatment. Patients with hydrosalpinges should thus be screened for concurrent CE before embryo transfer, and treated accordingly.

Trial registration number: Not applicable.

P-460 Effect of intra-uterine G-CSF on endomerial thickness in patients with persistently thin endometrium: A retrospective study of 244 thawed embryo transfer cycles

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Study question: Does intra-uterine G-CSF infusion increase endometrial thickness in women with earlier cancelled embryo transfer cycles due to thin endometrium despite using estradiol validate, pentoxiphylline,vitamin E and Sildenafil citrate?

Summary answer: Use of intra uterine G-CSF results in significant improvement in endometrial thickness from 7.19 ± 0.52 mm to more than 8.10 ± 1.09 mm.

What is known already: Persistently thin endometrium represents a therapeutic challenge for the physicians. It leads embryo transfer in suboptimal conditions, cancelled transfer and transfer to gestational surrogates; leading to emotional and financial burden.

The mainstream treatment for improving endometrial thickness is estrogen supplementation. However few patients are refractory to estrogen supplementation and require other modalities. Inconsistent response has been seen with sildenafil citrate, low dose aspirin, pentoxifylline, and tocopherol etc, with no established roles.

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Earlier case reports have been published showing significant improvement in endometrial thickness after intrauterine instillation of granulocyte colony stimulating factor.

Study design, size, duration: This is a historical case control study of 192 women with persistently thin endometrium in previous 2 estrogen replacement therapy(ERT).

All cases were recruited at Craft Hospital and Research Centre over a period of 3 years from January 2013 to December 2015.

All women had endometrial thickness less than 7 mm at day 20 of previous 2 estrogen replacement cycle and it failed to improve even with addition of above mentioned adjuvants

Participants/materials, setting, methods: After initial failed attempt all women were started on ERT (8 mg /day) from day 2 of period and serial ultrasound monitoring was performed from 10 th day onwards at interval of 3-5 days.

Intra uterine instillation of 300 mcg/1 ml of G-CSF (Endokine, Filgrastim, INTAS) was done if endometrial thickness was less than 7 mm after 18 days of high dose ERT.

Main results and the role of chance: Before infusion of G-CSF the mean ET of the cohort was 7.19 ± 0.52 on day 18.1 ± 3.7 of high dose ERT.

After 48 hours of infusion the mean endometrial thickenss of the cohort increased by 0.57 mm and reached 7.76 ± 0.96 mm.

At second review after 4.9 ± 2.7 days of G-CSF infusion the mean endometrial thickness of the cohort reached 8.10 ± 1.09 mm showing increase of 0.91 mm.

Out of 244 cycles, 115 cycles (47%) showed improvement in endometrial thickness upto and beyond 8 .0 mm. The average increase in endometrium seen in these 115 cycles was 1.75 ± 0.62 mm on last day of ERT.

In other 119 cycles the improvement in endometrial thickness was less than 1 mm and did not reach threshold for embryo transfer.

There were 10 more cycles where reduction in endometrial thickens was noted after G-CSF infusion.

Total 127 frozen-thawed embryo transfer was performed i.e. 52% of treated cycles. The implantation rate, clinical pregnancy and live birth rate per transfer procedure were 27.5%, 44% and 29.9% respectively for these women.

Limitations, reasons for caution: t is a retrospective study with small sample size.The women acted as their own controls.

Wider implications of the findings: This study supports the use of intrauterine -G-CSF for patient with persistently thin endometrium.

But overall improvement in endometrial thickness is less than 1.75 mm.

Further robust data in terms of large multicentric randomised controlled trials are needed to substantiate the findings.

Trial registration number: Not applicable as it is a retrospective analysis.

P-461 The reproductive outcome following Atosiban administration around the time of embryo transfer: a systematic review and meta-analysis

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Study question: Does administration of Atosiban around the time of embryo transfer improve the reproductive outcome in subfertile women undergoing assisted reproduction?

Summary answer: Atosiban administered around embryo transfer increases the clinical pregnancy rate and does not influence the miscarriage rate. There are insufficient data related to live birth.

What is known already: Uterine contractions at the time of embryo transfer are negatively correlated with the outcome of assisted reproduction treatment. Ovarian stimulation associates an increase in the frequency of uterine contractions, possibly because supraphysiological serum estradiol levels may induce endometrial production of oxytocin and formation of oxytocin receptors. Oxytocin antagonists (ie: Atosiban) are being used for the treatment of imminent premature birth with minimal side effects.

Study design, size, duration: The present study is a systematic review and meta-analysis of randomised controlled trials (RCTs) investigating the role of Atosiban administered around the time of embryo transfer. It was conducted according to the published Cochrane Protocol (CD012375) with the last systematic search performed on 26th of January 2017.

Participants/materials, setting, methods: Participants were women undergoing embryo transfer as part of their assisted reproduction treatment irrespective of the cause of subfertility, age, previous treatments, obstetric and gynaecological history or city/country of origin. Women from the intervention group received Atosiban around the time of embryo transfer and were compared to women from the control group who received placebo or no intervention. The endpoints were clinical pregnancy, miscarriage, live birth and adverse events rates.

Main results and the role of chance: Seven moderate quality RCTs met the inclusion criteria and were meta-analysed. There were 963 women in the intervention group and 1015 women in the control group. The administration of Atosiban around the embryo transfer increased the clinical pregnancy rate (RR 1.25, 95% confidence interval (CI) 1.10 to 1.41, seven RCTs, n = 1978, $I^2 = 30\%$, $p < 0.0004$, moderate quality evidence) and did not influence the miscarriage rate (RR 1.05, 95% CI 0.75 to 1.47, five RCTs, n = 1756, $I^2 = 0\%$, p = 0.79, moderate quality evidence). One study reported similar live birth rates between the intervention and control groups (RR 1.046, 95% CI 0.874 to 1.253, n = 800, p = 0.612, low quality evidence). None of the RCTs reported differences in adverse events between the study groups.

Limitations, reasons for caution: There was significant clinical (women characteristics) and methodological (Atosiban regimes, stage of embryo, embryo processing) diversity between RCTs. The effect of Atosiban on clinical pregnancy is promising; however, future studies should report on live birth as a primary outcome prior to the incorporation of the intervention in routine clinical practice.

Wider implications of the findings: Previous retrospective and non-randomised studies reported conflicting effects of the intervention. The present meta-analysis of seven RCTs presents the most up to date evidence and supports future research aiming to establish the optimal regime and identify the patient groups that could benefit the most from Atosiban around embryo transfer.

Trial registration number: Not applicable.

P-462 Next generation RNA sequencing on human trophectoderm cells reveals competent blastocysts for single embryo transfer in in vitro fertilisation cycles

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Study question: Can trophectoderm (TE) transcriptome patterns predict successful Day 5 blastocyst (BC) implantation?

Summary answer: Our transcriptome analysis revealed twenty-three RNAs significantly over-expressed in successfully implanted BCs, mainly involved in steroidogenesis and eight RNAs highly expressed in non-implanted BCs.

What is known already: Previous studies have attempted to establish the relationship between gene expression profile and BC implantation in in vitro fertilization (IVF) utilising microarrays (Jones et al., 2008) or a next generation RNA Sequencing (RNA-Seq) approach (Kirkegaard et al., 2015). None of these studies performed aneuploidy screening on the analysed BCs and there are currently no predictive molecular markers of IVF BC implantation competence.

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These findings suggested a principal difference in the gene expression related to clinical outcome, which would potentially allow for a new approach to refine and improve existing criteria for selecting viable BC for single embryo transfer (SET).

Study design, size, duration: To determine the TE transcriptome patterns linked to successful BC implantation, ten BCs were biopsied from 6 couples and subsequently transferred into the female uterus. The compared groups were: i. BCs that successfully implanted into the female uterus and ii. BCs that failed to implant. The samples were collected within a three-month period.

Participants/materials, setting, methods: Infertile couples (34-46 years) with no uterine abnormalities underwent fertility treatment in Genesis Athens IVF clinic, Athens, Greece. Day 5 TE biopsy were followed by fresh uterine transfer. RNA and DNA respectively of TE cells from each BC, underwent RNA-Seq using the latest Clontech single cell (SMART) approach and aneuploidy detection by QF-PCR for chromosomes X,Y and autosomes 13, 18 and 21 following whole genome amplification (WGA). Bioinformatic analysis involved Bioconductor packages in R.

Main results and the role of chance: The current study employed a more precise approach to reveal the patterns of the TE transcriptome that accompany successful implantation determined by the detection of CP. Following library construction and RNA sequencing, the data were processed and analysed statistically using the edgeR Exact test with an FDR of 0.05. Two of the analysed BCs were excluded as outliers. A total of thirty-one transcripts were significantly differentially expressed between the TE cells from embryos going on to generate successful versus unsuccessful CPs. Of these, twenty-three transcripts were significantly up-regulated in the successfully implanted BCs, including HSD17B1 and CYP11A1, while the remaining eight transcripts were significantly up-regulated in non-implanted BCs, including WNK3 and BAK3. Ontological analysis revealed pathways involved mainly in steroid biosynthesis and sterol metabolic process with high confidence. Samples with detected chromosomal aneuploidies were excluded from this study.

Limitations, reasons for caution: Sample size is the main limitation of the current study. However, RNA-Seq is a robust method and although embryo implantation is a multifactorial biological process it can address the study question for the particular samples analysed.

Wider implications of the findings: The current methodology is probably more reliable to investigate single TE biopsies, compared with the previous approaches employed. The detected transcripts could be used in a diagnostic aid for selecting the most viable BC, with the best potential to support full term pregnancy following SET, among a morphologically similar cohort.

Trial registration number: Not applicable.

P-463 Interactome between embryo trophectoderm cells and endometrial epithelial and stromal cells: novel insights into implantation process in human

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Study question: Which cell surface proteins mediate interactions between polar-trophectodermal blastocyst cells and the receptive endometrial epithelial and stromal cells during embryo implantation?

Summary answer: A complex network of surface proteins on endometrial epithelial/stromal cells and polar-trophectodermal cells participate in the first interactions between the developing embryo and receptive uterus.

What is known already: Human embryo implantation is a dynamic process that requires a dialogue between the receptive uterine lining (endometrium) and blastocyst-stage embryo. As it is ethically and technically impossible to study human embryo implantation *in vivo*, the molecular processes taking place during implantation are not well known. The few studies of embryo-endometrium molecular interactions conducted so far are based on endometrial whole-tissue, that consists of different cell types. This obvious limitation creates a need for cell-type specific approach to predict the very first molecular interactions between the surfaces of the blastocyst and receptive endometrial epithelial and stromal cells.

Study design, size, duration: Paired endometrial tissue samples from 17 women were obtained from pre- and receptive menstrual cycle phase. The biopsies were dissociated, CD9-positive epithelial cells and CD13-positive stromal cells were separated with FACS sorting and full transcriptome analysis was performed by RNA-sequencing. The interaction partners from embryo side were obtained from our previously published human embryo transcriptional map, which is based on RNA-sequencing of 1529 single cells of 88 embryos (Petropoulos et al., 2016).

Participants/materials, setting, methods: Protein-protein interaction (PPI) networks were constructed using data on upregulated genes from receptive endometrial epithelium/stromal cells and polar-trophectodermal cells. The detected mRNAs were converted into corresponding proteins, the subset of proteins localized in cell membrane and surface were extracted, and PPI networks were created based on interactions in STRING 10.0 database. The biological processes of cell-type specific interaction networks were identified by enrichment analyses and assembled together into super-clusters.

Main results and the role of chance: Endometrial epithelial-trophectodermal cell network contains 157 and endometrial stromal-trophectodermal network 138 interacting cell surface proteins. Several of the genes in the cell-specific networks were unique to the corresponding cell types, such as epithelial genes S100 calcium-binding protein P (*S100P*), mucin 20 (*MUC20*), prostasin (*PRSS8*) and CD44 antigen (*CD44*); and stromal genes laminin subunit beta 1 (*LAMB1*), lipid phosphate phosphohydrolase 3 (*LPP3*), supervillin (*SVIL*) and decorin (*DCN*). Biological processes related to cell attachment and proliferation like *single organismal cell-cell adhesion*, *cell division* and *establishment or maintenance of cell polarity* were detected in both blastocyst-epithelial and blastocyst-stromal cell-specific PPI networks. The largest super-cluster in stromal-specific cell network was related to the positive regulation of kinase activity and extracellular matrix organization, while in epithelial-specific cell network blood coagulation and cell junction assembly clusters were detected. The hyaluronan metabolism pathway, known to be important in embryo implantation, was uniquely enriched in epithelial cell network, underlining the cell type specific nature of human embryo implantation process.

Limitations, reasons for caution: As it is unethical and technically challenging to study human embryo implantation *in vivo*, we lack appropriate tools to validate *in silico* predicted interactions in humans.

Wider implications of the findings: New understanding about the molecular processes that lead to successful embryo implantation allow us to detect new relevant marker genes for receptive endometrium and maturing blastocyst, thus allowing to predict the best timing for embryo transfer in *in vitro* fertilization cycles.

Trial registration number: not applicable.

P-464 The impact of difficult embryo transfer on implantation, clinical pregnancy and live birth rates following single blastocyst replacement

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Study question: What is the impact of difficult embryo transfer (ET) on in vitro fertilisation (IVF) outcomes following the transfer of a single fresh blastocyst?

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Summary answer: This is the first study to show that difficult embryo transfer is more detrimental to a high-quality embryo than those with lower potential to implant.

What is known already: Difficult ET has been shown to decrease implantation and clinical pregnancy rates. It is unclear what defines a 'difficult' transfer as there are no universally agreed classification systems and because it is generally judged subjectively. The number and quality of embryos replaced is accepted to affect pregnancy rates, though the significance of these factors in the context of difficult embryo transfer is not well understood. The impact of difficult ET on IVF outcomes has largely been tested in cycles where multiple embryos of differing qualities have been replaced at different stages as opposed to when a single blastocyst is transferred.

Study design, size, duration: In this study 754 fresh single blastocyst transfers were undertaken between June 2009 and April 2014. Three different levels of difficulty were identified based on the steps taken to overcome resistance. A fourth group (Group M) was identified when clinicians anticipating a difficult ET and electively chose to insert the firm malleable stylet from the outset. Embryo quality was judged subjectively by the embryologist using our simplified grading system (A best – C worst).

Participants/materials, setting, methods: Embryo transfers were performed under ultrasound guidance using an 18-centimetre soft embryo replacement catheter. If the soft catheter could not be passed with ease, the outer sheath was advanced and if this did not overcome the resistance the catheter was withdrawn and a firmer malleable stylet catheter introduced. Each embryo transfer documentation record was classified by two experts. Primary clinical outcomes were implantation, clinical pregnancy and live birth rates.

Main results and the role of chance: Documentation of ET was incomplete in 16 cases (2.1%), and in 86 cases (11.4%) a malleable stylet was used electively before threading the soft catheter (group M): these cases were excluded from the analysis leaving 652 single blastocyst transfers, which formed the study group. There were no significant differences in patient characteristics. For all embryos, implantation rates and clinical pregnancy rates fell significantly ($p < 0.05$). Live birth rates fell from 51.4% to 43.2% when an embryo transfer was difficult but this was not significant ($p = 0.202$). Accounting for embryo grade, implantation rates (IR) fell by a magnitude of 25% from 84.3% to 62.0% for the highest quality (grade A) embryos during difficult transfers ($p = 0.001$). Whilst the decrease was significant for the best quality embryos it was not the same for the lower quality grade B and C embryos. Clinical pregnancy (CP) and live birth rates fell for all grades of embryo as transfer difficulty increased. Despite this clear fall in CP and LB rates the results did not reach statistical significance ($P< 0.05$).

Limitations, reasons for caution: The limitations of this research is the subjective interpretation of ET difficulty and embryo quality. A larger prospective study is needed with more accurate grading systems of ET and embryo quality to explore whether the prognostic potential of a single blastocyst is significantly changed by the difficulty of ET.

Wider implications of the findings: This study has shown that a difficult embryo transfer has a significant impact on the potential of single high quality (Grade A) blastocyst to implant. This fall in prognostic potential affects all embryos to some degree but the highest quality blastocysts seem most affected.

Trial registration number: Not applicable.

P-465 The optimal endometrial thickness to reduce miscarriage and maximise live birth in in-vitro fertilisation treatment: A multicentre cohort study

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Study question: What is the association between endometrial thickness and reproductive outcomes following in vitro fertilisation treatment and the endometrial thickness threshold that optimises the IVF outcome

Summary answer: An endometrial thickness threshold of 10 mm or more is suggested to maximise the chances of a live birth and minimise miscarriage.

What is known already: The NICE guideline on Fertility suggests performing an embryo transfer in in-vitro fertilisation (IVF) treatment if the endometrial thickness is more than 5 mm. However, there is uncertainty on the optimal endometrial thickness.

Study design, size, duration: Design Observational multicentre cohort study.

Participants/materials, setting, methods: Setting Data from CARE Fertility Group; including six main and seven satellite centres in the UK.

Participants 25,427 women undergoing fresh cycle of IVF in the CARE centres who have had an endometrial thickness measurement and a recorded reproductive outcome, between 2007 and 2016.

Main outcome measures Primary outcomes: Miscarriage and live birth; Secondary outcomes: Biochemical and clinical pregnancies, implantation rate, biochemical pregnancy loss, and clinical pregnancy loss.

Main results and the role of chance: Endometrial thickness is strongly associated with miscarriage and live birth in an IVF cycle. The risk of a miscarriage was 41.7% with 5 mm endometrial thickness and gradually decreased to 26.5% with an endometrial thickness of 10 mm or more. On the other hand, live births increased from approximately 15% to more than 33% with increasing endometrial thickness. Statistical modelling for optimal endometrial thickness threshold found 10 mm or more maximised live birth while minimising miscarriage.

Limitations, reasons for caution: Ultrasound measurements of endometrial thickness are inherently subjective even though the technique is standardised across the CARE units. We have not linked the fresh cycles to subsequent frozen-thawed cycles and they are not taken into account for the cumulative live birth rate.

Wider implications of the findings: Live births are recorded even with 5 mm maximum endometrial thickness. However, since the live birth rates double when the thickness is 10 mm or more may be preferable to decide not to transfer an embryo within this cycle and opt for a subsequent frozen embryo transfer.

Trial registration number: None.

P-466 Hysteroscopic placement of Tornado microinsert in paitients with hydrosalpinx before in vitro fertilization-embryo transfer

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Study question: To evaluate the feasibility and IVF outcomes of Tornado microinserts placement for infertile women with hydrosalpinx

Summary answer: Tornado placement is an effective method for occlusion of hydrosalpinges when laparoscopy is hard to be performed.

What is known already: Laparoscopic proximal tubal occlusion or salpingectomy is an option for infertile women with hydrosalpinx. However, less injured and safer method remains to be investigated.

Study design, size, duration: A prospective, single-arm, clinical study including 152 women with hydrosalpinges (unilateral or bilateral) from May 2014 to May 2016 was analyzed.

Participants/materials, setting, methods: A total of 152 women undergoing hysterscopic Tornado inserts placement were included in the analysis. The main outcomes were live birth rate in subsquent cycles

Main results and the role of chance: The success rate of Tornado microinserts placement was 98.4% (240/244). 118 women had a total of 159 embryos transferred. The clinical pregnancy rate was 57.63% (68/118) and the live-birth rate was 36.44 % (43/118). There were two cases of ectopic pregnancy even after Tornado microinserts placement.

Limitations, reasons for caution: Due to the small sample size, larger studies should be needed for the confirmation of effectiveness and safety of Tornado microinserts placement.

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Wider implications of the findings: The results suggest that Tornado microinserts placement might be an option for the management of women with hydrosalpinx before ART

Trial registration number: None.

P-467 The effect of endometrial injury on VEGF expression in patients with Repeated Implantation Failure: A randomized control trial

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Study question:: Does endometrial injury really effect on VEGF expression as a pivotal marker of implantation in patients with Repeated Implantation Failure?

Summary answer: Endometrial injury during proliferative phase of menstrual cycle before embryo transfer can improve VEGF gene expression in case group compared to control.

What is known already: Based on previous studies, local injury to endometrium in luteal and /or proliferative phases of menstrual cycle has conflicting effect on implantation and clinical outcomes, however, little is known about molecular aspects of injury in patients with Repeated Implantation failure preceding IVF/ICSI.

Study design, size, duration: A total of twenty women with repeated implantation failure (RIF) who failed to conceive during two or more IVF/ICSI cycles and embryo transfer (ET) participated in this randomized controlled trial (RCT) study. Pipelle endometrial sampling was done twice: One in the follicular phase and again in the luteal phase in case group (N = 10) but it was done once in control group (N = 10) just in the luteal phase for genomic evaluation.

Participants/materials, setting, methods: Total RNA were extracted from endometrial tissues, Then *VEGF* gene expression was investigated by quantitative real-time PCR.

Main results and the role of chance: *VEGF* gene expression was detected in endometrial samples of both groups. The mean relative expression of *VEGF* gene was higher in the case group compared with control group.

Limitations, reasons for caution: The number of subjects who participated in this study is low.

Wider implications of the findings: As a whole, our study provided molecular evidence that patients with repeated implantation failure can benefit from local injury during an ongoing IVF cycle, in addition, it seems VEGF as an angiogenic factor plays an important role in regulating human endometrial receptivity.

Trial registration number: NCT02480127.

P-468 Altered expression of Na+- and Cl- ion channels in endometrium of women with recurrent implantation failure (RIF)

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Study question: Is there any correlation between the expression of Na^+- and Cl^- ion channels in endometrium and implantation failure?

Summary answer: Result revealed increased expression of CFTR but decreased expression of SCNN1A, SCNN1B, SCNN1G (coding subunits of ENaC) in endometrium of RIF patients vs. fertile women.

What is known already: Successful implantation of the embryo is an important stage in human reproduction. During implantation, the uterine undergoes fluid absorption in preimplantation phase. The critical role of ion channels on reproduction, mainly on embryo implantation, have been reported in various aspects. The amiloride-sensitive epithelial sodium channel (ENaC) is a Na+ channel that encoded by SCNN1 genes and Cystic fibrosis transmembrane conductance regulator (CFTR) is a Cl^- channel that are expressing in the epithelial cells of a numerous kinds of tissues, such as human endometrium. ENaC and CFTR may play a main role in absorption of uterine fluid in embryo implantation period.

Study design, size, duration: A case control study with 20 patients in recurrent implantation failure and 9 fertile women(oocyte donors).

Participants/materials, setting, methods: Consent was obtained from patients according local ethical approval. Endometrial injury obtained from 20 infertile women (22-35 years old) in window of implantation (19th -24th days of menstrual cycle) by pipelle. As control group, endometrial injury obtained from 9 fertile women between 22-35 years old through oocyte donation procedure before stimulation in window of implantation phase by pipelle. expression of CFTR, SCNN1A, SCNN1B,SCNN1G in endometrial,all of the sampels were evaluated quantitatively by real-time PCR.

Main results and the role of chance: Quantitative PCR analysis showed that mRNA expression of CFTR gene was increased and SCNN1A, SCNN1B and SCNN1G genes was decreased significantly in endometrium of RIF patients vs. fertile group.(P-Value≤0.01)

Limitations, reasons for caution: For getting more information we need to investigate large number of RIF patients and control group.

Wider implications of the findings: These data imply the potential role of ion channels in implantation failure.

Trial registration number: -.

P-469 Assessment of endometrial receptivity using the endometrial receptivity array (ERA) in women affected by latent genital tuberculosis (LGTB) undergoing In Vitro Fertilization (IVF)

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Study question: Does latent genital tuberculosis diagnosed by DNA Polymerase chain Reaction (PCR) of endometrial tissue,affect endometrial receptivity in women undergoing IVF as evaluated by ERA

Summary answer: There is no significant difference in endometrial receptivity as assessed by ERA,in women with or without LGTB

What is known already: Genital Tuberculosis is an important cause of infertility and IVF failures in India. Studies suggest that LGTB affects endometrial thickness and causes changes at a molecular and biochemical level altering the window of implantation (WOI). This can lead to implantation failures in IVF. ERA is a customised microarray test which evaluates the transcriptomics of WOI using 238 genes that are differentially expressed.ERA is a superior test compared to other markers of endometrial receptivity having a sensitivity of 88.57 % and specificity of 99.7 % and helps us to understand the personalized window of implantation with a high accuracy.

Study design, size, duration: Retrospective study. Records of frozen embryo transfer (FET) patients between 2014-2016 were scanned.165 patients included in our study underwent endometrial biopsy for diagnosis of LGTB

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using DNA PCR,Koch's culture and ERA for assessment of endometrial receptivity before FET.Of these 25 patients were positive for LGTB. Comparison was done between LGTB positive patients (both treated and untreated) and controls(DNA PCR negative and no previous exposure to ATT).Kochs culture positive (active tuberculosis) were excluded.

Participants/materials, setting, methods: Patients were evaluated for LGTB using DNA PCR and Koch's culture and Endometrial receptivity was assessed by ERA in a hormone replacement cycle (HRT).Estrogen priming was started on cycle day 2/3 with estradiol valerate. When the endometrial lining was 7 mm or the maximal achievable thickness, vaginal progesterone 400 mg twice a day was added. Endometrial biopsy was taken after five days of progesterone. Patients with endometrium <5.5 mm were excluded from the study.

Main results and the role of chance: Patients divided into three groups based on DNA PCR results and exposure to ATT.Group A: DNA PCR positive, untreated (n = 11),Group B: DNA PCR positive, treated (n = 14) and Group C (controls): DNA PCR negative and no previous exposure to ATT (n = 140),Endometrial receptivity assay results interpreted as Receptive(P+5) or Non receptive. Patients with altered WOI(Pre or Post receptive) had repeat ERA with suggested progesterone replacement resulting in correction of the WOI.Group A: 7 patients (63.6%) showed no change in the WOI and 4 (36.3%) had a changed WOI Group B :11(78.5%)patients had no change in the WOI while 3 (21.4%) had a changed WOI. Group C: 109 (77.8%) had no change in WOI and 31 (22.1%) had changed WOI. Comparison amongst the 3 groups was done using Fischer Exact test. Group A vs Group B - p-valve 0.652., Group A vs Group C - p-valve 0.283, Group B vs Group C - p-valve 1.00, Group A vs Group B vs Group C p-valve-0.543.p valve of </0.05 -statistically significant.No significant difference seen in the endometrial receptivity of patients who had LGTB compared to controls

Limitations, reasons for caution: Small number of patient in group A & B

Wider implications of the findings: LGTB does not seem to alter the endometrial receptivity. Anti tubercular treatement for LGTB in endemic areas solely to improve the endometrial receptivity maynot be helpful,though larger prospective randomized studies are needed to confirm the above results.

Trial registration number: Nil.

P-470 iTraq proteomic analysis of endometrium from fertile donors, IUD carriers and patients with recurrent implantation failure (RIF) reveals differential proteomic profiles

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Study question: Are there any proteomic differences among a receptive endometrium, a refractory (IUD) one and an endometrium from RIF patients?

Summary answer: Our results revealed clear proteomic differences between RIF/IUD and receptive/ IUD comparisons but no differential profile was found in RIF/receptive comparison.

What is known already: The human endometrial transcriptome has been extensively investigated in the last decade resulting in the development of new diagnostic tests based on the transcriptomic signature of the window of implantation (WOI). Much less is known about the proteomics derived from the transcripts present during the WOI.

Study design, size, duration: This study is a basic proteomic analysis of human endometrial biopsies taken from twenty-eight IVF patients. Human endometrial samples (n = 28) were collected from fertile donors (n = 10), IUD carriers (n = 10), and RIF patients with at least 3 previous IVF failures (n = 8) at the same day during the window of implantation (WOI) (Hormonal Replacement Therapy; P+5).

Participants/materials, setting, methods: Protein identification and quantification were performed using isobaric tags for relative and absolute quantification analysis (TRAQ 8-plex) and mass spectrometry, followed up by 'in silico' functional analysis (KEGG database) and correlation networks using Gene Ontology (GO). Statistical analyses were carried using an ANOVA and T-test analyses. Validation by Western blot (WB) was performed for the Plastin 2 (PLSL), Lactotransferrin (TRFL) and Lysozyme C (LYSC) proteins.

Main results and the role of chance: iTRAQ analysis identified 1,873 differentially expressed proteins among the three groups. ANOVA analysis revealed 188 significant proteins, while t-test showed 133 statistically significant proteins between receptive vs. IUD endometria. RIF vs. IUD comparison revealed 158 significant proteins and 54 differentially expressed were shared between the two comparisons. Surprisingly, no differential expressed protein was found between receptive vs. RIF groups. Validation by WB for PLSL, TRFL and LYSC confirmed our iTRAQ results. KEGG functional analysis revealed 12 and 17 significant pathways for receptive vs. IUD and RIF vs IUD respectively (10 shared pathways). These 10 common pathways were related to aspects of amino acids and fatty acids metabolisms. RIF/IUD comparison's specific pathways were those related to the Complement and coagulation cascades and pathways related to the Ribosome and Spliceosome were the specific ones for receptive/ IUD comparison. RIF/IUD correlation network showed two main functional groups, the Immune System and the Ion Transport Process. In the case of receptive/IUD correlation network, one important group appeared related to transcription, translation and proteins regulation processes, supporting the results obtained by 'in silico" functional analysis by KEGG.

Limitations, reasons for caution: This is mainly a descriptive study with no functional studies on the proteins found. We also used a moderate number of human endometrial samples for the iTRAQ analysis.

Wider implications of the findings: Our study demonstrate a differential proteomic profile between receptive compared to IUD endometria, showing key proteins related to receptivity status. No differences were detected between RIF patients and fertile donors, suggesting that RIF´s endometrium is not pathological, reinforcing the hypothesis of a Window of implantation (WOI) displacement.

Trial registration number: 'not applicable'.

P-471 Novel cell-type specific RNA-seq analysis provides new insights into the molecular processes of receptive phase endometrium and identifies sensitive biomarkers

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Study question: Does RNA-seq analysis of single endometrial epithelial and stromal cells provide novel insights into the molecular processes of endometrial development from pre-receptive into receptive phase?

Summary answer: Cell-type specific RNA-seq is a powerful approach for unravelling molecular processes of endometrial receptivity, and to detect novel sensitive biomarkers of receptivity.

What is known already: Molecular profile of receptive phase endometrium has been extensively studied during the last decades, but most of the studies have focused on transcriptome analyses of endometrial whole-tissue biopsies. Endometrium, however, is a complex tissue composed of multiple cell types, epithelial and stromal cells being the most abundant among them. So far, only one group has analysed endometrial cell-specific transcriptome (Evans GE *et al*), and they clearly show cell-type-specific transcriptome profiles. They used laser capture microdissection for cell-type isolation, which has limitations,

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mainly the decrease of RNA integrity. Therefore, new state-of-the-art techniques should be applied in order to overcome these limitations.

Study design, size, duration: Paired endometrial tissue samples from pre-receptive (n = 20) and receptive phase endometria (n = 20) from Estonia and Spain were collected.

Participants/materials, setting, methods: 40 endometrial biopsies were dissociated, CD9-positive epithelial cells and CD13-positive stromal cells were isolated with fluorescent activated cell sorting (FACS) and full transcriptome analysis was performed by RNA-seq. The single-cell tagged reverse transcription (STRT) RNA-seq protocol was followed. The STRTprep pipeline version 3.0.0 was used for processing raw sequencing reads, aligning to the hg19 genome. g:Profiler software was used for enrichment analyses. RNA-seq results were validated using real-time PCR.

Main results and the role of chance: A total of 675 epithelial cell-specific genes and 756 stroma cell-specific genes were detected as up-regulated, while 130 epithelial cell-specific genes and 133 stroma-specific genes were down-regulated in receptive phase endometria when compared to pre-receptive endometria. Rigorous filtering was applied on the differentially expressed genes in order to identify true cell-type specific molecules (e.g. genes detected in 75% of samples, significant expression difference between two cell types). After the filtering, 141 epithelial-specific genes and 372 stroma-specific genes remained as significantly up-regulated, and 6 epithelial-specific genes and 99 stroma-specific genes remained as down-regulated in receptive endometria. The receptive epithelium marker genes with the highest score of cell-specificity were *TNSFF10*, *TGFB2*, *DDX52*, *GPR160*, *CLU*, *CDH1*, *ARHGEF38*, *MAP7*, *SGK1*, *SPP1* and *RORC*. The top stromal cell-specific marker genes of receptive endometrium were *LMOD1*, *CFD*, *APOD*, *PROK1*, *HAND2*, *NR2F1*, *IGF2*, *TEX40*, *ZEB1*, *ANGPTL1* and *APOC1*. Receptive phase epithelial cell-specific marker genes were enriched in processes related to multicellular organismal homeostasis, cell activation involved in immune response and plasma membrane organization. Receptive phase stroma-specific genes were enriched in rRNA metabolic process, activation of immune response, regulation of cell adhesion, apoptotic signalling pathway, and processes involved in smooth muscle cell proliferation.

Limitations, reasons for caution: Rigorous statistical analysis could result in the failure to identify some molecules and molecular pathways involved in the receptive phase endometria and some potentially relevant biomarkers of receptivity.

Wider implications of the findings: With our novel RNA-seq approach new molecules and molecular processes of receptive phase endometria were detected, but also already known molecules and pathways were confirmed. The cell-specific RNA-seq analysis could provide the long-sought-after sensitive biomarkers of endometrial receptivity.

Trial registration number: na.

P-472 The risk for extrauterine gravidity (EUG) in IVF treatment

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Study question: What is the general risk for EUG after fresh or frozen blastocyst transfer in IVF treatment and can we elucidate potential risk factors?

Summary answer: The risk for EUG after transfer was found to be as low as after spontaneous conception.

What is known already: The spontaneous occurrence of EUG in an unselected population after spontaneous conception is around 1-2%. EUG is a severe complication for woman, requesting immediate medical treatment or surgery. Suggested risks factors to develop an EUG are advanced maternal age, tubal surgery, appendectomy, cesarean section, inflammation of the fallopian tubes, smoking and last but not least ART. ART was reported to be associated with EUG rates ranging from 3.3% up to 8.6% of all clinical pregnancies. Among the risk factors in ART, suboptimal transfer techniques and time-point of transfer (cleavage stage transfer) are regularly discussed.

Study design, size, duration: A retrospective study was conducted in our fertility clinic in Bregenz / Austria, including all IVF-cycles with blastocyst transfer resulting in a positive pregnancy test between January 2010 and December 2015. Clinical data were extracted by using the DynaMed® software, statistics were performed using SPSS statistics.

Participants/materials, setting, methods: In total 4.003 IVF-cycles with fresh blastocyst transfer resulting in a positive pregnancy test were analyzed. Patient's history was analyzed in regard to IVF-cycle characteristics, previous EPs, type of infertility, previous in-house cycles and outcome as well as abdominal and tubal or uterine surgeries.

Main results and the role of chance: Out of 4.003 blastocyst transfers resulting in an elevation of urine or blood beta-hCG levels and a positive pregnancy test 43 patients (1,1%) were diagnosed with EUG. Thereby, 12 EUG occurred after vitrification/warming and 31 after fresh blastocyst transfer with no statistical difference between the two groups. In 74.4% blastocyst transfer was performed easily without using additional guidance. The mean female age of patients diagnosed with EUG was 37.1 years (37.3 in the reference group) with eight patients younger than 35 years. The reasons for infertility treatment in EUG patients were 30.2% female factors, 34.9% male factors, 16.3% a combination of male and female factors and 18.6% idiopathic infertility. The incidence of uterine malformations in the EUG group was 11.7%, abdominal surgery was even reported for 44.2% and 6 patients reported a previous EUG (14.0%). Interestingly, 55.8% of the EUG group revealed sub-optimal endometrium build-up in the transfer cycle.

Previous EUG, uterine malformations, abdominal surgeries and inadequate endometrium build-up are potential main risk factors for an ectopic pregnancy.

Limitations, reasons for caution: As EUG seems to be a rare situation in after ART when using blastocyst transfer, further analysis on large data sets should confirm our results.

Wider implications of the findings: Our data analysis shows that ART and blastocyst transfer per se does not increase the risk for EUG. Specific risk groups (including patients with previous EUG and abdominal surgery should be advised for the increased risk. Endometrial build-up should be continuously controlled and possibly improved in frozen/warmed blastocyst transfer.

Trial registration number: not applicable.

P-473 Endometrial thickness is associated with the clinical pregnancy rate in unstimulated menstrual cycles – a study based on Natural Cycle IVF

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Study question: Does the endometrial thickness (EMT) correlate with the pregnancy and live birth rate in unstimulated menstrual cycles?

Summary answer: EMT does correlate with the clinical pregnancy rate in unstimulated menstrual cycles – according to data generated in Natural Cycle IVF (NC-IVF) treatment cycles.

What is known already: Several IVF studies have indicated that pregnancy rate correlates with EMT. Thin endometrium has been described to result in lower pregnancy rates. However, all these findings are based on conventional, gonadotropin stimulated IVF therapies. One small study reported on unstimulated frozen embryo transfer cycles and described a decreased pregnancy rate in thin endometrium patients, suggesting EMT to be a stimulation independent predictive factor for pregnancy. Data based on unstimulated fresh embryo transfer are still missing.

Study design, size, duration: Retrospective, observational single center study, performed 2011 to 2016. In women 18-42 years of age with regular menstrual cycles (24-32 days) and basal FSH concentrations <10IU/L undergoing their first NC-IVF cycle were identified. Only the first cycle, leading to an

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embryo was considered for analysis. Couples with endometriosis > rASRM II° or sperm collection by Testicular Sperm Extraction (TESE) were excluded.

Participants/materials, setting, methods: 225 women undergoing a NC-IVF cycle were identified. 111 women (49.3%) were excluded due to missing transfer, 5 women (2.2%) due to severe endometriosis and 4 women (1.8%) due to TESE resulting in 105 women to be included in the analysis. EMT at the time of follicle aspiration as well as biochemical and clinical pregnancy rates and live birth rate were analysed. Furthermore, week of gestation, birth weight and birth weight percentiles were evaluated.

Main results and the role of chance: Age of participants was 34.7 ± 3.8 years (range 21-42 years). 20 women (19%) had performed previous IVF therapies without pregnancy. Infertility factors were male factors (n = 53, 50.5%), endometriosis rASRM I-II° (n = 15, 14.3%), tubal factors (n = 19, 18.1%) and idiopathic infertility (n = 17, 16.2%). AMH concentrations were 15.6 ± 14.4 pmol/L (0.5-94 pmol/L).

Follicle aspiration was performed on day 13.9 ± 2.3 (range 9-22 days) of the menstrual cycle. Endometrial thickness was 8.7 ± 1.7 mm (range 6-16 mm). Total biochemical pregnancy rate was 29.5%, clinical pregnancy rate 24.8% and live birth rate 15.2% per transfer.

To analyse the effect of EMT on clinical pregnancy rate, women were divided into three percentile groups (25th percentile group: endometrium ≤7 mm (n = 27), 25th -75th percentile group: endometrium >7 mm (n = 48) and < 10 mm, 75th percentile group: endometrium ≥10 mm (n = 30). The percentile groups did not differ regarding age, AMH and cycle day of aspiration.

Clinical pregnancy rate was in the lower percentile group 7.4%, in the medium group 27.1% and the upper group 36.7%. Pregnancy rates were significantly different in the three groups (P = 0.034) as analysed by Chi-Square test. In a multi-variate regression analysis, none of the candidate predictors was significantly associated with clinical pregnancy achievement.

Limitations, reasons for caution: The number of participants is limited due to strict inclusion and exclusion criteria. The number of participants is too small to identify EMT as a potentially independent predictor of success.

Wider implications of the findings: The study provides evidence that EMT not only correlates with pregnancy rate in stimulated but also in unstimulated cycles. Accordingly the thickness of the endometrium should be considered in any infertile couples even if ovarian stimulation is not performed.

Trial registration number: Not applicable.

P-474 Can mtDNA copy number assessment be a valid embryo selection tool over morphology-based embryo scoring?

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Study question: This study is designed to evaluate if embryo selection by mtDNA copy number can bring any additional benefit over classical embryo scoring on clinical outcome.

Summary answer: Our results can indicate that a better clinical outcome can be predicted if mtDNA copy number is prioritized for embryo selection in single embryo transfers.

What is known already: Numerous studies have been performed to analyze and document the importance of mitochondria in mammalian preimplantation development. Recently, besides comprehensive chromosomal screening (CCS), several groups have investigated the possibility of using mtDNA copy number assessment as a clinical viability score in cases undergoing preimplantation genetic screening (PGS). Although promising results obtained in these reports, new data also indicate that such an assessment may still be in its infancy and more studies are needed. There are still many unknowns regarding the methodology and clinical conditions in which the mtDNA copy number assessment should be performed.

Study design, size, duration: Our study has been designed as a retrospective cohort study including 230 consecutive SETs performed after PGS by next generation sequencing (NGS) between October 2015-December 2016 in Bahceci Fulya IVF Centre.

Participants/materials, setting, methods: In all cases, embryos were cultured to the blastocyst-stage and suitable embryos on day 5/6 of embryo development were biopsied and individually vitrified. According to their PGS results and the availability of mtDNA copy number data, SETs were performed as frozen embryo replacements. Data were grouped with respect to embryo selection strategy prioritized as group I (156 cases, based on known mtDNA copy number) and group II (74 cases, based on embryo morphology).

Main results and the role of chance: In total, 783 hatching-stage blastocysts were biopsied and processed for CCS analysis by next generation sequencing (NGS). Among 399 embryos that were found to be chromosomally normal, WGA material of 286 embryos subsequently used also for mtDNA copy number analysis by qPCR. Main case demographics, such as female age (35.0 ± 4,5 vs. 35.5 ± 4,4) and the number of oocytes collected (12.9 ± 7,6 vs. 12.3 ± 6,4) were found to be similar in both groups. Also, additional cycle and laboratory characteristics such as the number of previous ART trials, total dose of gonadotropins used and sperm concentration and motility were found to be statistically similar (p>0.05). Distribution of blastocyst grades in both groups also displayed very similar pattern. In group I, biochemical pregnancy, clinical pregnancy and ongoing pregnancy/live birth rates were found to be 80.1%, 72.6% and 65.6% respectively. In group II, these values were 67.5%, 63.9% and 47.8%. Although higher clinical pregnancy and ongoing/live birth rates were obtained in the former, the difference were found to be statistically not significant.

Limitations, reasons for caution: Number of cases included can be the main limitations of the study. Although our results indicate a better clinical outcome when mtDNA copy number is prioritized, further studies with higher number of cases are required to draw a firm conclusion.

Wider implications of the findings: Our study indicates that mtDNA copy number assessment can be a better embryo selection tool over classical morphology-based scoring systems in terms of predicting cycle outcome in SET cases after PGS.

Trial registration number: None.

P-475 Endometrial injury does not affect the endometrial transcriptomic profile during the window of implantation

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Study question: Is the endometrial transcriptomics analyzed by the molecular endometrial receptivity analysis (ERA) modified after an injury provoked by an endometrial biopsy?

Summary answer: Endometrial transcriptomic profile analyzed by ERA is not affected after a previous endometrial biopsy obtained 48 hours before in the same patient.

What is known already: Mechanical injury of the endometrial cavity or scratching is being used by many clinicians aiming to modify the endometrial conditions to improve embryo implantation. No scientific evidences of the effect of such intervention in endometrial transcriptomics has been demonstrated yet. The endometrial receptivity analysis (ERA) is a commercial available test based on the transcriptomic signature of 238 genes that allows to diagnose the personalized window of endometrial receptivity defining the profiles of pre-receptivity, receptivity and post-receptivity in each patient. Bioinformatics data on these transcriptomic profiles from >12,000 patients worldwide have been accumulated and curated in the last 6 years.

Study design, size, duration: In thirty-six patients, two endometrial biopsies from the uterine fundus were obtained two-days apart in the same patient under hormonal replacement therapy cycle (HRT). The first was taken after proper estradiol priming leading to a trilaminar endometrium measuring

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>6.5 mm adding 5 days of progesterone administration (P+5 - 120 hours), and the second 48 hours later (P+7 - 168 hours). Samples were collected from January to November 2016 at Oak Clinic, Japan.

Participants/materials, setting, methods: RNA was extracted and quality assessed by an A2100 Bioanalyzer (Agilent Technologies). Only samples with RIN (RNA Integrity Number) >7 were hybridized onto the customised ERA array (Diaz-Gimeno, et al., 2011), scanned in an Axon 4100 A scanner (Molecular Devices), and analysed by ERA computational predictor. Limma package was used to compute the analysis of differentially expressed (DE) genes of the endometrial samples investigated versus our curated data base obtained from >12,000 patients worldwide.

Main results and the role of chance: No relevant differentially expressed (DE) genes were found in the expected endometrial transcriptomic profiles obtained in two consecutive endometrial biopsies, in the same patient under the same HRT cycle at P+5 and P+7. They were not different from the expected transcriptomic profile at the indicated days obtained from single biopsies reported in our curated data base. Therefore, tissue injury provoked by an endometrial biopsy does not affect the transcriptomic signature of the second biopsy obtained 48 hours later.

Limitations, reasons for caution: Limitation for the present study is the number of patients analyzed with two consecutive endometrial biopsies.

Wider implications of the findings: Our study demonstrates that the injury provoked by an endometrial biopsy does not modify the expected transcriptomics and therefore, proteomics/function of the endometrial tissue. Clinical interventions based on modifications of the endometrium due to tissue injury must be considered with caution since no real biological effect is observed.

Trial registration number: Not apply.

P-476 Immunoregulatory effects of the Blastocyst Conditioned Media: focus on the control of the inflammatory response

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Study question: Could the human Blastocyst Conditioned Media (BCM) control the initial inflammatory response during the peri-implantation period toward a tolerogenic one?

Summary answer: Decidualized cell responded differentially to BCM, accordingly their quality, by reducing reticular stress-associated inflammation, recruiting regulatory T lymphocytes (Tregs) and allowing blastocyst-like spheroids invasion.

What is known already: The decidualization program of human endometrial cells involves a physiological reticular stress (RE) and unfolded protein response (UPR), allowing them to expand their endoplasmic reticulum and change their secretome increasing the production of immunomodulators. This physiological RE and UPR response would allow the activation of one of the 3 RE sensing proteins, IRE1, which induces kinase / RNase TXNIP expression, and activates the inflammasome generating a sterile inflammatory response with production of IL-1b. This sterile inflammatory response, associated to the implantation period, should be later controlled in favor of a tolerogenic microenvironment by maternal and blastocyst-derived factors.

Study design, size, duration: Human endometrial stromal cell line (HESC) were decidualized or not with medroxiprogesterone+dbcAMP during 8 days, and used to evaluated RE and UPR. Then, HESC cells were treated with human Blastocyst-condition-media (BCM), previously classified accordingly their quality, for 24 h. An *in vitro* implantation model based on co-culture of blastocyst-like spheroids (BLS) from Swan-71 trophoblast cells line over decidualized-HESC cells and a transwell migration system for Tregs recruitment were used to evaluate decidual functionality.

Participants/materials, setting, methods: Mature oocytes were inseminated (conventional IVF or ICSI was applied according to male evaluation). After 3 days, embryos were transferred individually to drops of G2 plus-medium. At day 5, BCM were retrieved and embryos classified following Istanbul concensus. Maternal monuclear cells were obtianed by pheripheral blood and Tregs frequency quantified by intracellular Foxp3 staining and FACS analysis. Gene and protein expression was evaluated by PCR or FACS respectively. BLS invasion was evaluated by fluorescence microscopy.

Main results and the role of chance: We observed a significantly increase in the expression of CXCL8, CXCL12 and IL-1b after the decidualization Since IL-1b can act as a 'double edge' mediator in early pregnancy, we evaluated BCM effect on IL-1b production. BCM reduced IL-1b intracellular production in decidualized cells ($p < 0.05$, Student T-test). This was accompanied by a decreased mRNA of reticular stress sensors as PERK and ATF6 expressions ($p < 0.05$, Anova, Sidak).

When we studied the inflammasome activation pathway, TXNIP was not modulated but NLRP3 and IL1b levels were both significantly decreased after BCM treatment ($p < 0.05$, Anova Sidak). These results, suggest that BMC is able to reduce the UPR on decidualized cells. Using the *in vitro* implantation model, we observed that BCM obtained from developmentally impaired blastocysts decreased the invasion index of BLS on decidualized cells and Tregs recruitment. Similar results were observed when UPR was previously inhibited using STF-083010, suggesting that decidual UPR may be relevant for trophoblast initial invasion ($p < 0.05$ Anova, Sidak).

Since, decidual cells are able to control immunological microenvironment partially by recruiting Tregs, we evaluated whether BCM was able to modulate this mechanism. We observed that decidualized-HESC treated with BCM from developmentally impaired blastocysts significantly reduced Treg recruitment ($p < 0.05$, Anova, Sidak).

Limitations, reasons for caution: The present results were obtained using immortalized cell lines and *in vitro* implantation and invasion models. Further studies are necessary to elucidate whether the mechanisms operate similarly *in vivo* and rule out any factor not contemplated *in vitro*.

Wider implications of the findings: The results presented provide new clues whether the blastocyst soluble factors (represented by BCM) might contribute to the cross-talk with decidualized cells controlling the inflammatory response and allowing blastocyst invasion accordingly with their quality. This could contribute to a better undestanding of reproductive dissorders such as *in vitro* implantation failures.

Trial registration number: N/A.

P-477 Reprogramming of the hCG signalling profile in human endometrial stromal cells from recurrent miscarriage patients

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Study question: To identify the LHCGR signalling pathways activated by hCG in the endometrial stroma, during deciudalization in control and recurrent miscarriage (RM) patients.

Summary answer: Collectively, our data strongly suggests that control and RM patients have opposing signalling pathways.

What is known already: Cross-talk between an implanting embryo and the uterine endometrium is vital for successful implantation. Locally secreted factors within the uterine environment play a vital role in this feto-maternal communication. Human chorionic gonadotrophin (hCG), a glycoprotein hormone secreted by the embryo, plays key functions in the ovary and endometrium via its cognate G protein-coupled receptor (LHCGR). The signal pathways activated by LHCGR within the endometrium are largely unknown. Perturbations in hCG action in endometrial stromal decidualization has been shown to be associated with recurrent miscarriage (RM), a key mechanism in perturbed embryo quality control at the feto-maternal interface in these patients.

Study design, size, duration: This is a laboratory based study performed on n = 6 patients in each group; normal versus recurrent miscarriage.

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Participants/materials, setting, methods: Human endometrial stromal cells (HESCs) from either control patients or RM patients were decidualised for 72 hours, or left undifferentiated. Expression of LHCGR was confirmed in both patient groups. Cells were stimulated at 0, 5 and 30 minutes with hCG. The resulting protein lysates were used to probe phospho-MAPK array membranes that detect 26 different phospho-MAPKs. Fold change over basal for each target at the 5 and 30 minutes time points was calculated.

Main results and the role of chance: Collectively, the array data strongly suggests that control and RM patients have opposing signalling pathways. In control, many pathways activated by hCG in undifferentiated cells are decreased in decidualised cells and only a subset of pathways are activated. In RM, the same pathways are either not activated or not deactivated following decidualisation.

Limitations, reasons for caution: None

Wider implications of the findings: Our data shows that decidualisation reprograms the signalling profile in control but not recurrent miscarriage HESCs. The failure of RM HESCs to reprogram their hCG signalling upon decidualisation could be an important defect which contributes to erroneous embryo selection at the time of implantation that can lead to pregnancy loss.

Trial registration number: This was not a trial.

P-478 New transcriptomic insight improves endometrial Recurrent Implantation Failure (RIF) diagnosis and distinguishes clearly between a displaced and a disrupted Window Of Implantation (WOI)

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Study question: Endometrial Recurrent Implantation Failure (RIF): a matter of timing or a disrupted Window of implantation signature? Is it possible to diagnose both types of RIF?.

Summary answer: The proposed transcriptomic prediction design resolves the understanding of endometrial RIF diagnoses and distinguishes at least two RIF main causes: displaced and disrupted WOI.

What is known already: Endometrial transcriptomics prediction has been applied to human WOI in two main works: Simon (Diaz-Gimeno et al., 2011) and Macklon groups (Koot et al., 2016). Diaz-Gimeno considers endometrial RIF as a displacement and is not distinguishing a disrupted WOI signature. Nerveless, Koot considers endometrial RIF as a disrupted WOI removing the LH timing effect. Both of the models are considering LH as gold standard in the prediction design, however, Diaz-Gimeno signature clinical application have been demonstrated approximately 25% of RIF discrepancies between transcriptomics profile and LH in the expected WOI (LH+7) (Ruiz-Alonso et al., 2013).

Study design, size, duration: Retrospective analysis comparing WOI endometrial transcriptomics and prediction in controls (n = 72) versus RIF patients (n = 43) in samples collected from LH+5 to LH+8. Raw data was downloaded from GEO database (GSE58144) (Koot et al, 2016). In the same dataset we compared both predictor methodologies, Diaz-Gimeno and Koot, and we proposed a new transcriptomics insight to fill the gap between both studies and be able to detect and distinguish both types of RIF.

Participants/materials, setting, methods: Data was pre-processed and normalized using quantile method from limma R-package. Different designs for SVM predictors using caret R-package were performed: one considering RIF versus Controls removing and not removing LH variations, and the other one stratifying samples by transcriptomics and predicting RIF displacement. The predictive value was compared between Diaz-Gimeno and Koot signatures and a new diagnostic algorithm that distinguishes between both types of RIF in the same sample cohort is proposed.

Main results and the role of chance: WOI samples were classified by unsupervised transcriptomic methods (K-means) in ER: Early Receptive, R: receptive and LR: Late-Receptive profiles. Comparing transcriptomics with LH as gold standard we found some discrepancies and displaced samples, but LH+5 were mostly in ER, LH+8 were mostly in LR, LH+6 were mainly in ER and LH+7 were many of them in R but some samples were displaced to ER and PR profiles. WOI displacement predictor supervised by transcriptomic profiles classified RIF samples in ER, R or PR for Koot signature with an accuracy (ACC) of 0.90 and with Diaz-Gimeno with an ACC of 0.98.

RIF samples that were not displaced could be detected using a second model called disrupted WOI which is supervised only by control and RIF R samples. With this design, Koot signature obtained an ACC=1 (Specificity (Sp) of 1, Sensibility (S) of 1); and Diaz-Gimeno signature an ACC=0.789 (Sp 1, S 0.38).

Macklon statistical procedure for removing LH time effect is not as efficient as using transcriptomics as gold standard. Transcriptomic menstrual cycle timing is a confounding variable that should be controlled for distinguishing clearly between disrupted and displaced RIF samples.

Limitations, reasons for caution: The main objective of this study is to show both types of RIF causes to understand the gap between the both hypotheses suggested. Therefore, the prediction parameters in absolute values are not important. By other hand, clinical relevance of each transcriptomic profile has not been analysed in this study.

Wider implications of the findings: Besides of understanding the gap between both works, the main insight of this study is how to design predictors to distinguish clinically between the patient that could receive a personalised embryo-transfer day and the patient with a disrupted WOI that should be studied for developing new treatments.

Trial registration number: not applicable.

P-479 New approach for assigning endometrial receptivity using RNA sequencing

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Study question: Can low-read whole-transcriptome RNA-seq approach be used for endometrial receptivity biomarker analysis?

Summary answer: The results suggest that Single-cell Tagged Reversed Transcription (STRT) RNA-seq approach can be used as a sensitive and cost-effective method for endometrial receptivity assignment.

What is known already: Up-to-date microarray-based gene expression analysis of endometrial tissue has been the only clinically applied technique to predict the receptiveness of uterine lining in women undergoing IVF. The microarray approach is limited to previously well-described genes with no respect to isoform variation. It highlights that RNA-seq is the potential assay for routine gene expression analysis. Recent study on individual endometrial cells of different types introduces new RNA-seq approach for whole transcriptome analysis of endometrial tissue using low read count, that significantly reduces the cost for RNA-seq.

Study design, size, duration: In this study we investigated transcriptome profiles of early and mid-secretory endometrial tissue from healthy women using STRT RNA-seq method and compared it with Illumina Paired-End RNA-seq (PE RNA-seq). STRT method was preferred as a high-plex RNA sequencing technique that is beneficial in decreasing batch effect.

Participants/materials, setting, methods: Endometrial biopsy was obtained from a cohort of 35 healthy fertile volunteers (aged 23-36) on day one and day seven post ovulation, confirmed by LH testing. STRT 48-plex RNA-seq library was synthesized from whole-tissue total RNA using oligo-T primer, Unique Molecular Identifiers (UMI), and sequenced by Illumina HiSeq2000. Independently of this study, PE RNA-seq analysis was performed using Illumina Stranded TruSeq technique with participant inclusion criteria and RNA isolation procedures similar to the present study.

Main results and the role of chance: The differential expression analysis of mid-secretory and early secretory phase endometrium showed up to three-fold difference in the number of significantly differentially expressed genes

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(DEGs) between two approaches. Quantitative analysis showed that 724 DEGs identified by STRT (64% of STRT DEGs) overlapped with PE RNA-seq.

Functional analysis revealed similar within-dataset distribution of molecular functions and biological processes affected by DEGs, with the prevalence of genes associated to cellular processes (44,7% in STRT and 43,8% in PE RNA-seq) and metabolic events (36,3% in STRT and 34,7% in PE RNA-seq). Among the genes with the highest expression rate change were many recognized endometrial receptivity markers, such as *PAEP*, *GPX3*, *LAMB3*, *DPP4*, *SERPING1*. The abundance of DEGs identified by STRT (>40%) appeared to be common endometrial markers or genes previously associated with endometrial functioning.

Limitations, reasons for caution: STRT use in endometrial gene expression profiling is most justified for the analysis of well-described transcriptomic markers for endometrial receptivity, while low sequencing depth may be an issue in *de novo* marker analysis.

Wider implications of the findings: Prospectively, the method can be adopted for RNA isoform analysis that augments its potential in diagnostic field.

Trial registration number: not applicable.

P-480 Idiopathic recurrent pregnancy loss - role of pre implantation genetic screening

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Study question: Is there a role for preimplantation genetic screening (PGS) in management of idiopathic recurrent pregnancy loss(RPL)?

Summary answer: Considering high mean aneuploidy rate in young (<35years) women with RPL, PGS identifies euploid embryos for transfer resulting in better ongoing pregnancies and live births.

What is known already: Chromosome abnormalities are extremely common in human oocytes and embryos and are associated with a variety of negative outcomes for both natural cycles and those using assisted conception techniques. Aneuploid embryos may fail to implant in the uterus, miscarry, or lead to children with serious medical problems (e.g., Down syndrome). Preimplantation genetic screening (PGS) is suggested in patients with advanced maternal age or RIF to improve the outcomes of assisted reproductive treatments by ensuring that the embryos chosen for transfer to the uterus are chromosomally normal.

Study design, size, duration: Observational study of fifteen couple under age of 35 years with idiopathic recurrent miscarriage who were managed at our institute from 1/6/2016 to 31/12/2016.

Participants/materials, setting, methods: Women under 35years and couples with normal karyotyping and normal RPL profile investigations were included in study. Couples who had two or more first trimester miscarriages were considered as RPL.

All oocytes were subjected to ICSI. All embryos were cultured till Blastocyst stage and were subjected to trophectoderm biopsy. PGS was done through Next Generation Sequencing. Mean percentage of Anueploidy per case was calculated and compared against age matched aneuploidy rates of embryos reported in literature.

Main results and the role of chance: Mean embryo anueploidy rate in RPL group (N = 15) was 79.25% with mean clinical pregnancy rate and ongoing pregnancy rate of 71.4%. All the cases except one had atleast one euploid embryo for transfer. One woman had a early miscarriage. All these women have ongoing pregnancies that have successfully crossed the first trimester. Zachary et al., 2016 reported embryo aneuploidy rates in women under the age of 35 to be around 55% in general population. Results in the study group seem to show higher incidence of embryo aneuploidy in women under the age of 35 when compared against the general population. This further emphasizes the role of PGS in RPL women to optimize reproductive outcomes.

Limitations, reasons for caution: Limitation of this study was low numbers of women included. These are preliminary results and we are continuing follow up of these women with ongoing pregnancies to assess the livebirth rate. Further broad based clinical application of PGS in RPL is required to define its significance.

Wider implications of the findings: Considering the higher mean aneuploidy rate in young (<35yrs) RPL group, this data supports the use of PGS to select euploid embryo for transfer that could result in better ongoing pregnancies and live birth.

Women with RPL seem to benefit from PGS of embryos.

Trial registration number: not applicable.

P-481 Does urinary luteinizing hormone surge provide more accurate pregnancy dating than last menstrual period in women with Recurrent Pregnancy Loss?

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Study question: Determine if use of urinary luteinizing hormone surge corresponds better with crown rump length than last menstrual period for dating pregnancies under 10 weeks in women with recurrent pregnancy loss.

Summary answer: Study reveals that urinary LH surge corresponds better with CRL than LMP in dating pregnancies before 10 weeks in women with a history of RPL.

What is known already: The use of LMP is the gold standard for pregnancy dating. Recently, the reliability of LMP to accurately predict the estimated due date (EDD) has been called into question. The use of LMP relies on maternal recall, which can often be problematic. Many women do not recall their LMP or are unsure of the date of LMP. Differences in cycle length also affect the reliability of LMP. The use of urinary LH surge has not been well studied in regards to pregnancy dating, it could more reliably predict the EDD since it is an accurate indicator of date of conception.

Study design, size, duration: Observational cohort study from two academic RPL clinics. Women seen between July 2005 and September 2015 were eligible for inclusion. First ultrasound with CRL≥ 5 mm was used to estimate gestational age (GA), by using the Hadlock Formula. GA by LH surge was calculated as the number of days from LH surge to date of ultrasound + 13 days. GA by LMP was calculated as the number of days from LMP to date of ultrasound.

Participants/materials, setting, methods: IRB approval was obtained. Inclusion criteria included: women with ≥2 "unexplained" pregnancy losses <10 weeks size and 1 or more subsequent pregnancy(ies) of at least 10 weeks size; subsequent pregnancies were conceived using timed intercourse to LH surge, without the use of fertility medications. Continuous variables were compared with a student's t-test. Correlation coefficients were compared using a Fisher's Z-test.

Main results and the role of chance: 120 women met inclusion criteria with 122 subsequent ongoing pregnancies. The mean number of prior pregnancy losses <10 weeks was 3.6 (SD 1.6, range 2-10). The mean maternal age at time of subsequent pregnancy was 35 years (SD 3.6, range 26-46). The mean BMI at initial ultrasound was 25.5 kg/m^2 (SD 5.2, range 16.8-45.8). Of the 122 subsequent pregnancies, the mean GA by CRL was 49.9 days (SD 4.9, range 41-65). The mean absolute difference between GA by CRL and GA by LH surge was 1.6 days, whereas the mean absolute difference between GA by CRL and GA by LMP was 2.6 days, P< 0.0001. GA by LH surge more closely correlated to the CRL compared to LMP, with a correlation coefficient of 0.81 versus 0.69, P = 0.02.

Limitations, reasons for caution: Generalizability should be determined by evaluating a cohort of fertile women without a history of RPL.

Wider implications of the findings: This preliminary data suggests that urinary LH surge rather than LMP should be the gold standard in pregnancy dating if LH surge is known.

Trial registration number: Not applicable.

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P-482 Does follicular phase length impact pregnancy outcome in women with a history of Recurrent Pregnancy Loss?

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Study question: To determine whether follicular phase length could impact pregnancy outcome in women with Recurrent Pregnancy Loss (RPL).

Summary answer: In this study, the length of the follicular phase in the conception cycle did not significantly impact pregnancy success in women with RPL.

What is known already: During the follicular phase, the endometrium undergoes reconstruction and growth in preparation for implantation in the luteal phase. In a typical 28-30 day cycle, the follicular phase is usually 13-15 days, although it can have considerable variability. Much of the observed inter-cycle variability is secondary to differences in the follicular phase since the luteal phase, in comparison, is more constant. The relationship between follicular phase length and pregnancy outcome has not been well studied.

Study design, size, duration: Observational cohort study from two academic RPL clinics. Women seen between July 2005 and September 2015 were eligible for inclusion. Ovulation was defined as the day after luteinizing hormone surge. The follicular phase was defined as the number of days from the 1st day of menses to ovulation. Pregnancy was defined as a positive hCG at time of missed menses. Pregnancy success was defined as a term, preterm or an ongoing pregnancy >10 weeks size.

Participants/materials, setting, methods: Inclusion criteria included: 1) women seen between July 2005 to September 2015 with a history of ≥2 "unexplained" pregnancy losses <10 weeks size and 1 or more subsequent pregnancy(ies); 2) subsequent pregnancies were conceived using timed intercourse to the luteinizing hormone (LH) surge, without the use of fertility medications.

Main results and the role of chance: 175 women met inclusion criteria with 195 subsequent pregnancies. The mean number of prior pregnancy losses <10 weeks was 3.8 (SD 1.8, range 2-13). The mean maternal age at time of subsequent pregnancy was 35.6 years (SD 3.8, range 26-46). The mean BMI at initial pregnancy visit was 25.4 kg/m^2 (SD 4.9, range 16.8-45.8). Of the 195 monitored pregnancies, 68% resulted in a success and 32% resulted in a pregnancy loss <10 weeks size. The mean follicular phase length in the cycle of conception was 15.1 days (SD 3.9, range 5-39 days). The pregnancy success rate was 74% (62/84) in women with a follicular phase length of 13-15 days, compared to 60% (26/43) in women with a follicular phase of <13 days and 65% (45/69) in women with a follicular phase of >15 days, P = 0.2.

Limitations, reasons for caution: In order to demonstrate a difference of 15% in the ongoing pregnancy rate between groups, a sample size of 350 pregnancies would be required. Therefore, a larger cohort is needed to definitively answer this question.

Wider implications of the findings: Although the length of the follicular phase in the conception cycle did not significantly impact pregnancy success in women with RPL, there was a trend towards a higher pregnancy success rate with a follicular phase length of 13-15 days. As stated above, a larger cohort is needed.

Trial registration number: Not applicable.

P-483 Determining embryo developmental competence by measuring expressivity of the paternal epigenome

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Study question: To profile the transcriptome of men with unexplained infertility and to identify key sperm-specific RNAs that contribute to post-fertilization embryonic developmental competence.

Summary answer: Transcriptome profiling of human spermatozoa using next generation sequencing (RNA-Seq) can reveal genes products that are critical to embryonic development and assisted reproductive treatment outcomes.

What is known already: Although semen analyses are useful in diagnosing male infertility, a normal semen analysis does not guarantee fertility and does not provide functional information about the sperm. While new infertility biomarkers such as SCSA, TUNEL, FISH and Comet assays may provide additional information, they do not reflect an individual's fertility potential. Upon fertilization, the sperm provides a complete, highly structured, and epigenetically marked genome into the oocyte. It is thought that characterization of sperm RNAs may be suited to better understand the contribution of the paternal epigenome, and the regulatory roles these RNA transcripts play in post-fertilization embryo development.

Study design, size, duration: During a 15-month period, RNA-Seq was performed on ejaculated semen samples provided by 25 consenting men undergoing infertility screening. All men had normal semen parameters and their female partners <35 years had normal infertility screening. Following RNA extraction, the relative abundance of RNA transcripts was compared between men who were or not able to achieve conception with intracytoplasmic sperm injection (ICSI). The profiles of these RNA transcripts were then related to embryo development characteristics.

Participants/materials, setting, methods: RNA was isolated from $25x10^6$ spermatozoa using a hybrid isolation protocol with TRIzol® Reagent (ThermoFisher Scientific, USA) and a spin column kit (RNeasy Mini Kit, QIAGEN, Germany). Pilot sequencing was carried out at 2×36 bp to determine library quality and expanded to 50-60 M reads at 2×76 bp utilizing an Illumina platform (NextSeq 500). Data was processed and analyzed using the Tuxedo Protocol. Expression values were calculated in FPKM (Fragments Per Kilobase of Exon Per Million Fragments Mapped).

Main results and the role of chance: The 25 consenting men had the following semen parameters: 37.3 ± 17 x 10^6/mL (concentration), 46.6 ± 10% (motility), and normal morphology. The average concentration of RNA extracted was 14.3 ± 6 ng/μL. The age demographics were comparable among couples who conceived *i.e.*, fertile (men = 37.6 ± 3 years; women = 34.8 ± 3 years) and did not conceive *i.e.*, infertile (men = 38.3 ± 5 years; women = 33.6 ± 7 years) with ICSI. The fertilization rates with ICSI and number of embryos transferred were also similar. RNA expression analysis revealed 86 differentially expressed genes between the groups ($P < 0.001$) – 24 overexpressed and 62 unexpressed. The expression profiles of these RNA transcripts were compared with embryo development profiles. The blastomere cleavage characteristics was similar between the two groups and was consistent with the expression of *RANBP2*, a gene involved in the assembly of cytoskeletal elements. A higher embryonic fragmentation rate (14.3%) was noted in the infertile group that corresponded to a higher magnitude of *BOK* RNA transcripts ($P < 0.001$), a gene which is known to regulate apoptosis during the cell cycle. As expected, *PLK4* and *BUB1*, which dictate chromosome stability and mitotic segregation by regulating centrosome development, had decreased expression in the infertile group ($P < 0.001$), therefore leading to increased aneuploidy and impaired implantation.

Limitations, reasons for caution: The small sample size of our study represents a limitation, and therefore, our findings need further validation in a larger cohort. The contribution of the paternal genome in embryonic aneuploidy needs confirmation via genomic sequencing of pre-implantation embryos or cytogenetic analysis of miscarriage specimens.

Wider implications of the findings: The application of sperm RNA-Seq can extend beyond microscopic evaluation, morphometric assessment, and diagnostic assays to help predict the contribution of the male gamete to early pre-implantation development. This novel technique can further classify the transcripts that play an influential role in pre and post fertilization and embryo developmental competence.

Trial registration number: Not Applicable.

P-484 Effect of raised serum progesterone level on the day of ovulation trigger on micro RNA expression in endometrium in stimulated cycles

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Study question: Is there a difference in expression of m RNAs in the endometrium in patients with high progesterone on the day of ovulation trigger in controlled ovarian hyper stimulation cycles?

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Summary answer: There is a difference in expression of micro RNAs in the endometrium in patients with high progesterone on the day of ovulation trigger.

What is known already: Elevated serum progesterone (P) on the day of human chorionic gonadotrophin (hCG) has been reported to occur in 20%-40% of in vitro fertilization (IVF) cycles. The impact of this is still being debated. Lower pregnancy rates with high progesterone concentration on the day of hCG administration has been reported. Elevated progesterone might induce premature endometrial maturation. MicroRNAs (miRNAs) are small, non-coding RNAs that are able to regulate gene expression at the post-transcriptional level. miRNA expression profile reflects the receptivity status of human endometrium. Therefore, miRNAs are potential biomarkers for endometrial receptivity and may help optimize the protocol for IVF treatment.

Study design, size, duration: It is a prospective study with 4 patients undergoing IVF/ICSI,divided them into 2 groups. First group being patients with P ≤1.5 ng/ml and second group with P > 1.5 ng/ml on the day of ovulation trigger. An endometrial biopsy was taken on the day of oocyte retrieval and a second one 5 days after retrieval for each patient i.e. a total of 8 biopsies.

Participants/materials, setting, methods: Controlled ovarian hyperstimulation was done for all the patients with antagonist protocol. Serum Progesterone was measured on the day of ovulation trigger and oocyte retrieval was done 36 hours later. The biopsies were divided in two halves for histological staining and to evaluate for the expression of micro RNA.MicroRNA (miRNA) sequencing was carried out using Illumina NextSeq500 Single-end sequencing. Alignment against miRBASE database, reporting known and novel miRNA with expression analysis and comparison was done.

Main results and the role of chance: In the retrieval day biopsy,1007 miRNAs were expressed in both the groups. 182 miRNAs were expressed only in group I whereas 186 miRNAs were expressed only in group II. 26 miRNAs were significantly upregulated in the Group II when compared to Group I. Similarly, in the Day 5 biopsy, a total of 961 miRNAs were expressed in both the groups. 137 miRNAs were expressed only in group I whereas 250 miRNAs were expressed only in group II. 36 miRNAs were significantly upregulated in the Group II when compared to Group I. Sequences not showing hits with known miRNA's were extracted and were considered for novel miRNA prediction. In the retrieval day biopsy, 50 novel miRNAs were expressed in both the groups, 165 only in Group I and 361 only in Group II. Whereas in the day 5 biopsy, 41 novel miRNAs were expressedin both the groups, 186 only in Group I and 374 only in Group II, MicroRNAs with copy number > = 10 were considered for target prediction. These miRNA sequences were fed as input along with reference mRNA sequences to miRanda 3.3a6 tool. miRNA hits having minimum free energy < = -20 are assumed to be the targets for reported miRNA.

Limitations, reasons for caution: The main limitation of this study is the small sample size. A study with more samples is required for validation. Also, in this study only patients with antagonist protocol were recruited.There may be differences in the endometrial receptivity profile according to various stimulation protocols

Wider implications of the findings: Through this study we were able to demonstrate that raised progesterone affects endometrial receptivity. Hence, it would be ideal to proceed with fresh embryo transfer in cases with P ≤1.5 ng/ml. However, when P > 1.5 ng/ml, freezing the embryos and transferring in a later cycle would give a better pregnancy rate.

Trial registration number: Not applicable.

P-485 Predictive value of plasma human chorionic gonadotropine measured 14 days after Day-2 single embryo transfer

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Study question: How does plasma human chorionic gonadotropine (p-hCG) measured exactly 14 days after day-2 single embryo transfer (SET) predict pregnancy, delivery and perinatal outcome?

Summary answer: Plasma-hCG is a significantly better predictor of clinical pregnancy than of ongoing pregnancy and delivery and was neither related to birth weight nor gestational age.

What is known already: Prediction of pregnancy outcome as soon as possible after Assisted Reproductive Technologies (ART) is important for patients and clinicians. Early p-hCG levels are the best known predictor of pregnancy outcome, but no studies investigating p-hCG after ART have been restricted to SET of Day-2 embryos.

Study design, size, duration: This is a sub-study to an RCT comparing the short GnRH-antagonist protocol with the long GnRH agonist protocol including 1050 women undergoing their first IVF/ICSI treatment. The first patient was enrolled in 2009. In the current study we included all women with SET, who had p-hCG measured exactly 14 days after Day-2 SET (n = 466).

Participants/materials, setting, methods: A total of 466 women, 243 (52.1%) in the GnRH-antagonist group and 223 (47.9%) in the GnRH-agonist group, fulfilled the inclusion criteria of fresh Day-2 SET and p-hCG measured exactly 14 days after ET. Receiver operating characteristic (ROC) curves were generated using a nonparametric distribution method to assess the predictive value of p-hCG for reproductive outcomes.

Main results and the role of chance: The p-hCG levels 14 days after SET were similar after GnRH-antagonist (282.8 (198.6) IU/L) and GnRH-agonist (266.0 (206.3) IU/L) protocol (p = 0.48); after IVF (291.3 (218.3) IU/L) and ICSI (249.2 (169.2) IU/L) (p = 0.28); and whether (278 (238.5) IU/L, n = 152) or not (278 (238.5) IU/L, n = 33) a high quality embryo was transferred. P-hCG predicted clinical pregnancy (AUC=0.953; 95% CI 0.915-0.992) significantly better than it predicted ongoing pregnancy (AUC=0.803, 95% CI; 0.717-0.890) and delivery (AUC=0.772, 95% CI; 0.691-0.854). Women with p-hCG levels in the lowest quartile had significantly lower clinical pregnancy, ongoing pregnancy, and delivery rates ($p < 0.001$), whereas the pregnancy outcome and post-clinical pregnancy loss remained similar throughout the three highest p-hCG quartiles. The p-hCG level was neither related to birthweight nor gestational age at delivery.

Limitations, reasons for caution: The use of prospectively collected data derived from an RCT limits the sample size.

Wider implications of the findings: A clinical pregnancy was optimally predicted by a p-hCG value of 123 IU/L (sensitivity 86.1%, specificity 96.3%). Discriminatory cutoff values are useful in guiding clinicians identifying pregnancies with high risk of adverse outcomes and instituting more intensive pregnancy surveillance in this population.

Trial registration number: For the initial randomized controlled trial (Toftager et al.,2016): EudraCT #: 2008-005452-24, ClinicalTrial.gov: NCT00756028.

P-486 Proteomic assessment of the signatures underlying human placental vascular development

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Study question: Can proteomics inform us of the molecular signatures underlying human placental vasculogenesis and maturation?

Summary answer: 3586 proteins were identified and quantified from placental arterial biopsies and 1078 were differentially expressed between 1st and 3rd trimester of pregnancy.

What is known already: The regulation of human placental organogenesis is crucial for ensuring normal fetal growth and successful pregnancy outcome.

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This depends on the *de novo* formation in first trimester, and maturation throughout gestation, of the placental vasculature. It is of interest, therefore, to establish the breadth of molecular signatures that may underlie placental vasculogenesis. Much information regarding this comes from transcriptomic studies or non-human model systems. As such, we have performed a proteome-wide examination of the molecular changes that occur during the in vivo maturation of human placental blood vessels.

Study design, size, duration: We performed proteome-wide assessments of chorionic plate arterial samples isolated from placental biopsies obtained following delivery (LREC 10/H0906/71) in normal pregnant women.

Participants/materials, setting, methods: Biopsies were obtained from women undergoing termination of pregnancy in 1st trimester (7-12 weeks, n = 9) and vaginal delivery at term of 3rd trimester (39-40 weeks, n = 7) of normal pregnancy. Arterial homogenates were trypsin-digested and the resultant peptides examined by LC-MS-based, label-free quantification using SWATH (Sciex TripleTOF 6600). Proteins were quantified on the basis of at least two, and at most five, unique peptides per protein.

Main results and the role of chance: Dynamic regulation of the human placental vascular proteome was evident with 1078 proteins differing between 1st and 3rd trimester (t-test with multiple comparison correction, FDR < 0.01). 710 proteins were up-regulated at term and pathway analysis revealed many linked to components of myofilament/cytoskeletal integrity and of metabolic-contraction coupling (e.g. glycolysis). 368 proteins were down-regulated with many key to gene splicing and protein synthesis including ribosomal proteins, spliceosome proteins and translation initiation/elongation factors. There was a trend towards increased expression of 50 proteins as gestation progressed between 6-12 weeks of 1st trimester pregnancy; 39 of which were differentially expressed in between 1st and 3rd trimesters and included proteins potentially involved in extracellular matrix deposition (LAMA4, LAMB2), dense body formation (PDLIM3, ACTN1) and myofilament integrity (MYH11, CNN1).

Limitations, reasons for caution: Future work that enabled collection of samples from broader gestational settings will be beneficial for further examining chronological trends.

Wider implications of the findings: Our study gives a comprehensive description of major proteome changes accompanying human placental vascular development. This provides a useful platform from which to (i) investigate how these may be altered in situations of placental-mediated disease and (ii) examine if other situations of human vasculogenesis/vascular remodelling involve similar proteomic changes.

Trial registration number: not applicable.

P-487 Assessment of endometrial receptivity by Endometrial Receptivity Array (ERA) in patients with adenomyosis and previous implantation failure

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Study question: Does adenomyosis alter endometrial receptivity(ER) leading to displaced window of implantation (WOI) in patients with previous implantation failure in IVF,as determined by ERA.

Summary answer: In patients of adenomyosis with implantation failure, Endometrial Receptivity (WOI) is significantly altered (47.22%) compared with controls 21.60% (patients with no adenomyosis).

What is known already: Adenomyosis is known to be associated with subfertility but there is paucity of studies on alteration of endometrial receptivity with adenomyosis.ERA is a customised microarray test evaluating the transcriptomics of WOI using 238 genes that are differentially expressed. ERA is accurate and reproducible compared to other markers of endometrial receptivity with sensitivity of 88.57 % and specificity of 99.7 %. Studies have shown WOI displacement in RIF patients leading to the concept of personalized embryo transfer(pET) as a therapeutic strategy but so far there are no studies evaluating endometrial receptivity in patients with adenomyosis with ERA.

Study design, size, duration: Retrospective study. 374 patients with one or more previous IVF failures who underwent ERA test between 2013-2016 were enrolled in the study. Patients were grouped according to the presence (Group 1, n = 36) or absence of adenomyosis (Group 2, n = 338 (controls). Alteration in endometrial receptivity determined by ERA, between the two groups was compared. Further analysis was done between patients with previous one IVF failure and RIF.

Participants/materials, setting, methods: All patients underwent ERA test in hormone replacement cycle (HRT). Estradiol valerate was started in a dose of 2 mg, this was increased to 6 mg or more till an appropriate endometrial thickness (≥7 mm) was achieved. Vaginal progesterone suppository 400 mg twice a day was then started for a period of 5 days (*P* + 5). Endometrial biopsy for ERA was taken after complete 5 days of progesterone and sent to igeonomix for analysis

Main results and the role of chance: Endometrial receptivity assay results were interpreted as Receptive(P+5) or Non receptive (displaced window of implantation, Pre/Post Receptive)

Basic demographic features between the two groups were similar. Mean age of patients with adenomyosis group was 34.5 years while in non adenomyosis it was 33.5 yrs (p-.166) WOI was displaced (Non Recptive ERA result) in 47.2% (17/36) patients with adenomyosis (group 1) compared to only 21.6% (73/338 pts) without adenomyosis (group 2) which was statistically significant (p < 0.001, CI-8.7%-42.5%) with risk ratio of displaced WOI in adenomyosis vs non adenomyosis to be 2:1 Subgroup Analysis was done to compare incidence of RIF between Adenomyosis group and Non Adenomyosis group. Incidence of RIF was 66.67% (24/36) in adenomyosis group while only 35% (118/338) had RIF in non adenomyosis group which was statistically significant (p<0.001, CI-15.5 to 47.9%).

Limitations, reasons for caution: retrospective nature of the study

Wider implications of the findings: Results indicate patients with adenomyosis have higher chance of having displaced WOI (altered endometrial receptivity) which could be responsible for implantation failure in IVF. ERA can be used electively in patients with adenomyosis for personalized embryo transfer (pET).

Trial registration number: not applicable.

P-488 Impact of airplane travel after an embryo transfer procedure in the reproductive outcome of oocyte donation cycles

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Study question: Does airplane travel have any impact after an embryo transfer procedure in the reproductive outcome of oocyte donation cycles?

Summary answer: Taking a plane within 72 hours after an embryo transfer appears to have a negative impact on the clinical pregnancy rate in oocyte donation cycles.

What is known already: Cross-border ART is rapidly increasing worldwide, especially within European countries. A recent Cochrane Systematic Review suggests that there is insufficient evidence to support any specific length of time for women to remain recumbent, if at all, following embryo transfer (ET). However, there is a paucity of information on the possible impact of airplane travel after an embryo transfer procedure.

Study design, size, duration: This is a prospective, observational, single center, cohort study. The hypothesis assumes a 15% decrease in reproductive outcomes in patients flying back to their countries using air transportation within 72 hours following embryo transfer. A sample size of 134 patients per group was estimated for the primary outcome: pregnancy / clinical pregnancy rates per ET. The study was performed during years 2015-2016.

Participants/materials, setting, methods: In total, 402 ET cycles were analyzed. The study group (A) (n = 134) included recipients using airplane transportation within 72 hours after ET; control group B (n = 134) included recipients flying back to their countries >72 hours post-ET and control group C (n = 134) included patients whose transportation post-ET did not involve air travel. Patients were 1:1 matched according to: type of ET (fresh/frozen) and

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number of transferred embryos (one/two). The procedures were done at the blastocyst stage.

Main results and the role of chance: Globally, pregnancy rates were similar among groups (57.5, 61.2 and 60.4; in groups A, B and C respectively). However, clinical pregnancy rates were significantly lower in the study group (37.6%) vs controls (52.2%, p = 0.01 and 54.1% p = 0.007; groups B and C, respectively). The clinical pregnancy rates remained similar between groups B and C. Logistic regression analysis showed that patients taking air transportation within 72 hours after ET were 1.83-1.99 times more likely to experience a decrease in clinical pregnancy outcome when compared either to patients taking a plane >72 hours (CI:1.12-3.00) and local recipients (CI:1.21-3.27) respectively.

Limitations, reasons for caution: The biological plausibility of a factor existing in the study group at the peri-implantatory period affecting only clinical pregnancy but not implantation remains controversial. Larger studies including a wider range of residual or other possible confounders (e.g. embryo quality; travel distance) are needed in order to confirm our data.

Wider implications of the findings: Cross-border reproductive treatments are steadily increasing especially within European countries. The information from this study may have important logistic implications for recipients and may also help clinicians to properly counsel patients about the impact of airplane travel after ET. Finally, our conclusions might boost controversies regarding post-ET interventions for ART.

Trial registration number: Not applicable.

P-489 Subsequent Pregnancy outcomes following Tubal Ectopic pregnancy Management- A 2 year follow up study in a University Hospital

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Study question: Does the method of management of tubal ectopic pregnancy affect the subsequent pregnancy outcome including the past obstetric history

Summary answer: High Intra-uterine pregnancy rates across the management groups.

No increase in repeat ectopics in non-surgical groups.

No co-relation between previous livebirth and subsequent pregnancy outcomes.What is known already: The incidence of ectopic pregnancy is 11/1000 pregnancies with tubal ectopic being the most common. A range of options are available for managing a tubal ectopic including expectant; methotrexate and surgical methods. NICE guidelines on ectopic pregnancy and miscarriage (CG154) however, does not recommend expectant management where as recently published RCOG/AEPU guideline on Diagnosis and Management of Ectopic Pregnancy (No. 21)considers this as an option.

Increased availability of EPAU units and improvement in scanning the diagnosis of ectopic pregnancies happens at an earlier stage of pregnancy. Surgical method is a favoured option followed by medical management.

Study design, size, duration: Retrospective follow up cohort study of subsequent pregnancy outcomes following tubal ectopic pregnancy. Data was obtained from electronic reporting systems and clinical documentation.

PUL was recorded as ectopic outcomes. Patients with a scan diagnosis of ectopic pregnancy between 01/01/2013 and 31/12/2-14 were included in the study

Subgroup analysis was undertaken and comparison made between previous livebirth and no previous pregnancy prior to the ectopic pregnancy.

Participants/materials, setting, methods: The Early pregnancy unit is a 5-day service and accepts referrals from GP and self referrals managed according to the trust guideline.

Expectant management is offered to asymptomatic women with hcg less than 2000iu/l and mass less than 3 cm.

Medical management is offered to women with hcg between 2000iu to 4000iu/l and mass less than 3 cm and who are compliant.

Any patient with live ectopic, free fluid, pain or hcg>4000iu/l are managed surgically.

Main results and the role of chance: Conservative group: 55/95 (57.9%) became pregnant. 8 had repeat ectopic, 7 miscarriages and 40 had a live birth.

The ectopic recurrence rate was 14.54% (8/55) with IUP rate of 85.45%.

Medical group: 50/89(56.17%) achieved pregnancy. 9 had repeat ectopic, 2 miscarriages and 39 had a live birth.

The ectopic recurrence rate was 18% (9/50) with IUP rate of 82%.

Surgical group: 48/101(47.52%) had a subsequent pregnancy. 9 had repeat ectopic, 3 miscarriages and 36 had a live birth.

The ectopic recurrence rate was 18.75% (9/48) with IUP rate of 81.25%.

The outcomes from our study are comparable between the management groups and to the published evidence (1, 2).

Subgroup analysis: There were 79 patients within the total of 126 patients (62.69%) in 2013 whose index pregnancy was an ectopic pregnancy. Their subsequent outcomes were:

8/79 had a repeat ectopic (10%)
32/79 patients did not achieve a subsequent pregnancy until 2016.(40.50%)
39/79 had a subsequent IUP(49.36%)
47 patients within the ectopic pregnancy had a previous live birth. however their outcomes were similar to the patients with an ectopic pregnancy as the index pregnancy. The repeat ectopic rate was 10.63% and the subsequent IUP rate was 46.80%

Limitations, reasons for caution: Retrospective study with loss to follow up.

Data is unavailable regarding patients who voluntarily chose not to have further pregnancies.

Subgroup analysis was performed for only one year.

Will need to extend the study period of subgroup analysis to be confident of the lack of correlation with past obstetric history.

Wider implications of the findings:

- High subsequent IUP rates across management groups with no increase in repeat ectopics and no influence from previous pregnancy outcomes.
- Reassuring that 40.3% had a live birth within 2-3 years
- The data will reassure women and will be useful for counselling women regarding their options of management and aid decision making

Trial registration number: Not applicable.

P-490 Influence of the ABO blood group of males and females on the results of in vitro fertilization cycles

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Study question: Does the AB0 group of males and females influence the results of in vitro fertilization (IVF/ICSI) cycles?

Summary answer: Cycles with embryo transfers in couples whose blood types are both 0, have a higher rate of clinical pregnancy, live births and implantation rate

What is known already: In the literature a possible association has been suggested between the antigens of the ABO groups of females and the results of the IVF/ICSI cycle, with contradictory results. Group A has been associated with an increased risk of early hyperstimulation and group 0 as a risk factor for low ovarian reserve. Likewise, the presence of A or B antigens in spermatozoa has been associated with an increased risk of infertility. We do not know of any studies concerning the results of IVF/ICSI cycles in relation to the ABO blood group of males and females.

Study design, size, duration: A retrospective descriptive observational study in which 2.414 IVF/ICSI cycles were carried out in couples with primary infertility between January 2007 and December 2015. Among them, embryo transfer was performed in 1.873 cycles. Two groups were defined according to the combination of the AB0 groups of males and females. Group O: Both women and men are blood group 0. Group No0: Women or men with blood group AB.

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Participants/materials, setting, methods: Of 2414 IVF/ICSI cycles fresh transfers were performed in 1.873. Of these, 286 (15.27%) belonged to group 0 and 192 (10.25%) to group No0. The main variables studied were: clinical pregnancy rate per transfer, biochemical pregnancy rate per transfer, newborn rate per transfer and implantation rates. Other variables studied were women's age, oocytes obtained, fertilized oocytes, embryos at +2 or +3, and number of embryos transferred.

Main results and the role of chance: The groups 0 and No0 were similar for possible confounding variables: age (34.37 vs 34.41 years, ns), oocytes recovered (10.9 vs 10.4 ns), fertilized oocytes (4.5 vs 4, 8; ns), total embryos (4.35 vs 4.68; ns) and number of embryos transferred (1.83 vs 1.82; ns).

The live birth rate (20.6% vs 13.7%, p = 0.05) and the clinical pregnancy rate (36.4% vs 27.5%, p = 0.046) was higher in group 0 than in the Group No0, although the biochemical pregnancy rate was similar in both groups (46.2% vs 39%, p = 0.287). These results could be explained by the fact that the implantation rate was significantly higher in group 0 (25.35% vs 17.8%, p < 0.001). Similarly, the newborn rate per embryo transferred was higher in group 0 (13.7% vs 8.5%, p <0.001).

Limitations, reasons for caution: This is a retrospective descriptive study with a limited sample size. The study has only focused on the combination of 0/0 couples with pairs having at least one individual AB blood group. In the future it will be necessary to extend this study to all possible combinations.

Wider implications of the findings: This study reveals that embryos from couples whose blood group is 0 have higher implantation rates than others whose progenitors are carriers of A or B alleles. This may be related to immune response due to fact the presence of antigen A or B results in lower embryo implantation rates.

Trial registration number: Non applicable.

P-491 Cutoff points of serum beta-hCG levels to predict pregnancy after different in vitro fertilization treatments

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Study question: Can different predictive values of β-hCG levels be stablished depending on the in vitro fertilization treatment and the pregnancy outcome?

Summary answer: Highly predictive beta-hCG levels cutoff points can be proposed for pregnancy outcomes.

What is known already: Pregnancies that occurred after in vitro fertilization treatment present an increased risk for an adverse outcome. Thus, there is a necessity for markers that precisely diagnose the pregnancy helping to determine appropriate follow-up guidelines during the first trimester. In this sense, levels of beta-hCG are highly predictive for pregnancy after in vitro fertilization treatment, albeit prognostic values and beta-hCG thresholds are not consistent. We hypothesized that some threshold levels of serum beta-hCG could be stablished for different conditions (fresh or frozen embryo transfer) with accurate predictive value of pregnancy outcomes (no pregnancy, single or twin, first trimester abortion).

Study design, size, duration: A single-centre retrospective cohort study was performed in the Assisted Reproduction Unit of Hospital Quironsalud Málaga (Spain). A total of 435 in vitro fertilization cycles in the time period from 2011 to 2016 were included. Only couples undergoing fresh or frozen-embryo transfer with positive of beta-hCG levels measured in our laboratory on day 14 after eggs collection (EC) and with available data on pregnancy outcome were included regardless of age.

Participants/materials, setting, methods: Serum beta-hCG concentration on day 14 after EC was measured using VIDAS® kit. Pregnancy was assessed by ultrasonic examinations at 6th and 12th week of pregnancy. Analyses were performed using the R software. Receiver operating characteristic (ROC) curve analyses were performed to determine optimal cutoffs values. Student's-t test was used to assess quantitative variables. Chi-squared test or Fisher exact test were used to compare differences in frequencies. Statistical significance was set at p <0.05.

Main results and the role of chance: A total of 435 Embryo Transfer (ET) with positive value of b-hCG were included. There were 288 (66%) ongoing pregnancies (12 weeks after ET), 83 (19%) biochemical pregnancies and 64 (15%) miscarriages (before 12 weeks). According to the ROC curve, a concentration of beta-hCG ≥ 117.5mIU/mL predicted ongoing pregnancy with a sensitivity of 80% and specificity of 70% (AUC = 0,82, p < 0.0001). A beta-hCG level ≥ 170.5 mIU/mL sensitivity 80% and specificity 62% (AUC = 0.78, p < 0.0001) predicted the probability of twin pregnancies. In order to study the treatment effect, cycles were grouped into fresh ET (n = 198) and frozen ET (n = 237), there were no differences in ongoing pregnancy rates 69% for fresh cycles and 64% for frozen cycles. A cuttof point of ≥123.5.00 mIU/mL with a sensitivity of 80% and specificity of 68% (AUC=0.82, p < 0.0001) and a value of ≥116.5 mIU/mL sensitivity or 82% and specificity or 70% (AUC=0.82, p < 0.0001) can predict ongoing pregnancy for fresh s and frozen cycles respectively. Finally, a cutoff level of beta-hCG < 66.5 mIU/mL can predict miscarriage outcome before 12 weeks with a sensitivity of 67% and specificity of 43% (AUC=0.77, p<0.0001).

Limitations, reasons for caution: Our study is limited by its retrospective design and the possible selection bias, due to the restriction of data available for b-hCG levels at day 14 after EC.

Wider implications of the findings: The results of this study provide data on the use of beta-hCG as reliable early indicator of pregnancy after in vitro fertilization treatment, which could be useful in counseling patients after ET, not only regarding conception, but also about the prognosis of such pregnancy.

Trial registration number: not applicable.

P-492 The natural history of pregnancy related enhanced myometrial vascularity

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Study question: What is the natural history of pregnancy related enhanced myometrial vascularity (EMV)?

Summary answer: We have shown that EMV after a pregnancy resolves in the majority of cases without the need for surgery, those surgically managed had minimal complications.

What is known already: Enhanced myometrial vascularity (EMV) is a term used to describe an abnormal connection between the arterial and venous system within the uterus leading to high velocity blood flow and risk of haemorrhage. Currently there is no clear guidance on the management of such cases. Recent evidence suggests that those with a high peak systolic velocity (PSV) may require intervention with embolization, whereas the lower velocity cases could be managed conservatively. Nevertheless, the lack of robust evidence surrounding this condition means the information given to women regarding outcome is often limited.

Study design, size, duration: A prospective audit of practice in a London teaching hospital between October 2015 to January 2017. The clinical course of twenty-six women was documented and followed up until discharge from the unit.

Participants/materials, setting, methods: Women attending the early pregnancy unit with abnormal bleeding and ultrasound features of EMV were included. The diagnostic criteria involved the subjective identification of an area in the myometrium with increased vascularity and a PSV greater than 20 cm/sec within the collection of vessels. The clinical presentation, bleeding score, PSV, intervention (if required) and time to resolution were all documented.

Main results and the role of chance: Twenty-six patients with a diagnosis of EMV were followed up. Mean age was 33 (range, 22-43), twelve women had at least one previous miscarriage. The initial presentation included incomplete miscarriage (n = 11), missed miscarriage (n = 4), retained products of conception following failed surgical management of miscarriage (n = 5), failed medical termination of pregnancy (n = 4), failed expectant management of miscarriage (n = 1) and postpartum retained products (n = 1). 24/26 presented with bleeding at the time of diagnosis, with 15/26 reporting heavy bleeding with clots (bleeding score 4). The outcome data for twenty-three patients is available and the mean number of days taken to resolve was 41 (range 2 to 140). The majority of patients (15/26) reported minimal bleeding whereas two patients had a blood loss of 500 ml. One patient required a blood transfusion. In patients surgically managed the average PSV associated with minimal blood loss was 34 cm/sec and 66 cm/sec when blood loss reached 500 ml blood loss. Those

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that underwent expectant management took 60 days (range 14 to 140) to resolve. 8/26 cases opted for surgery to remove retained products in the first instance, and resolution in this group took a mean of 23 days (range 2 to 59).

Limitations, reasons for caution: This is a small observational review of clinical cases. However, it is the largest prospective case series in the current literature. The subjective assessment involved in highlighting those with enhanced myometrial vascularity is a potential bias.

Wider implications of the findings: These findings suggest expectant management is successful but can take up to 60 days to resolve. EMV itself may be an iatrogenic diagnosis that is associated with the pathophysiology of miscarriage, and resolves with resolution of bleeding. However, more work is required to establish the underlying mechanism at play.

Trial registration number: not applicable.

P-493 Chronic endometritis therapy following autologous platelet-rich plasma treatment

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Study question: To explore whether autologous intrauterine platelet-rich plasma (PRP) treatment could eliminate uterine stroma inflammation, offering an alternative therapeutic approach to women suffering from chronic endometritis.

Summary answer: Our data demonstrated the successful effect of autologous intrauterine PRP treatment in women suffering from chronic endometritis with subsequent positive IVF outcomes and live births.

What is known already: Chronic endometritis, a persistent inflammatory condition of the inner lining of the uterine cavity, is caused by ascending infections from organisms normally present in the indigenous vaginal flora, sexually transmitted infections, tuberculosis and medical procedures that permit bacteria intrusion to the uterus through the cervix. Chronic endometritis therapy is based on oral antimicrobial treatment, however not all cases respond to the above medications, rendering necessary the need of alternative therapeutic approaches. The antimicrobial and anti-inflammatory effects of platelet-rich plasma have led clinicians to administer PRP as an efficient treatment for several medical issues, including osteoarthritis and tendinitis.

Study design, size, duration: Seven women, aged 42.17 ± 5.34 years, with unexplained infertility or ≥2 recurrent implantation failures referred to our IVF centre from January to July 2015. Chronic endometritis was diagnosed by hysteroscopy and microbiological analysis. Following the appropriate antibiotic therapy, 4 patients underwent in vitro fertilization (IVF) with embryo transfer (ET) resulting in only one clinical pregnancy which was spontaneously aborted. All women underwent a second hysteroscopy that certified the persistence of chronic endometritis.

Participants/materials, setting, methods: All women underwent autologous intrauterine PRP treatment. The autologous PRP was prepared using the RegenACR®-C Kit according to manufacturer's instructions. The endometrial infusion was performed using a non-surgical, transvaginal ultrasound-guided injection with an appropriate catheter. After the PRP treatment the patients underwent a third hysteroscopy and a simultaneous endometrial sampling at the luteal phase of the menstrual cycle in order to perform microbiological analysis. Within the next three months all women underwent new embryo transfers.

Main results and the role of chance: Following the autologous intrauterine PRP treatments, hysteroscopy and microbiological analyses revealed no remaining signs of chronic endometritis in 5 women (71.43%) of which four (80%) achieved 2 singleton and 2 twin clinical pregnancies resulting four live births and one ongoing twin pregnancy.

The delivery of high concentrations of platelet-derived factors to damaged tissues is considered to promote tissue healing and regeneration. These bioactive factors, including proteins with antibacterial and fungicidal effects, coagulation factors and membrane glycoproteins that control the synthesis of interleukins and chemokines, are capable of confronting the inflammation processes. Furthermore, dense granule-derived factors, such as adenoside diphosphate, adenoside triphosphate, serotonin, histamine, dopamine and calcium ions, can promote tissue homeostasis, whereas alpha granule-released growth factors play fundamental roles in wound healing and tissue regeneration. The intrauterine PRP treatment has modulated the local inflammatory response of mares with chronic degenerative endometritis, while it has promoted the endometrial growth in patients with thin endometrium improving the pregnancy outcome. In the light of the above analysis, we could assume that the autologous, intrauterine PRP treatment of our patients probably modulated the aberrant inflammatory processes of chronic endometritis and promoted the endometrial stroma healing.

Limitations, reasons for caution: Our investigation was based on seven women of advanced maternal age suffering from chronic endometritis. Further studies in larger population groups, analyzing chronic endometritis patients of all ages are needed to confirm our findings.

Wider implications of the findings: After the verification of our finding in larger patient groups, PRP could be used as a first line treatment for chronic endometritis patients, especially for those that do not correspond to conventional antibiotic schemes.

Trial registration number: none.

P-494 Impact of embryo group culture on early ICSI outcomes

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Study question: Could embryo development, be better if embryos were cultured in small groups rather than individually in a medium drop?

Summary answer: Group culture has positive impact on early embryo development. This could promote embryo quality, especially if transfer is planned in early cleavage stage.

What is known already: Embryo culture is one of the most delicate links in Intra-Cytoplasmic-Sperm-Injection(ICSI) cycles. As they grow in culture medium, embryos are being deprived of different growth factors naturally present in women genital tract. Some authors, suggested that grouping embryos for culture could enhance their devolpement by providing autocrine or paracrine factors.

Study design, size, duration: This prospective randomized study, conducted in a single center, took place between April 2015 and July 2016.

Patients who had less than 6 fertilized oocytes were included.

A total number of 291 ICSI cycles were randomly divided into two groups depending on the day of oocyte retrieval.

Participants/materials, setting, methods: -Groupe A (retrieval took place on: Monday, Wednesday or Friday) included 141 couples whom zygotes were cultured, in groups of 2 to 3 in the same drop.

-Group B (retrieval took place on: Tuesday, Thursday or Saturday) included 150 patients whom zygotes were cultured, in individual drops.

Embryo development was evaluated in both groups after culture with regards to: CRs, EQ, BRs and BURs.

Main results and the role of chance: Grouped embryos had significant higher CRs when compared to individual embryos (respectively 96.10% vs 91.80%; **p = 0.012**). Same results were noticed for embryo quality on day 2 or day 3, where group culture provided significant better embryo grades, and the mean number of good quality embryos was greater for embryos gathered in a same drop (EQ: respectively 40% vs 31.76%, p = 0.067; mean number of good quality per cycle: 1.29 vs 0.94, **p = 0.009**).

Although better BRs and BURs were obtained for group culture (respectively 20.95% vs 19.81% and 54.17% vs 44.74%), no statistical differences were found for both parameters.

This study suggests that group culture can promote embryo development at the early stages. Those findings could be beneficial for patients who had previous failure in ICSI cycles, or patients who had reduced number of oocyte retrieval.

Limitations, reasons for caution: Because of the difficulties found for morphological follow up of embryos, some could be reticent to adopt embryo group culture. Though, some devices have been invented to combine group culture with embryo identification.

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Wider implications of the findings: Identifying the different autocrine/paracrine factors could be intersting for producing culture medium that are well adapted for embryo culture.

Trial registration number: None.

P-495 Quantitative βHCG concentrations at defined outcome points are predictive of likelihood of ongoing pregnancy

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Study question: To determine the predictive power of serum βHCG concentrations regarding the likelihood of ongoing pregnancy.

Summary answer: Serum βHCG concentrations on trigger+17 have a strong predictive power for pregnancy outcome, leading to earlier identification of a non-continuing pregnancies and improved patient preparation.

What is known already: Serum βHCG levels 2 weeks after embryo transfer are a reliable marker to predict the pregnancy outcome in ART patients. Although the range is considerable, lower βHCG levels are associated with pregnancy failure. It is known that there is a predictive capacity related to quantitative βHCG concentration and the eventual outcome of the pregnancy. Following implantation, the βHCG concentrations increase rapidly in the mid-late luteal phase and the test signal strength obtained in both serum and urine can guide the likelihood of outcome.

Study design, size, duration: Serum βHCG concentrations on day-17 after trigger in 1846 consecutive ART cases where the pregnancy test was "positive" (βHCG > 10 IU/L) were examined. Categories of βHCG concentration were matched to the clinical outcomes at scan (7-8 weeks). The defined outcomes were: 'biochemical' pregnancy (bleeding and failure prior to scan), non-continuing pregnancy (no bleeding but empty sac/absent fetal heartbeat at scan) and clinical pregnancy (single positive heartbeat at scan). Twin pregnancies were excluded from analyses.

Participants/materials, setting, methods: The absolute βHCG concentrations were assessed for sample values from ART cycles resulting in "positive" pregnancy (defined as (βHCG > 10 IU/L), effected using one commercial platform (Beckman Coulter Access 2®) within an ISO accredited testing laboratory.

Main results and the role of chance: When the βHCG concentration on trigger+17 was <30 IU/L (n = 82), 93% resulted in a biochemical pregnancy, 4% in a non-continuing pregnancy and only 2% in an ongoing clinical pregnancy. With βHCG concentrations of 30-50 IU/L (n = 68), these figures were respectively 59%, 15% and 24%. If the βHCG concentration was 50-70 IU/L these figures were respectively 36%, 11% and 52%. With βHCG concentrations >70 IU/L these figures were 8%, 5% and 86% (P < 0.01, Anova), respectively.

Limitations, reasons for caution: Further analyses are required for natural and constructed frozen embryo transfer cycles, and also for days other than trigger+17.

Wider implications of the findings: Further investigations, based on βHCG concentrations wουλδ improve our understanding of implantation, perhaps related to embryo quality or endometrial receptivity. When lower concentrations are identified, patients can be advised of likely outcomes with more precision and can be psychologically better prepared for the greater likelihood of a non-continuing pregnancy.

Trial registration number: N/A.

P-496 Uterine contractility is the only ultrasound marker of implantation at the moment of the embryo transfer in substituted cycles

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Study question: Can we determine some ultrasound marker at the moment of embryo transfer (ET) at the blastocyst stage in substituted cycles?

Summary answer: High uterine contractility correlates with lower pregnancies rates. Endometrial flow velocimetry and endometrial volume were no correlated with outcomes of the treatment.

What is known already: It is known that uterine contractions (UC) at the time of the ET adversely affect IVF outcomes when fresh embryos are transferred during the stimulated cycle. Some studies have found that uterine contractility is much higher in stimulated cycles than in natural or substituted cycles. To date, influence of UC in substituted cycles has not been studied. Other functional ultrasound data like endometrial flow velocimetry and endometrial volume have been proposed as predictive markers of endometrial receptivity at the moment of the ET.

Study design, size, duration: We recovered prospectively ultrasound data of 89 egg recipients receiving embryos at the blastocyst stage in our center between February 2015 and September 2016. Patients included fulfilled medical and legal criteria for oocyte donation and followed the standard preparation protocol using transdermal estradiol and progesterone (subcutaneous or vaginal). All the embryo transfers were performed at the blastocyst stage after 5 days of progesterone.

Participants/materials, setting, methods: The same morning of the embryo transfer an ultrasound evaluation was performed.For the UC assessment 2D scan of the uterus was recorded while a 6 min video.The records were analysed at 20× regular speed using a VLC media player and examined. Endometrial volume and subendometrial and endometrial vascularization 3D parameters (VI,FI,VFI) were measured.The association between Pregnancy Test and uterine contractions and 3D parameters above mentioned was assessed by Student's T-test and Logistical Regression analysis.

Main results and the role of chance: Mean number of UC per minute was significantly lower in the group with positive pregnancy test, 1,22 UC/min in patients with positive test (PT) and 1,55 UC/min in patients with negative test (NT) p = 0,023 OR 2.806 (CI 95%: 1.11-7.08). Results of fluxometric index didn't achieve statistical differences in patients with PT or NT. (see table 1)

Table 1.

	Positive Test (Mean ± SD)	Negative Test (Mean ± SD)	p
Endometrial volume (ml)	4.89 ± 4.57	3.93 ± 2.65	0.386
Uterine contractions (per minute)	**1.22 ± 0.49**	**1.55 ± 0.67**	**0.023**
Endometrial-uterine volume	9.69 ± 12.93	8.68 ± 5.67	0.744
Endometrial Flow Index (FI)	23.45 ± 8.2	22.90 ± 8.30	0.803
Subendometrial Flow Index (FI)	23.65 ± 8.95	23.49 ± 8.33	0.947
Endometrial vascularisation-flow index (VFI)	0.41 ± 0.84	0.36 ± 0.42	0.82
Subendometrial vascularization-flow Index (VFI)	0.67 ± 1.43	0.56 ± 0.59	0.748
Endometrial Vascularization Index (VI)	1.26 ± 2.42	1.01 ± 1.14	0.659
Subendometrial Vascularization Index	2.00 ± 4,27	1.77 ± 2.04	0.823

Limitations, reasons for caution: Sample size was limited.

Wider implications of the findings: Evaluation of UC is useful for endometrial receptivity assessment in substituted cycles. Interventions to minimize

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uterine contractility could be helpful to improve clinical outcomes in egg donation or frozen embryo transfers.

Trial registration number: Data were extracted from clinical trial registered. EudraCT: 2014-004784-20; ClinicalTrials: NCT02363127.

P-497 Endometrial Immunophenotype Profiles in Patients with Adverse Reproductive Outcomes

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Study question: Reproductive immunology is an evolving, and controversial field. This study aims to assess the endometrial lymphocytes populations in patients with poor reproductive outcome, and correlate levels with aetiology.

Summary answer: Different endometrial immunophenotype profiles are seen in patients with recurrent miscarriage or repeated implantation failure. Further research is needed to determine potential clinical applications.

What is known already: Miscarriage and implantation failure are frequently occuring undesired outcomes of assisted reproduction.Causes include embryo genetic abnormalities or an abnormal endometrial environment. PGS has revolutionised embryo screening, but there is still debate and controversy about the gold standard test to assess endometrial function. An endometrial biopsy has the ability to simultaneously test both receptivity and hostility, however, a complete uterine immunophenotype is not currently well described, and there is no fully validated technique to completely assess the cellular populations.

Study design, size, duration: An observational study was design to assess endometrial populations in patients with poor reproductive outcomes 250 patients attending for endometrial scratch prior to commencing an ART cycle between Jan 2015 and October 2016 agreed to have their endometrial immunophenotype analysed in addition to the procedure Patients were stratified based on their reproductive outcome, and centile ranges were developed for the overall population and separate subgroups.

Participants/materials, setting, methods: An endometrial biopsy immunophenotype was developed to investigate by flow cytometry the various lymphocyte populations: peripheral blood type NK (pNK): [CD16+,CD56^{dim}], decidual/uterine tissue specific type (uNK):CD16-,CD56^{bright}], natural killer-Tcell type (NK-T):[CD16-,CD3+,CD56^{dim}], B-cells (CD5+,CD19+), Plasma cells(CD45+,CD138+), T cells (either CD4+,CD8+), T-regulatory cells (Treg, CD4+,CD127^{dim},CD25+), Th1-type Tcells (CD4+,CD183+) and Th2-type Tcells (CD4+,CD183-). Centile based reference ranges were established, and sub-groups determined for repeated implantation failure (RIF, >3 unsuccessful ETs) and recurrent miscarriage (RM, > 2 consecutive miscarriages).

Main results and the role of chance: Reference ranges for the lymphocyte populations were established in 250 endometrial biopsies. The overall population median levels were pNK 1.2%, uNK 41.3%, and NKT 2.7%. The patients were then characterised by reproductive history, and divided into subgroups for further analysis. Different lymphocyte levels were seen in these groups. In patients with recurrent miscarriage, mean levels were: pNK 6.4%, uNK 32.0% and NKT 6.6%.Patients with repeated implantation failure had different results: pNK 3.3%, uNK 43.1% and NKT 2.4%. interestingly, overall Treg and B Cell results were similar between groups, which is different to some existing data.T Cells were predominantly the Th1 subtype in all groups (50.8% Th1: 8.0% Th2 overall)

Cell Type	Overall	Recurrent Miscarriage	Implantation Failure
pNK	1.2	6.4	3.3
uNK	41.3	32.0	43.1
NKT	2.7	6.6	2.4
Treg	2.6	4.0	4.9

Limitations, reasons for caution: This study is limited by the lack of randomisation, as patients were recruited based on attendance for endometrial scratch. Analysis of the endometrium from fertile controls would further validate this data.

Wider implications of the findings: Patients with recurrent miscarriage demonstrated a different endometrial immunphenotype profile to the overall population. Further work is needed to assess if these differences are causative or incidental, and whether the application of personalised treatment regimes can improve outcomes

Trial registration number: Not applicable.

P-498 Recurrent pregnancy loss evaluation combined with 23-chromosome testing of miscarriage tissue explains the cause of pregnancy loss in over 90% of all miscarriages

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Study question: Will the addition of 23-chromosome microarray testing on miscarriage tissue combined with the standard evaluation for recurrent miscarriage explain most losses?

Summary answer: Over 90% of recurrent miscarriages can be explained when combining genetic testing on miscarriage tissue with the standard evaluation for recurrent miscarriage.

What is known already: Recurrent pregnancy loss (RPL) is estimated to occur in 2 to 4% of reproductive age couples. Various etiologies, either alone or in combination, have been demonstrated to contribute to RPL including parental chromosomal translocations, congenital and acquired uterine anomalies, endocrine imbalances, autoimmune factors including antiphospholipid syndrome, as well as infectious and thrombophilic causes. We sought to determine if the evaluation of miscarriage tissue at the time of the second or subsequent loss would explain the majority of "unexplained" losses. Also, we desired to determine if our proposed algorithm of evaluating all miscarriage tissue after the second loss was valid.

Study design, size, duration: Single-center, prospective study included patients seen in a private Recurrent Pregnancy Loss clinic from 2013 to 2016. All sixty-five women had two or more consecutive losses and had a complete evaluation for RPL as defined by the ASRM, and had miscarriage tissue evaluated by 23-chromosome micro array after their second or subsequent miscarriage.

Participants/materials, setting, methods: Frequencies of abnormal results for evidence-based diagnostic tests considered definite or probable causes of RPL (karyotyping for parental chromosomal abnormalities, and 23-chromosome microarray on miscarriage tissue; pelvic sonohysterography, hysterosalpingogram, or hysteroscopy for uterine anomalies; immunological tests for lupus anticoagulant, anticardiolipin and antiphosphatidyl serine antibodies, and beta2 glycoprotein antibodies; hormonal tests for TSH, mid-luteal progesterone, and hemoglobin A1c; cultures for microbial infection). We excluded cases where there was maternal cell contamination of the miscarriage tissue.

Main results and the role of chance: The majority of losses 42/65 (64.5%) were aneuploid. In those women with an aneuploid loss, the majority 29/42 (69%) had a normal RPL workup. However, 13/42 (31%) had an evidence-based test result that would have been overlooked if the recommended evaluation was eliminated. Conversely, 23/65 (34.5%) of women had a euploid loss. Of these, 21/23 (91%) had an abnormal finding on the evidence based testing for RPL. Overall, 29/65 (44.6%) had an aneuploid loss and a normal RPL workup; 13/65 (20%) had an aneuploid loss and an abnormal RPL workup; 21/65 (32.3%) had a euploid loss and an abnormal RPL workup; and only 2/65 (3.1%) had a euploid loss and a normal workup. Thus only 2 out of 65 women who were evaluated in this study had a completely unexplained pregnancy loss. All other losses were explained by aneuploidy in the miscarriage tissue or an abnormal finding on an evidence-based test for RPL or both. In this study we

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were able to explain 96.7% of pregnancy losses based on the testing algorithm. If evidence-based tests were not performed and only genetic testing of miscarriage tissue took place, 13/65 or 20% of proven causes for RPL would not have been identified

Limitations, reasons for caution: This is a single-center study on a small group of well-characterized women with RPL. The age of patients varied from 20 to 40 years. More aneuploid pregnancy losses would be expected in older women. A larger study is ongoing to confirm and expand these results.

Wider implications of the findings: Genetic evaluation on miscarriage tissue obtained at the time of the second and subsequent pregnancy losses should be recommended to all couples with two or more consecutive pregnancy losses. The combination of a genetic evaluation on miscarriage tissue with an evidence-based evaluation for RPL will explain over 90% of miscarriages.

Trial registration number: Not applicable.

POSTER VIEWING SESSION
MALE AND FEMALE FERTILITY PRESERVATION

P-499 The influence of letrozole on oocyte quality in breast cancer patients

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Study question: Does letrozole affect oocyte quality in breast cancer patients undergoing fertility preservation?

Summary answer: Oocytes retrieved from breast cancer patients stimulated with letrozole exhibit higher degree of central granulation, increased number of refractile bodies and increased perivitelline space.

What is known already: Controlled ovarian stimulation using letrozole and gonadotropins for oocyte cryopreservation is a well-established procedure aiming at fertility preservation in breast cancer patients with reproductive wishes after chemotherapy treatment. Letrozole has been demonstrated to be effective as ovulation inductor and was shown to have comparable results to conventional ovarian stimulation protocols.

Study design, size, duration: Observational retrospective study conducted from January 2015 to December 2016. We analysed 651 oocytes obtained from 69 breast cancer patients undergoing ovarian stimulation for fertility preservation. In addition, 698 oocytes from 92 aged-matched healthy women undergoing ovarian stimulation for ICSI indicated for male factor infertility were studied for comparison.

Participants/materials, setting, methods: The present study was performed at a public tertiary hospital. In breast cancer patients, ovarian stimulation was carried out with letrozole, recombinant FSH and a GnRH antagonist. oocytes obtained were analysed for the presence of refractile bodies, ooplasm colour and regularity, degree of central granulation, zona pellucida thickness, size of perivitelline space, presence of vacuoles in the ooplasm and oocyte retraction. The results obtained were compared with the age matched control group.

Main results and the role of chance: In comparison with oocytes obtained from healthy women undergoing conventional ovarian stimulation, the oocytes retrieved from breast cancer patients stimulated with letrozole and recombinant FSH exhibited a higher degree of central granulation ($p = 0.005$), an increase in the number of refractile bodies ($p = 0.003$) and increased size of perivitelline space ($p = 0.019$). No statistically significant differences were found between the groups with respect to ooplasm colour and regularity, presence of vacuoles in the ooplasm, zona pellucida thickness and oocyte retraction.

Limitations, reasons for caution: It is not clear whether we can extrapolate results obtained in healthy women to breast cancer patients. Furthermore, some patients of the study group started ovarian stimulation in the luteal phase, which could be a confounding factor.

Wider implications of the findings: Even if there is evidence in medical literature suggesting that the use of letrozole throughout ovarian stimulation is an efficient procedure, our findings alert to the possibility of a deleterious influence of letrozole on oocyte quality. Further studies are needed to clarify this fact.

Trial registration number: Not applicable.

P-500 Preservation of ovarian follicle by concomitant administration of GnRH agonist or GnRH antagonist during cyclophosphamide or paclitaxel chemotherapy in mice

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Study question: There was evaluated whether GnRH-induced ovarian function suppressing preserves primordial follicles in the administration of cyclophosphamide or paclitaxel.

Summary answer: GnRH analogues block destruction of primordial follicles caused by cyclophosphamide and paclitaxel in mouse ovaries.

What is known already: Although chemotherapy improves the survival rate of women in reproductive age who have malignant cancers, it has been reported that cytotoxic chemotherapeutic agents may induce premature ovarian failure and infertility among patients of reproductive age.

Study design, size, duration: The degree of destruction of primordial follicles was analyzed following the administration of cyclophosphamide or paclitaxel respectively in mouse ovaies. In addition, there was evaluated whether GnRH-induced ovarian function suppressing preserves primordial follicles in the administration of cyclophosphamide or paclitaxel.

Participants/materials, setting, methods: Saline or cyclophosphamide (50, 75 mg/kg), paclitaxel(12.5, 19 mg/kg)were intraperitoneally injected into seven-week old female ICR mice. GnRHa (Leuplin®) and GnRH antagonist (Cetrotide®) were injected into mice, and administered with 50 mg/kg and 75 mg/kg of cyclophosphamide following 9 days treatment with GnRH analogues. H&E staining and TUNEL assay was performed. Apoptotic index was defined as the proportion of stained nuclei to total of 400 granulosa cells that were counted.

Main results and the role of chance: Cyclophosphamide and paclitaxel cause destruction of primordial follicles in mouse ovaries. The number of primordial follicles decresed in the group of high dose compared to the group of low dose treated with cyclophosphamide or paclitaxel(13.3 ± 2.1 vs 16.0 ± 2.4, 11.5 ± 2.9 vs 8.4 ± 2.6). Treatment with GnRHa and GnRH antagonist significantly increased the number of primordial follicles at a low concentration of cytotoxic agents ($p < 0.05$), whereas the number of primoridal follicle increased only in GnRHa I antagonist treated group at a high concentration of cyclophosphamide or paclitaxel($p = 0.030$, $p = 0.043$). Decreased apoptotic indices were shown in all types of GnRH analogues co-treated groups ($p < 0.05$). For the comparisons of GnRHa vs. GnRH antagonist GnRHa was relatively effective in their follicle preserving effects.

Limitations, reasons for caution: By assessing the number of primordial follicles in each experiment, we compared the protective effects of specific GnRH analogues, but we could not find the significant differences among each other. The comparisons of apoptotic index showed the significant differences among each type of GnRH's.

Wider implications of the findings: GnRH analogues block destruction of primordial follicles caused by cyclophosphamide and paclitaxel in mouse ovaries, suggesting that GnRH analogues may be applicable to increase fertility opportunity in malignant cancer patients of reproductive age planning future pregnancies.

Trial registration number: N-R

P-501 Letrozole supplementation during ovarian stimulation alters oocyte maturation rates in breast cancer candidates for fertility preservation

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Study question: To investigate whether controlled ovarian stimulation with letrozole supplementation (COSTLES) modifies ovarian response and follicle responsiveness to exogenous FSH in breast cancer (BC) patients?

Summary answer: Despite similar response to exogenous FSH, BC patients having undergone COSTLES show reduced oocyte maturation rates in comparison with those having received standard stimulation regimen.

What is known already: Oocyte and/or embryo vitrification after controlled ovarian stimulation (COS) represents the most established and efficient method of fertility preservation (FP) before cancer treatment. However, traditional COS regimens are associated with supraphysiologic serum estradiol and are therefore not recommended in estrogen-sensitive diseases such as BC. To protect the patients from the potential deleterious effects of elevated estrogen levels during ovarian stimulation for FP, protocols using aromatase inhibitors (letrozole) were developed. However, COSTLES outcomes and follicle responsiveness to exogenous FSH in comparison with conventional protocol have been poorly evaluated in BC patients candidates for FP.

Study design, size, duration: This is the first investigation comparing COSTLES and standard ovarian stimulation in BC patients. From July 2013 to December 2016, we prospectively studied 133 breast cancer patients, 25 to 40 years of age, candidates for FP using oocyte and/or embryo vitrification following COS. Of these, 68 patients underwent COSTLES while 65 had standard GnRH antagonist protocol.

Participants/materials, setting, methods: All women had 2 ovaries, no history of chemotherapy, BMI < 30 Kg/m^2 and a breast tumor that was surgically removed. Measurement of serum anti-Müllerian (AMH) levels and antral follicle count (AFC) were systematically performed before exogenous FSH administration (d0). Follicle responsiveness to FSH was assessed by the Follicular Output Rate (FORT) calculated from the ratio between the preovulatory follicle count (PFC, 16-20 mm) on the day of oocyte triggering (dOT) and the d0 AFC.

Main results and the role of chance: Women in the COSTLES and control groups were comparable in terms of age 33.4 ± 4.7 vs. 33.8 ± 3.5 years, respectively), BMI (21.6 ± 3.1 vs. 22.6 ± 2.4 Kg/m^2) and ovarian reserve tests (AMH: 2.2 ± 1.8 vs. 2.7 ± 2.3 ng/mL; AFC: 18.5 ± 10.5 vs. 17.9 ± 10.7 follicles). After comparable starting doses and total amount of exogenous FSH (3046 ± 1332 vs. 2996 ± 1344IU, respectively), the FORT index did not differ significantly between groups (33.8 ± 24.6 vs. 37.2 ± 19.8%, respectively), leading to a similar number of oocyte recovered (12.3 ± 8.3 vs. 12.5 ± 8.7 oocytes, respectively). However, oocyte maturation rates were significantly lower in COSTLES compared to standard protocol (67.3 ± 19.7 vs. 76.3 ± 20.1%, $p<0.02$). As a result, the number of metaphase 2 oocyte vitrified was lower in patients having received letrozole supplementation (8.1 ± 2.6 vs. 9.6 ± 3.4 oocytes, $p<0.05$ respectively).

Limitations, reasons for caution: Notwithstanding the limited sample size, our findings provide new data on COSTLES in BC patients. Whether the present results will impact the overall success rates of the FP procedures after oocyte thawing is still unclear. A particular vigilance on this issue will be required.

Wider implications of the findings: At present, there is no evidence that standard COS promotes the proliferation of residual tumoral cells following breast surgery. If not, the confirmation of our results would lead reconsidering the real interest of COSTLES before adjuvant chemotherapy.

Trial registration number: Not applicable.

P-502 Six children born after 15 ovarian tissue transplantations: the French protocol for the development of ovarian tissue autograft in order to restore ovarian function (DATOR)

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Study question: To assess the efficiency of cryopreserved ovarian tissue autograft in terms of reproductive outcome in women who underwent gonadotoxic treatments.

Summary answer: Between December 2007 and September 2016, ovarian tissue transplantation was performed in 15 patients, and 5 women gave birth to 6 children.

What is known already: Ovarian cryopreservation is one of the available options for preserving fertility prior to potentially sterilizing treatments; it's even the only possible option in prepubertal girls or when there is an urgent need to start the treatment. The prospect of restoring fertility by retransplantation is becoming increasingly real: in France, the first live birth after orthotopic ovarian autograft, was obtained by Roux et al. in 2009; the exact number of live births is unknown but according to a recent update from Jensen et al. (2016), worldwide approximately 95 children have been born or will be born in the near future.

Study design, size, duration: This is a prospective multicentric cohort study of 15 women who had frozen-thawed ovarian tissue transplanted since 2007. None of the patients were lost to follow-up.

Participants/materials, setting, methods: Patients aged from 18 to 43 years, who have cryopreserved their ovarian tissue, cured from their initial disease, with child desire but premature ovarian failure, were included. A two-step orthotopic autotransplantation was performed, and a monthly follow-up was carried out during a period of one year. The data collected concerned the restoration of ovarian function and live births, the need for assisted reproductive techniques, the pregnancy follow-up and the perinatal outcome of the children.

Main results and the role of chance: Fifteen patients benefited from cryopreserved ovarian tissue autograft.

The indications for ovarian cortex autoconservation were Hodgkin's lymphoma (9 patients), non-Hodgkin's lymphoma (2), Ewing's sarcoma (1), systemic mastocytosis (1), sickle cell disease (1) and periarteritis nodosa (1).

The patient mean age (SD) at the time of grafting was 34 (5) years.

Seven patients succeeded in becoming pregnant. Five women delivered a child/children (6 children in total); this included one patient who gave birth to 2 children two years apart. A first trimester miscarriage occurred in one case. Two patients in 5 conceived with the assistance of *in vitro* fertilization.

Among the 5 patients who conceived, 3 had received chemotherapy before ovarian tissue cryopreservation, and the longest time of tissue harvesting was 8 years and 4 months.

All the babies born were healthy; one congenital diaphragmatic hernia was diagnosed and successfully cured. No relapse of the initial disease occurred.

Limitations, reasons for caution: Autograft of cryopreserved ovarian tissue must be performed with caution in women suffering from malignancies that may metastasize to the ovaries. Ischemic tissue damages occurring after retransplantation are currently another major problem. The full lifespan of grafts is still being evaluated.

Wider implications of the findings: In our series, the success rate was 33.3% with a majority of natural conceptions. These results constitute a good argument in favor of cryopreservation of ovarian tissue and its subsequent reuse by orthotopic autograft to restore ovarian function and fertility in women who experience ovarian failure due to gonadotoxic treatments.

Trial registration number: The study is registered in ClinicalTrials.gov under the number NCT02846064.

P-503 Residual ethylene glycol (eg) and dimethyl sulfoxide (dmso) concentration in human ovarian tissue during warming steps of vitrification method and slow freezing method

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Study question: Is there concern whether all the cryoprotectants, which may be toxic in the human body, can be washed out at the time of transplantation in vitrification and slow freezing methods?

Summary answer: Around 10 mg/g DMSO and 10 mg/g EG in vitrification method whereas 0 mg/g in slow freezing method remained in ovarian tissue after warming just before transplantation.

What is known already: There have been 60 births after transplantation of cryopreserved ovarian tissue (Donnnez J and Dolmans MM, 2015): 58 using the slow freezing method, and two using the vitrification method. In the warming protocols of vitrification method, the concentration of cryoprotectants is four times higher and warming time is four times shorter than in slow freezing. We have discussed time, cost, histology of follicles and stroma, viability test, and follicular development in both methods; however, we did not examine the residual amount of cryoprotectants in human ovarian tissue after warming just before transplantation and did not discuss about safety of patients.

Study design, size, duration: This study was approved by the ethics committee of our clinics. Four patients who cryopreserved by vitrification method had Gender Identity Disorder and one who cryopreserved by slow freezing method had uterine cancer. We measured the amount of cryoprotectants before warming (I), after TS (II), after DS (III), and after WS2 (IV) in the vitrification method, and before warming (I), after TS3 (II), and after WS2 (III) in the slow freezing method.

Participants/materials, setting, methods: Four ovarian tissue (10x10×1 mm) were cryopreserved by the vitrification method using 6.4 mol/l EG and DMSO (Kagawa et. al., 2009) and one ovarian tissue (8x4×1 mm) was frozen by the slow freezing method using 1.5 mol/l DMSO (Isachenko V., et al., 2012). The concentrations of DMSO and EG were analyzed by Shimadzu Techno-Research Inc., Kyoto, Japan. Concentrations in the ovary tissues were quantified using GCMS-QP2010Ultra (Shimadzu) with InertCap Pure-WAX column (0.25 mm×30 m, 0.50μm thickness; GL Science).

Main results and the role of chance: The maximum amount of DMSO and EG in vitrification method vs. slow freezing method was 130.0 ± 8.2 mg/g and 112.5 ± 15.0 mg/g vs. 70 mg/g and < 0.050 mg/g before warming,respectively. In the slow freezing method, most of the cryoprotectants were washed out and the amount reached zero after thawing. In the vitrification method, there was a 30 to 50% reduction in after each step in warming, however, never reaching undetectable limits. We found around 10 mg/g DMSO and 10 mg/g EG in ovarian tissue after warming just before transplantation. The amount of cryoprotectant of ovarian tissue when cryoprotectants were not used was <0.050 mg/g. We believe that cryoprtectants should be completely washed out when we transplant ovarian tissue into the human body after warming. Safety is crucial in clinical medicine.

Limitations, reasons for caution: We found most of the residual cryoprotectants in ovarian tissue were washed out and approached the detection limit in the case of slow freezing method. However, in the vitrification method by Cryotissue kit, around 10 mg/g DMSO and 10 mg/g EG remained in ovarian tissue after warming just before transplantation.

Wider implications of the findings: We do not know the risk of toxicity of the cryoprotectants in detail, however, we are afraid of toxicity for mother and child. So, we use slow freezing method in our human ovariantissue preservation enterprise 'HOPE" for fertility preservation.Further research for improvement of cryopreservation method are needed.

Trial registration number: None.

P-504 Comparison of the rate of intact embryos after thawing and clinical outcomes in the human embryo vitrification using Cryo-loop and Hemi-straw

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Study question: Can Cryo-loop increase the rate of intact embryos after thawing and clinical outcomes by complementing the weakness of Hemi-straw?

Summary answer: Cryo-loop with high thermal conductivity significantly improved the rate of intact embryos after thawing and clinical outcomes compared to Hemi-straw.

What is known already: To increase the success rate of vitrification technology, a carrier with high thermal conductivity that can minimize vitrification solution volumes is essential. With the Hemi-straw, it is difficult to adjust vitrification solution volumes, and thermal conductivity is affected by the external environment and the skill level of embryologists. On the other hand, Cryo-loop has ultra-rapid cooling rate (>20,000/min) even when it is directly exposed to LN2, and the volumes can be adjusted to the minimum volume (<1ul). In our study, we aimed to determine whether Cryo-loop can improve the survival rate and clinical outcomes of embryos compared to the Hemi-straw.

Study design, size, duration: The non-donor in the transfer cycles of vitrified-thawed embryos performed from January 2015 through January 2016 (n = 208) was included in this study. A total of 101 patients underwent the procedure using Cryo-loop while 107 patients underwent the procedure using Hemi-straw.

Participants/materials, setting, methods: After day 3 embryos transfer, the surplus embryos were randomly vitrified with Cryo-loop or Hemi-straw. After thawing, the embryos were cultured in vitro for 24 hours to select the embryos showing subsequent development for transfer. The survival rate of the vitrified-thawed embryos was assessed with the proportion of the embryos that has intact blastomeres without any damage. After transfer, the pregnancy and implantation rates were compared between the Cryo-loop and Hemi-straw groups.

Main results and the role of chance: Patient characteristics were similar in the two groups. In each group, age (Cryo-loop: 35.7 ± 3.9 vs. Hemi-straw: 35.5 ± 3.4 P = 0.38), endometrial thickness (9.6 ± 2.1 vs.9.7 ± 1.7 mm $p = 0.40$) and number of embryo transferred (1.9 ± 0.4 vs. 1.9 ± 0.3 $p = 0.17$) did not show significant differences. However, the number of thawed embryos (3.0 ± 1.3 vs. 5.1 ± 2.1 $p < 0.001$) revealed a significant difference. In the Cryo-loop group, the rate of intact embryos after thawing (77.9% vs. 62.4% $p<0.001$), the rate of subsequent development (60.5% vs. 31.6% $p<0.036$), pregnancy rate (56.4% vs. 42.1% $p = 0.038$) and ongoing pregnancy rate (50.0% vs. 36.4% $p = 0.041$) were all significantly higher than those in the Hemi-straw group.

Limitations, reasons for caution: Since our clinic mainly performs day 3 cryopreservation, they were only a small number of day 5 cryopreservation cases, and the results have not been compared.

Wider implications of the findings: The Cryo-loop group showed significantly higher rates of survival, pregnancy and subsequent development than that of Hemi-straw group. Cryo-loop can freeze a small number of embryos, and the survival rate of embryos is higher. Therefore, Cryo-loop can reduce unnecessary freezing of embryos and provide more chances of conception.

Trial registration number: none.

P-505 Freezing and thawing of ovarian cortical patches induces differential profiles of microRNAs in the holding media to non frozen patches

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Study question: Does the process of cryopreservation alter the expression profile of microRNAs in the holding media for ovarian cortical patches?

Summary answer: Cryopreservation alters the expression profile of microRNAs in the holding media for ovarian cortical patches

What is known already: Ovarian cortical patch (CP) cryopreservation has been suggested as a novel approach to preserving fertility in women suffering from cancers. However the effects of freezing-thawing are not fully understood and ways to assess the tissue's potential to perform normally after thawing, are an important research goal. MicroRNA expression pattern in holding media may be useful in determining variation in tissue suitability which may obviate the need for further analysis of tissue itself. Specific miRNAs have already been identified, distinguishing disease states from normal tissue. Recently miRNAs, have been recognised in human ovarian follicles thought to play fundamental roles in folliculogenesis.

Study design, size, duration: CPs were collected from ovine ovaries. Some (n = 20) were fixed and stained, some (n = 20) were cultured and some

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(n = 20) were freeze-thawed, cultured in rotatory cell culture system (RCCS) for 5 days and then fixed. Each day, holding media from RCCS were collected for MiR-24, 193b and 320a determination and compared between non-frozen and frozen cultured groups. Follicles were counted on fixed patches and compared between three groups (fresh, non-frozen, cultured and frozen-thawed, cultured).

Participants/materials, setting, methods: After collection, some CPs were fixed in 4% paraformaldehyde (PFA) and stained with haematoxylin and eosin (H&E). Some were cultured and some were freeze-thawed, cultured in RCCS for 5 days followed by fixing and staining. Each day, 6mls of holding media were collected and replaced by fresh medium. MicroRNAs were determined in holding media using QIAGEN kits and were evaluated using real time polymerase chain reaction (PCR). Follicles were counted in all fixed, stained patches.

Main results and the role of chance: MiR-24 was upregulated on day 1, decreased until day 3, then remain almost constant until day 5 for both frozen and non-frozen cultured patches. MiR-193b decreased till day 3, then increased from day 4 till day 5 for non-frozen cultured patches. In frozen-thawed, cultured patches, MiR-193b was detected on day 1 only. MiR-320 A decreased from Day 1 till day 3, increased sharply on day 4, again decreased on day 5 in non-frozen cultured patches. In frozen-thawed, cultured patches, it constantly decreased until Day 5. Significant difference was noted between two groups for MiR-24, 193b and 320 A ($P < 0.05$). Total number of follicles counted in fresh, non-frozen cultured and frozen-thawed, cultured patches were 884 (primordial 68%, transitory 20.39%, primary 6%,secondary 2.9% antral 20.17%), 779 (primordial 48%, transitory 33.78%, primary 6%,secondary 7.13% antral 4.77%)and 526 (primordial 37%, transitory 37.32%, primary 13%,secondary 7.8% antral 5%) respectively. Significant differences were noted in case of primordial, transitory, secondary and antral follicles ($P > 0.05$) for non-frozen cultured patches and in case of primordial follicles ($P > 0.05$) for frozen-thawed, cultured patches when compared with fresh patches.

Limitations, reasons for caution: This study has been limited to comparing the effects of treatment over time due to difficulties of normalising against the differences in size of patches and variability of follicle numbers and stages of development. Use of ovine ovaries resulting in species-specific differences, causing inability to directly compare to humans.

Wider implications of the findings: Down-regulated MiR-193b together with up-regulated MiR-24 could have a synergistic role in cell apoptosis /damage. Thus, there appears to be an interesting interplay between these miRNAs and cell injury/damage which could further explored as novel markers of cell damage following cryopreservation.

Trial registration number: N/A.

P-506 SLUSH VS LIQUID NITROGEN VITRIFICATION OF HUMAN OVARIAN TISSUE PRESERVES GENE EXPRESSION AND IMPROVES POST WARMING IN VITRO CULTURE

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Study question: Does slush (SN) vs liquid nitrogen (LN) vitrification unalter stress/toxicity gene expression, and improve follicle health and progression during human ovarian tissue in vitro culture?

Summary answer: Data demonstrated a substantially lower up-regulation of stress-toxicity genes and better follicle survival, quality, and progression after SN compared to LN vitrification (LNV, SNV).

What is known already: Although vitrification emerged as an optimal method for oocyte cryopreservation, it was only recently applied to ovarian tissue and two live births have been reported. We previously demonstrated that histology, ultrastructure, and viability of human ovarian follicles and stromal cells were better preserved after SNV compared to LNV. However, damage to the follicle developmental potential by toxicity of cryoprotectants and the stress of vitrification/warming procedure could affect gene expression and recovery of ovarian function during reimplantation or long term in vitro culture.

Study design, size, duration: Human ovarian cortical strips 5x2x1 mm were vitrified/warmed (V/W) in SN or LN as previously reported. Fresh, SNV and LNV strips 1×1×0.5 mm per patient (n = 6) were cultured in oxygen-permeable dishes 1-9 days (D1, D9). Single-strand cDNA of fresh, and V/W tissues, were processed for analysis of 84 genes involved in stress and toxicity pathways and results were validated through qRT-PCR. Follicle quality, progression and viability were assessed through histology and live/dead assay under confocal microscopy.

Participants/materials, setting, methods: Ovarian biopsies, from six consenting patients (aged 20-39) were dissected using a tissue chopper, for V/W and culture in alpha-MEM plus supplements. Samples were fixed for histology or labelled with live-dead and Hoechst 33342 for confocal analysis or processed for analysis of gene expression. mRNAs, extracted through NucleoSpin RNA kit, were retro-transcripted by RT^2 First Strand kit, and analysed by Stress and Toxicity RT^2 Profiler^{TM} PCR Array The data of interest were validated by qRT-PCR

Main results and the role of chance: The impact of vitrification on ovarian tissue function was evaluated through comparison of gene expression in V/W samples cultured 24 h vs fresh tissue. RT-PCR arrays demonstrated a general up-regulation of genes (up-regulated more than 3-fold: threshold-value) in LN (DDB2, RAD51, IL6, IL1A, IFNG, TNF, CD40LG, CFTR, CRP, AQP2, CA9, MMP9, EPO) and not in SN samples. qRT-PCR confirmed high upregulation of 13 genes after LNV and showed a markedly slighter up-regulation of only 5 genes after SNV (LN vs SN upregulation: CD40LG, 29.1 vs 4.3; CFTR, 13.1 vs 4.11; CA9, 35.6 vs 6.5; AQP2, 9.7 vs 3.6; EPO, 23 vs 7.8).

Overall 3840 follicles were analysed. In fresh tissues (D9 vs D0), follicle quality (grade 1: 41 vs 54.2%) and viability (70.8 vs 92.7%) decreased and progression to the secondary stage increased (26.8 vs 2.6%). In V/W tissues (D9), SNV better preserved follicle quality (SNV vs LNV: 55 vs 16.4, $P < 0.001$) and viability (SNV vs LNV: 55.5 vs 34.8, $P < 0.01$). Interestingly, vitrification improved follicle progression compared to fresh samples at D9 (SNV and LNV vs Fresh: 51.4, 42.6 vs 26.8, $P < 0.001$)

Limitations, reasons for caution: Data has been collected on a limited number of patients and should be clinically validated.

Wider implications of the findings: Data confirmed that SNV better preserves ovarian follicles. SNV could be applied for fertility preservation of oncological patients. The culture system adopted promotes the progression of a high number of follicles to the secondary stage and could be used for in vitro folliculogenesis.

Trial registration number: none.

P-507 Ovarian stimulation and oocyte cryopreservation in women with Turner syndrome: Is it safe and effective?

Abstract withdrawn by the author

P-508 Selection of the most suitable ovarian cortex fragments for autotransplantation by non-invasive imaging using Reflectance Confocal Microscopy (RCM)

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Study question: Is it possible to use RCM for the selection of ovarian cortex fragments that contain the highest number of primordial follicles?

Summary answer: Using RCM we were able to visualize follicles in living ovarian cortex fragments of bovine and human origin without compromising the vitality of the tissue.

What is known already: There is an extreme variation in the distribution of primordial follicles across the surface of the ovary. As a result individual ovarian cortex fragments cryopreserved for fertility preservation purposes may vary

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considerably with respect to the number of primordial follicles they contain. Assessing the number of follicles in a cortex fragment by standard histology renders the fragment unsuitable for subsequent auto transplantation purposes. This emphasizes the need for a non-invasive technique to select the most promising ovarian cortex fragments before auto transplantation.

Study design, size, duration: Cortex fragments of bovine and human ovaries were prepared using standard techniques. The tissue fragments were cryopreserved using DMSO. After thawing the tissue fragments were subjected to imaging using RCM. To validate the RCM imaging technique, the RCM images were compared to the haematoxylin eosin stained sections of the same tissue fragments. The possible adverse effects of RCM were determined by assessing the vitality of the tissue and follicles before and after RCM imaging.

Participants/materials, setting, methods: Experiments were performed with ovarian tissue from patients that had donated their ovarian tissue after informed consent. Bovine tissue was derived from an abattoir. For *en face* RCM imaging a VivaScope 1500 was used. The vitality of the ovarian tissue was determined by a glucose-uptake assay and the survival of ovarian follicles was assessed by staining with Neutral Red.

Main results and the role of chance: The results show that with the use of RCM it is possible to image follicles in living ovarian tissue to a depth of maximal 200 μM. By comparing RCM images and haematoxylin eosin stained sections of the same ovarian tissue fragment we show that RCM is capable of visualizing all stages of follicle development, including primordial follicles. However, follicle visibility was generally better in bovine tissue compared to human tissue. Analysis of tissue and follicle viability before and after RCM indicate that the imaging procedure has no detectable negative effects on the ovarian cortex fragments.

Limitations, reasons for caution: The maximum depth at which follicles could be visualized with RCM is 200 μM, leaving some follicles undetected. RCM had no effect on viability of the tissue. However, it is unknown whether RCM has negative effects on follicular development after auto transplantation.

Wider implications of the findings: Our results indicate that it is possible to assess the number of follicles by RCM in cortex fragments intended for fertility preservation. By using this non-invasive imaging technique, fragments can be identified with the highest number of follicles. These fragments are the best candidates for reproductive success after autotransplantation.

Trial registration number: not applicable.

P-509 Effect of human recombinant vascular endothelial growth factor (VEGF-165) on testicular tissue xenografts from prepubertal boys

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Study question: Does human recombinant VEGF-165 have an impact on the integrity of seminiferous tubules, germ cell survival and differentiation in human prepubertal testicular tissue xenografts?

Summary answer: VEGF improves the integrity of seminiferous tubules through an enhanced vasculature after long-term xenografting. It has no significant effect on germ cell survival and differentiation.

What is known already: Testicular tissue autotransplantation has been proposed as potential fertility preservation strategy for prepubertal boys undergoing gonadotoxic treatments. Before any clinical application, further optimization of the technique is required. So far, xenografting of human prepubertal tissue resulted in limited spermatogonial survival and lack of complete spermatogenesis. Hypoxia, as a result of insufficient blood supply, could be the aetiology for the spermatogonial loss observed in testicular grafts. VEGF stimulates the formation of new blood vessels and promotes neovascularisation. Studies in other species indicate a role for VEGF on the improvement of germ cell survival and proliferation in immature testicular grafts.

Study design, size, duration: Frozen-thawed testicular tissue fragments from three prepubertal boys were cultured for five days with VEGF-165, followed by xenografting into the testicular parenchyma of 48 Swiss Nu/Nu mice. Retrieval of the grafts and evaluations were performed after a short (four-month) and a longer (nine-month) time-period.

Participants/materials, setting, methods: Histological assessment and immunohistochemical analysis for specific markers, including melanoma-associated antigen 4 and von Willebrand factor, was performed and seminiferous tubule integrity, number of spermatogonia and vascularisation were assessed. Most advance germ cell type present was identified morphologically and meiotic activity was determined using the meiotic marker BOLL. Maturation and functionality of the niche were evaluated by the expression of anti-Müllerian hormone (AMH), androgen receptor and inhibin a, by Sertoli cells.

Main results and the role of chance: Treatment of testicular tissue fragments from prepubertal boys prior to xenografting with 100 ng/ml of human recombinant VEGF-165 resulted in higher percentage of intact tubules ($P = 0.006$) after nine months. The result was accompanied by a positive long-term effect of VEGF on the percentage of vascular surface ($P = 0.020$) and vessel density ($P = 0.001$) of the grafts. The number of spermatogonia did not differ significantly between the treated and untreated group. While four months after grafting, spermatogonia were the most advanced germ cells in the grafts, pachytene spermatocytes were present after nine months irrespective the treatment. Sertoli cells were still expressing AMH after nine months, but a lower level of expression was noticed in tubules containing spermatogonia and spermatocytes indicating higher maturation level.

Limitations, reasons for caution: Scarcity of human immature testicular tissue for research purposes does not permit a more comprehensive study. Additionally, the different age of the donors and the treatment that two of them received before the biopsy may have influenced the outcome of grafting experiments.

Wider implications of the findings: The present study demonstrates for the first time the long-term effectiveness of VEGF on the tubular integrity of human prepubertal testicular tissue xenografts by enhancing the vascularisation of the grafts. Although further studies are required, VEGF may be considered as a potential candidate for the improvement of testicular tissue grafting.

Trial registration number: N/A.

P-510 Comparison of in vitro fertilization (IVF) and in vitro maturation (IVM) in a fertility preservation (FP) program for cancer patients

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Study question: To compare IVF and IVM results in terms of collection's parameters and, where applicable, pregnancy rates in a fertility preservation program.

Summary answer: IVF seems to be more efficient than IVM regarding collection's parameters, but no statistical difference appears in pregnancy rates despite tendency in favor of IVF.

What is known already: FP offers to young women with cancer in case of gonadotoxic therapy the potential to become pregnant with their own oocytes. Currently, cryopreservation of oocytes and/or embryos after controlled ovarian hyper stimulation (conventional IVF) represents the established method. Nevertheless, IVM is an interesting alternative method when chemotherapy can not be delayed or when ovarian stimulation is contraindicated. Although a few studies have reported clinical outcomes between IVF and IVM cycles in polycystic ovary syndrome (PCOS) patients, to date there is no report on the clinical outcomes comparing the two procedures in women with cancer undergoing FP.

Study design, size, duration: Retrospective cohort study from January 2003 to April 2016 in a University teaching hospital; 394 cycles of IVF and IVM treatments in 353 women with cancer.

Participants/materials, setting, methods: Three hundred fifty three women underwent 394 FP cycles: 187 IVF cycles (47.5%) and 207 IVM cycles (52.5%). Oocyte cryopreservation was performed in 236 cycles (60%), embryo cryopreservation in 147 cycles (37.2%), and oocyte + embryo cryopreservation in 11 cycles (2.8%). Out of the 353 patients, 23 patients returned (6.5%) to use

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oocytes or embryos cryopreserved in 34 cycles (19 after IVF cycles and 14 after IVM cycles).

Main results and the role of chance: The IVF group consisted of 187 cycles for 171 patients and the IVM group of 205 cycles for 182 patients. The two groups were comparable in terms of FSH level and antral follicle count, but a significant difference appeared in median [25th-75th] in age (29 [25-33] in IVF group versus 30 [27-35] in IVM group). Between IVF and IVM treatments, significant differences were found regarding the number of oocytes collected (12 [8-18] and 7 [5-12.5]; respectively), the total number of metaphase II oocytes (10 [7-15] and 5 [2-8]; respectively) and the number of oocytes cryopreserved (10 [6-15]; 5 [2-8]; respectively). Similarly, where applicable, the number of embryos cryopreserved (5 [3-7] and 3 [2-5]; respectively) was significantly different, whereas no significant difference was found in fertilization rates (79 [67-88] and 75 [58-100]; respectively). Concerning the comparison of the obstetrical outcomes, implantation rates were significantly lower in the IVM group (21.9 and 3.7, $p = 0.04$) but no statistical difference appeared in clinical pregnancy (36.8 and 14.3, $p = 0.15$) and live birth rates (31.6 and 7.1, $p = 0.09$). When comparing the type of material thawed (oocyte or embryo) according to the procedure (IVF or IVM), no difference was found in obstetric outcome.

Limitations, reasons for caution: The main limitation is the small size of IVF and IVM populations concerning obstetric data. Other limitations include the low use of frozen material, the large number of lost to follow-up and the study's retrospective and single centre nature leading to possible treatment and referral bias.

Wider implications of the findings: Oocyte and embryo cryopreservation after both IVF and IVM procedures are viable options in a FP program in women with cancer, even if IVF seems to be more efficient than IVM. However, more data, a longer follow-up and better interdisciplinary communication are needed.

Trial registration number: 16-089 MUHC

P-511 Experience in women with cancer: obstetrical outcomes after twelve years of oocytes and embryos cryopreservation following in vitro fertilization and in vitro maturation treatments

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Study question: To evaluate the use and the obstetric outcomes of oocyte and embryo cryopreservation following in vitro fertilization (IVF) and in vitro maturation (IVM) treatments.

Summary answer: Oocyte and embryo cryopreservation after both IVF and IVM treatments are effective strategies in women with cancer.

What is known already: Fertility preservation (FP) offers to young women with cancer the potential to become pregnant with their own oocytes. FP has being used routinely for relatively few years. Consequently, little is known about the proportion of patients who come back to use the frozen material and about the performance of these techniques in terms of pregnancy rates. To date, besides some case-reports, only few cohort studies were published after conventional IVF. All agree on a low use of frozen material and satisfactory pregnancy rates. Two live births have been reported after FP with IVM.

Study design, size, duration: Retrospective cohort study from January 2003 to April 2016 in a University teaching hospital; 394 cycles of IVF or IVM treatments were analyzed in 353 women with cancer.

Participants/materials, setting, methods: Three hundred fifty three women underwent 394 FP cycles: 187 IVF cycles (47.5%) and 207 IVM cycles (52.5%). Oocyte cryopreservation was performed in 236 cycles (60%), embryo cryopreservation in 147 cycles (37.2%), and oocyte + embryo cryopreservation in 11 cycles (2.8%).

Main results and the role of chance: Out of the 353 patients, 23 patients came back (6.5%) to use oocytes or embryos cryopreserved in 34 cycles. Only 21 patients underwent an embryo transfer. The median lapse of returning to attempt pregnancy was 4.6 [3.1-6.1] years. On the 34 embryo transfers, the cumulative clinical pregnancy rate per patient was 47.6% (10/21) and the live birth rate per patient was 38.1% (8/21). The miscarriage rate was 20% (2/10). Among the 8 deliveries, 5 followed embryo cryopreservation after IVF treatment, 1 followed oocyte cryopreservation after IVF treatment, 1 followed embryo cryopreservation after IVM treatment and 1 followed embryo cryopreservation after IVF+IVM treatment. Nine babies were born: one twin pregnancy who delivered prematurely and 7 term healthy babies after singleton pregnancy. The twin pregnancy rate was 12.5%.

We report the third live birth after IVM procedure in patients with cancer, and the first one after IVM and oocyte cryopreservation in cancer.

Limitations, reasons for caution: Due to the recent nature of FP techniques and the poor use of the frozen material, obstetric data are still limited. The performance of these techniques needs further follow-up. Other limitations include number of lost to follow-up and the retrospective and single centre nature responsible for treatments bias.

Wider implications of the findings: Our study shows that in case of FP for cancer, 6.5% women come back and pregnancies occur. Oocyte and embryo cryopreservation after both IVF and IVM procedure are viable options in a FP program in cancer women. However, more data, a longer follow-up combined with interdisciplinary communication are needed.

Trial registration number: 16-089 MUHC

P-512 Anti-Müllerian hormone, antral follicle count and number of oocytes retrieved in cancer patients compared to healthy women: A multiple linear regression model

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Study question: Are markers of ovarian reserve associated with the number of oocytes retrieved after controlled ovarian hyperstimulation in women with cancer?

Summary answer: Ovarian reserve markers are positively correlated with the total number of oocytes and MII retrieved in cancer patients. There were no differences with healthy women.

What is known already: The number of oocytes retrieved and cryopreserved for fertility preservation is associated with clinical pregnancy and life birth rates.

Medical literature describe a suboptimal ovarian response after controlled ovarian hyperstimulation (COH) in cancer patients.

Ovarian reserve markers (age, FSH, and specially Anti-Müllerian hormone -AMH- and antral follicle count -AFC) are associated with ovarian response and correlated with de number of oocytes retrieved in ART patients.

The correlation of these ovarian markers and the number of oocyte retrieval in cancer patients is unknown.

Study design, size, duration: Retrospective cohort study from 01/01/2014 to 31/12/2016.

A total number of 107 women were included. There were no losses to follow-up.

The number of total oocytes and MII retrieved in the cohort of healthy women and cancer patients were analyzed using a multiple linear regression model, accounting for disease condition, age, stimulation and trigger protocols, basal FSH, AMH and AFC.

Participants/materials, setting, methods: We included 55 women planning to undergo oocyte criopreservation for social reasons and 52 cancer patients at high risk of sterilizing treatments in a university hospital.

Exclusion criteria: Previous ovarian surgery; previous radio/chemotherapy treatment; diagnosis of infertility, endometriosis or polycistic ovaries; life threatening condition.

Ovarian stimulation was performed using gonadotophins (FSHr–HMG) within a GnRH antagonist protocol. In hormone dependent tumors, we added Letrozole. The final trigger was done with either HCG or agonist GnRH.

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Main results and the role of chance: Baseline characteristics in the 52 cancer patients (CP) before oncological treatment versus the 55 healthy women that cryopreserved oocytes for social reasons (SR) were: mean age: 30.4 years (SD 6.0) vs 37.7 (2.0), p <0.001; FSH: 5.3 mUI/ml (3.1) vs 6.7 (4.6) p 0.116; AMH: 23.5 pmol/l (18.7) vs 14.9 pmol/l (17.5), p <0.037; AFC: 16.2 (11.7) vs 12.0 (7.8), p<0,030. All patients were stimulated with a gonadotrophin/antagonist protocol, except for 44% of CP that also received Letrozole. In CP, 44% were triggered with HCG vs 89% in SR group (p<0.001).

Mean number of oocytes retrieved was (CP vs SR): 12.7 (SD 11.4) vs 10.0 (7.2), p 0.143. The mean number of M-II oocytes was: 9.8 (9.1) vs 7.7 (6.1), p 0.176.

Results of multiple linear regression models for total number of oocytes retrieved and for MII oocytes were similar. We report here beta coefficients (SE) and p values for each of the covariates included in the MII model: AFC 0.325 (0.089) p 0.001; AMH 0.152 (0.055) p 0.008; FSH -0.414 (0.176) p 0.022; age 0.238 (0.167) 0.159; cancer patients 3.384 (2.134) p 0.118; Letrozole -9.612 (2.399) p <0.001; HCG -5.575 (1.870) p 0.004.

Limitations, reasons for caution: It is a retrospective cohort with a relatively small number of patients due to the characteristic of included participants. Nevertheless, this study is important for the design of prospective studies that could confirm results.

We excluded patients with life threatening conditions, so results do not apply to these patients.

Wider implications of the findings: Oocyte reserve markers were significantly associated with oocytes retrieved in cancer patients (specially AFC and AMH). There were no differences in the adjusted number of MII retrieved in cancer patients compared to healthy women.

Interestingly, the use of Letrozole and final trigger with HCG significantly affected oocyte retrieval.

Trial registration number: -.

P-513 Comparison of residual dimethyl sulfoxide (DMSO) and ethylene glycol (EG) concentration in bovine ovarian tissue during warming steps between slow freezing and vitrification methods

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Study question: How much cryoprotectant is removed from cryopreservation ovarian tissue during warming steps of slow freezing and vitrification?

Summary answer: Slow freezing had more cryoprotectant removed from cryopreserved ovarian tissue than vitrification, and was considered safer for transplantation.

What is known already: There are slow freezing and vitrification methods for ovarian cryopreservation. In vitrification methods, the concentration of cryoprotectants is higher and warming time is shorter than in slow freezing. Therefore, with the warming protocols in vitrification, there is concern that all the cryoprotectants, which may be toxic in the human body, should be washed out at the time of transplantation. Our aim is to measure residual DMSO and EG concentration in ovarian tissue during warming steps following the slow freezing and vitrification method (Cryotissue and Ova Cryo Kit TypeM).

Study design, size, duration: In this study, we used five bovine ovaries with an average age of 24.2 months. We measured the residual DMSO and EG concentrations ① before warming, ② during warming, ③ after warming in slow freezing, Cryotissue and Ova Cryo Kit Type M.

Participants/materials, setting, methods: We analyzed the residual DMSO and EG concentration in slow freezing (EG is not included), Cryotissue and Ova Cryo Kit Type M (DMSO is not included) in the bovine ovarian tissue by gas chromatograph mass spectrometer (GC-MS) (Shimadzu Techno-Research, Inc.).

Main results and the role of chance: Residual DMSO concentration: slow freezing ①52.2 ± 7.6 mg/g ②1.2 ± 0.5 mg/g ③0.6 ± 0.9 mg/g, Cryotissue ①84.8 ± 12.0 mg/g, ②34.6 ± 4.7 mg/g ③10.3 ± 2.7 mg/g. Residual EG concentration Cryotissue: ①83.2 ± 11.2 mg/g ②34.2 ± 6.3 mg/g ③10.7 ± 2.7 mg/g, Ova Cryo Kit Type M ①124.0 ± 18.2 mg/g ②82.2 ± 14.7 mg/g ③33.4 ± 9.7 mg/g.

Slow freezing was significantly lower in residual DMSO in all steps compared to Cryotissue(①52.2 ± 7.6 mg/g vs 84.8 ± 12.0 mg/g ②1.2 ± 0.5 mg/g vs 34.6 ± 4.7 mg/g ③0.6 ± 0.9 mg/g vs 10.3 ± 2.7 mg/g; p<0.01). Ova Cryo Kit TypeM was significantly higher in residual EG in all steps compared to Cryotissue.(①124.0 ± 18.2 mg/g vs 83.2 ± 11.2 mg/g ②82.2 ± 14.7 mg/g vs 34.2 ± 6.3 mg/g ③33.4 ± 9.7 mg/g vs 10.7 ± 2.7 mg/g; p<0.01).

Limitations, reasons for caution: We found residual cryoprotectants when warming cryopreserved ovarian tissues. However, we do not know the degree of toxicity of the residual cryoprotectants we found in this study.

Wider implications of the findings: Of the three methods compared in this study, slow freezing had more cryoprotectants removed from the ovarian tissue. However, it was suggested that further studies such as the effect of residual amount of cryoprotectants on ovarian tissue should be made.

Trial registration number: None.

P-514 Evaluation of a novel rotary cell culture system for primordial activation of ovarian cortical patches

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Study question: Can a rotary cell culture system provide an effective culture environment for primordial and pre-antral follicles within the cortical patches to survive and develop?

Summary answer: The rotary cell culture system offered high survival rates for follicle within patches and sustained high levels of primordial and transitory population.

What is known already: Optimizing the culture system for the maturation of primordial follicles within ovarian tissue has been considered a promising strategy of fertility preservation for cancer survivors at pre-pubertal and reproductive ages. Rotary cell culture systems have been achieved success in culturing stem cells and cancer cells, which indicates prospective systems for the survival and growth of follicles enclosing in ovarian tissue.

Study design, size, duration: The experiment was replicated three times. A total of 8 ovine cortical strips were retrieved from the same ovary, in which 2 patches served as fresh controls, while others were cultured 7 days in rotary cell culture system.

Participants/materials, setting, methods: Cortical patches were allocated in four groups including fresh control group and three culture groups: i) base media, ii) base media plus 5 μM PS48, and iii) base media plus 5 μM PS48 and 25 μg/mL 740Y-P. Following 7 day culture period, cortical strips underwent histological analysis to examine follicle count. Immunocytochemical analysis were also performed to assess the expression of Ki67, AMH, GDF-9 and BMP-15.

Main results and the role of chance: Significant increases in the survival rate of follicles and in the density of transitory follicles were found in ovarian patches cultured with PS48 plus 740Y-P as compared to those other groups ($p > 0.05$). The expressions of proliferating marker (Ki67) and recruitment marker (AMH) of granulosa cells were also higher in this treatment group as opposed to control group. There were no statistically significant difference in the number of follicles expressed positive staining for GDF-9 and BMP-15 between control and cultured groups.

Limitations, reasons for caution: Small sample size and conducting on ovine model were main limitations of this study, leading to restrict the ability to compare and generalize results to humans.

Wider implications of the findings: The present findings provided evidences for *in vitro* follicular growth that may generate a large supply of mature oocytes for future assisted reproduction of infertile women with premature ovarian failure. This culture system, if it works effectively, could replace

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conventional stimulation protocols for IVF, which revolutionizes the treatment of infertility.

Trial registration number: Non.

P-515 A clinical protocol for isolating human preantral follicles using a good manufacturing practices grade collagenase NB6

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Study question: Is collagenase NB6 good manufacturing practices (GMP) grade able to isolate viable human preantral follicles for ovarian reconstructions after gonadotoxic treatments in case of cancer?

Summary answer: A high yield of viable preantral follicles, free from leukemic cells, has been isolated with collagenase NB6 GMP grade.

What is known already: Ovarian tissue transplantation is associated with a risk of reintroducing malignant cells (i.e. in case of leukemia).

This risk could be eliminated by grafting isolated follicles.

Isolation techniques following GMP are available but evaluating the contamination of the follicular suspension by leukemic cells is a crucial step.

Study design, size, duration: The efficiency of collagenase NB6 GMP grade was evaluated, in comparison with collagenase type IA, in terms of yield, morphology, viability and short-term in vitro culture of isolated follicles in a prospective experimental design. The presence of leukemic cells in follicular suspensions was determined using fluorescence microscopy and multicolor flow cytometry (MFC).

Participants/materials, setting, methods: Frozen-thawed ovarian biopsies obtained from 16 consenting women (25-37 years old) undergoing laparoscopic ovarian drilling for polycystic ovary syndrome were used. Enzymatic digestion of ovarian biopsies (n = 26) was performed using either collagenase type IA or collagenase NB6 (200UI/mL). Leukemic cells from blood or bone marrow of acute leukemia patients (n = 8) were added to initial follicular suspensions. Follicles were manually isolated. The 24 follicular suspensions thus obtained were analyzed by MFC before and after 3 washes.

Main results and the role of chance: Collagenase NB6 GMP grade has allowed the isolation of a high number of human preantral follicles (n = 1365), in comparison to collagenase type IA (n = 828) for 50 mg of tissue (p = 0.37). The survival rate of isolated follicles was 93.4% (n = 254/272) using collagenase NB6 GMP grade versus 92.6% (n = 276/298) using collagenase type IA, and their mean diameter was 31.66 ± 6.79 µm with collagenase NB6 (n = 317 follicles) versus 36.77 ± 7.69 µm with collagenase type IA (n = 273 follicles).

Even after 3 days of in vitro culture in a fibrin scaffold (50 mg/ml of fibrinogen and 10 UI/ml of thrombin), most of the isolated follicles were still alive (90.7% of viable follicles on 339 analyzed). The level of minimal residual disease was negative in 23 suspensions analyzed out of 24 after 3 washes. In 23 out of 24 follicular suspensions analyzed, less than 20 events corresponding to leukemic cells were present even if 10^6 of leukemic cells were previously added (in the 24th suspensions, 22 events were detected). Washes had no significant impact on follicles viability (96.1% on 323 follicles analyzed).

Limitations, reasons for caution: The efficiency of collagenase NB6 and the level of minimal residual disease have not been performed on cryopreserved ovarian biopsies from leukemia patients. We still have to work on the best way of reusing isolated preantral follicles, either by direct injection or seeding in a hydrogel for ovarian reconstruction.

Wider implications of the findings: Collagenase NB6 is a GMP clinical-grade enzyme which, in combination with the present isolation technique, will allow the use of isolated human preantral follicles for future clinical applications.

Trial registration number: Not applicable.

P-516 Effects of vitrification on functional morophology and viability of ovarian tissue

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Study question: What is the effect of vitrification on viability and functional morphology of ovarian tissue (OT) after thawing and *in vitro* cultivation in the incubation medium?

Summary answer: The comprehensive histological and morphofunctional analysis revealed a good preservation of follicles in vitrified OT and a quick restoration of the follicle viability after thawing

What is known already: Preservation of the reproductive function in women having cancer diseases in anamnesis is of current importance and remains one of challenging areas of oncology. It was found that chemotherapy and radiotherapy can severely affect the ovarian follicle reserve and lead to fertility loss and premature menopause. Cryopreservation of ovarian tissue before cancer therapy followed by autotransplantation of *thawed* ovarian tissue was found to be effective to preserve the ovarian reserve in female patients with malignant neoplasms. Currently, the main method of cryopreservation of ovarian tissue is slow freezing. Vitrification method is experimental

Study design, size, duration: The study was performed on *cortical ovarian fragments* obtained from 17 patients ranging from 20 to 47 years old during diagnostic laparoscopy after getting their written informed consent. Research material comprising native tissue as a control was obtained immediately, at 30 min, 1, 2, 4, 8 and 22 h after thawing and fixed then during 24 h in Bouin's acid liquid and *10% neutral buffered formalin* with subsequently standard histological processing and embedding in Histomix.

Participants/materials, setting, methods: General histology of OT, follicle count, verification of their stage and morphology were studied on haematoxylin and eosin. For investigating functional morphology and viability of follicles, fibroblasts and endothelium of OT immunohistochemical studies were performed by using antibodies to proliferating cell nuclear antigen (PCNA), a marker of endothelial cells of capillaries (CD34), a protein of intermediate filaments of cells of mesenchymal origin – vimentin, a cyclin (Ki-67), and a marker of vascular endothelium (CD31).

Main results and the role of chance: Investigations of vitrified tissue after thawing and cultivation for up to 4 hours in the incubation medium indicated that cortical architectonics remained *essentially unchanged*. The results of a comparative analysis demonstrated that the percentage of morphologically normal follicles decreased to 64%. However, the relative pool of structurally unchanged primordial follicles exceeded 90%.

The study of functional morphology of native OT revealed an intense positive reaction of nuclei of fibroblasts, vascular endothelium and granulosa cells in secondary follicles with antibodies to proliferation markers: PCNA and Ki-67. Expression of CD31 and CD34 was observed in endothelium of vessels and capillaries. Vimentin was found in fibroblasts of the ovarian stroma and endothelium. Up to 4 hours of *cultivation* of thawing *tissue* proliferative activity of endothelial and stromal cells differed little from that in native control. CD31 and CD34 immunostaining provided evidence of a good preservation of tissue vascularization. Expression vimentin showed high viability of fibroblasts and vascular endothelium. A cultivation more 8 h resulted in irreversible morphological changes in OT. Death of most cortical cells with *pyknotic nuclei* occurred. *Detachment of endothelial cells* was observed. Almost all *follicles were* classified as *degenerated*. *Vimentin expression* was *dramatically reduced* in the cytoplasm of stromal cells.

Limitations, reasons for caution: None

Wider implications of the findings: The comprehensive histological and morphofunctional analysis revealed a good preservation of follicles in vitrified OT and a quick restoration of the viability of its cellular components after

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thawing. These data clearly demonstrate that preserving fertility by vitrification OT is a successful and safe clinical option for cancer patients.

Trial registration number: NA.

P-517 The majority of women who bank their oocytes conceives naturally

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Study question: How many women conceive after oocyte banking for fertility preservation and with which mode of conception?

Summary answer: After a mean follow-up of 34 months, 31% of women who banked their oocytes reported a pregnancy of which 79% were achieved through natural conception.

What is known already: Women at risk for premature ovarian insuffiency (POI) due to a medical condition or gonadotoxic treatment and women who face age-related fertility decline may increase their chances of future childbearing by banking oocytes. Follow-up of reproductive outcomes of women who had fertility preservation through oocyte banking is limited.

Study design, size, duration: We performed a prospective follow-up study of a cohort of 327 women who banked oocytes for all fertility preservation indications between July 2009 and August 2015 in a University Hospital.

Participants/materials, setting, methods: We retrieved medical data, i.e. clinical indications for fertility preservation and outcomes of ovarian stimulation from medical files. We asked all women to fill out an additional questionnaire on demographics, diagnosis, gonadotoxic treatment or ovarian surgery received, menstrual cycle and contraception, status of their relationship, pregnancy attempts and intended use of banked oocytes at time of follow-up.

Main results and the role of chance: A total of 234 of the 326 women (72%) responded and 219 of the 326 women (67%) consented to participate and returned the questionnaire. Mean time to follow-up of respondents was 34.1 months. Indications for fertility preservation were age-related fertility decline 96/219 (44%), gonadotoxic therapy 86/219 (39%), genetic predisposition or POI 19/219 (9%), ovarian surgery 11/219 (5%) and chemotherapy in the past 7/219 (3%). Almost half of the women responding (44%) were trying or had tried to become pregnant after oocyte banking. Sixty-seven of the 219 women (31%) reported 84 pregnancies after oocyte banking. Sixty-six of these pregnancies (79%) were naturally conceived. The remaining 18 pregnancies (21%) were conceived with ART of which six pregnancies (7%) were conceived with banked oocytes. The 84 pregnancies resulted in 39 (46%) live births, 18 (21%) ongoing pregnancies at time of follow-up, 23 (27%) miscarriages, three (4%) induced abortions and one (1%) termination of pregnancy.

Limitations, reasons for caution: Large-scale follow-up studies, including natural conception rates, from other centres are needed to conclude if our results are generalizable to other settings and populations. A longer follow-up of these women is needed.

Wider implications of the findings: Our results suggest that most women wish to become pregnant in the next few years after cryopreservation of oocytes. Considering that nearly 80% of the women in our study conceived naturally, the chances of natural conception should be discussed during the counseling of oocyte banking.

Trial registration number: Not applicable.

P-518 Gender and educational disparities underlying elective egg freezing: results from the first major qualitative study of oocyte cryopreservation in the United States and Israel

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Study question: Why are educated professional women in the United States and Israel increasingly turning to oocyte cryopreservation as a way to preserve their fertility?

Summary answer: Highly educated professional women undergo oocyte cryopreservation for lack of partners to marry, not for educational or career ambitions, as media reports suggest.

What is known already: In the United States and Israel, so-called "social" (elective) egg freezing is increasingly being adopted by professional women as a means of fertility preservation. Extensive media coverage suggests that educational and career ambitions are the main determinants of professional women's fertility postponement, especially as they "lean in" to their careers. But is this true? To date, no major qualitative study of women's egg freezing experiences has been undertaken to assess whether women are using egg freezing to postpone their fertility in pursuit of their professions, or whether other factors in women's lives are more important in egg freezing decisions.

Study design, size, duration: This study is the first binational, qualitative comparison of women's egg freezing decisions and experiences. It was undertaken from June 2014 to August 2016 in the United States and Israel, and involved in-depth interviews conducted by senior medical anthropologists in both countries. An identical interview guide was used in both countries, but translated into Hebrew in Israel.

Participants/materials, setting, methods: In-depth, ethnographic interviews were conducted with 150 women who had undertaken elective egg freezing in the United States (114) and Israel (36). Women were recruited from a total of 8 IVF clinics (4 in each country; 3 academic, 5 private), located in several major American and Israeli cities (e.g., New York, San Francisco, Washington, DC, Haifa, and Tel Aviv).

Main results and the role of chance: In both the US and Israel, women freezing their eggs for elective reasons were highly educated professionals (>80% with graduate degrees) in their late 30 s/early 40 s. Most of these women (>90%) were not intentionally "postponing" their fertility because of education or careers. Rather, they were desperately "preserving" their fertility beyond the natural end of their reproductive lives, because they were single without partners to marry. In most cases, these women were unable to find educated men willing to commit to family life—the reflection of a growing, but little-discussed gender trend, with women increasingly outnumbering male college graduates in both countries. Because of this dearth of educated men to marry, women resorted to egg freezing as a technological concession to the "man deficit." However, egg freezing was not seen by most women as a "revolutionary" technology—one that creates new gender norms or family formations (e.g., single motherhood "by choice"). Rather, almost all of the women in this study who employed egg freezing were heterosexual and wanted to become married mothers. Women lamented the "missing men" in their lives, viewing egg freezing as a way to buy time while on the continuing (online) search for a committed partner.

Limitations, reasons for caution: Participation in this study was voluntary, sampling was not random, and the response rate was unquantifiable. Still, the study sample was heterogeneous in terms of ethnicity and religion, particularly in the US, where egg freezing does not appear to be limited to professional white women, as media reports would suggest.

Wider implications of the findings: Increasing numbers of highly educated professional women around the world are single. They may turn to egg freezing to preserve their fertility in the absence of male partners to marry. Egg freezing is thus a technological "fix" to a growing problem of "leftover" educated women and "missing" educated men.

Trial registration number: Not applicable.

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P-519 Comparison of ovarian reserve and ovarian response to hyperstimulation in women undergoing oocyte vitrification according to the type of malignancy

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Study question: Is there a difference between women suffering from breast cancer, lymphoma or other solid cancers in terms of ovarian reserve and ovarian response to hyperstimulation?

Summary answer: The type of cancer did not significantly affect ovarian reserve and ovarian response to controlled ovarian hyperstimulation (COH) in women undergoing oocyte vitrification cycle.

What is known already: Cancer treatment, and particularly chemotherapy, is generally associated with ovarian reserve alteration failure. However, whether cancer itself affects ovarian function in women remains controversial in the literature. While some studies reported lower ovarian reserve and/or ovarian response to COH in women with cancer compared with controls, this was not definitively confirmed, as some studies suffered from some limitations such as limited population or heterogeneous stimulation protocols. Moreover, no study evaluated the potential impact of the type of cancer on ovarian response to COH.

Study design, size, duration: This retrospective cohort study was conducted in 105 women aged 18-40 years referred to our Department of Reproductive Medicine for fertility preservation (oocyte vitrification) between 2013 and 2016. All of them had just been diagnosed with cancer with gonadotoxic treatment scheduled.

Participants/materials, setting, methods: Women were grouped according to cancer type: 58 women with breast cancer, 25 with lymphoma and 22 women with other solid cancer. Each woman underwent ovarian reserve evaluation with AMH and Antral Follicle Count. Ovarian stimulation was performed with an antagonist protocol with random-start to reduce risk of delay in treatment of their malignancy. Oocyte retrieval was performed 36 hours after final oocyte maturation by hCG or GnRH agonist administration. All mature oocytes were vitrified.

Main results and the role of chance: Baseline AFC and AMH were not different between women with breast cancer, lymphoma or other cancer (AFC: 23,6 ± 13,6 vs 17,7 ± 7 vs 22,9 ± 13,4 respectively, p = 0,35; AMH: 4,9 ± 3,4 vs 4,1 ± 3,2 vs 4,7 ± 3,1 respectively, p = 0,8). The number of mature oocyte (MII) was also comparable between the three groups (11 ± 6 vs 10,6 ± 5,4 vs 10,2 ± 5,6, p = 0,96). As well as oocyte maturity rate defined as MII/oocyte retrieved (0,7 ± 0,2 vs 0,7 ± 0,2 vs 0,7 ± 0,2, p = 0,96). The number of FSH units per mature oocyte was comparable in the three groups (274,9 ± 254,9 vs 272,2 ± 210,5 vs 285,0 ± 228,5, p = 0,9). We also reported comparable number of MII (10,5 ± 6,6 vs 11 ± 4,3, p = 0,7) and oocyte maturity rate (0,7 ± 0,2 vs 0,8 ± 0,2, p = 0,4) between conventional start and random-start ovarian stimulation protocols.

Limitations, reasons for caution: As most women in this study still do not have permission for pregnancy, we could not analyze subsequent embryo development and pregnancy rates after ART cycle with frozen-thawed oocytes

Wider implications of the findings: As the type of cancer does not appear to impact significantly ovarian reserve and ovarian response to COH, our results do not support the relevance of integrating this parameter when establishing ovarian stimulation protocol for oocyte vitrification cycle in women with cancer.

Trial registration number: N/A

P-520 Novel technology identifies significant rise in sublethal single strand DNA damage in oocytes following chemotherapy exposure which correlates with increased fetal malformations and pregnancy failure

Abstract withdrawn by the author

P-521 Prediction of ovarian function recovery after ovarian protection with gonadotropin-releasing hormone agonist during chemotherapy in young breast cancer patients

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Study question: What factors are predictive for recovery of ovarian function after ovarian protection by gonadotropin-releasing hormone (GnRH) agonist during chemotherapy in young breast cancer patients?

Summary answer: Age, tamoxifen use, pretreatment follicle-stimulating hormone (FSH) and anti-Müllerian hormone (AMH) are useful markers for predicting recovery of ovarian function after GnRH agonist.

What is known already: Growing evidence indicates that treatment with GnRH agonist during chemotherapy is a reliable option for fertility preservation in breast cancer. However, no study to date has investigated the predictive factors for recovery of ovarian function after GnRH agonist treatment for ovarian protection.

Study design, size, duration: Prospective cohort study. Between January 2013 and May 2015, 105 young breast cancer patients were included.

Participants/materials, setting, methods: Participants were diagnosed with breast cancer and received GnRH agonist during cyclophosphamide-based chemotherapy for ovarian protection. Serum levels of AMH, FSH, and estradiol were measured before and after completion of chemotherapy. Associations between pretreatment hormones, clinical factors, and recovery of ovarian function (resumption of menstruation or AMH ≥ 1 ng/mL) were evaluated at 12 months and long-term follow-up after completion of chemotherapy.

Main results and the role of chance: Mean age was 32 years (range: 23-42 years). Ninety-four patients (89.5%) experienced resumption of menstruation at 12 months, and proportion of patients with AMH ≥ 1 ng/mL at 12 months was 71.4%. After long-term follow-up (mean: 737 days, N = 69), 98.6% patients resumed menstruation, and 78.2% had AMH ≥ 1 ng/mL. In multivariate analyses, tamoxifen use (P = 0.035) and pretreatment FSH (P = 0.032) were predictive of resumption of menstruation, and age (P = 0.019), tamoxifen use (P = 0.022), pretreatment FSH (P < 0.001) and AMH (P = 0.040) were predictors for AMH ≥ 1 ng/mL at 12 months. In addition, pretreatment AMH was a predictor for AMH ≥ 1 ng/mL after long-term follow-up. Receiver-operator characteristics curve analyses gave area under curve of 0.805 for resumption of menstruation and 0.903 for serum AMH concentration ≥ 1 ng/mL at 12 months, when age, tamoxifen use, pretreatment FSH and AMH were combined.

Limitations, reasons for caution: Although mean follow-up duration was 2 years, long-term data are needed to confirm our results. Larger study is needed to establish meaningful cut-off values for prediction. Pregnancies and live births are not addressed although ovarian function is not a perfect surrogate marker for fertility.

Wider implications of the findings: This finding can support decision making about options for fertility preservation. Based on the prediction for successful recovery of ovarian function by GnRH agonist for ovarian protection, whether cryopreservation is necessary or not could be determined.

Trial registration number: None.

P-522 Optimizing in vitro activation and maturation of primordial follicles in transgender and oncological patients

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Study question: What are the targets of optimization to make the in vitro culture of ovarian cortex clinically useful in a fertility preservation setting?

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Summary answer: The focus for optimization of in vitro maturation of follicles starting from ovarian tissue is the limited preantral follicle development following primordial follicle activation.

What is known already: The ability to activate and grow human primordial follicles in vitro has been shown possible using a two–step culture system. In this model, ovarian cortical strips were cultured for 6 days, from which secondary follicles could be isolated for further growth in a 4 day culture period (E. Telfer, 2008). Although very promising, the current model is not efficient enough for implementation in a clinical setting. The proposed system still requires improvement, for which the targets need to be defined. Since the follicle pool is heterogeneous, we want to know what follicle classes are not supported in their development.

Study design, size, duration: Cryopreserved ovarian tissue of 3 trans men (23.3+/-1.53 years) and 3 oncological patients (29.29+/-4.34) was thawed and prepared for in vitro culture. Trans men underwent a hysterectomy with oophorectomy after a period of intramuscular testosterone undecanoate (55.3 +/-1.15 weeks). Oncological indications for fertility preservation comprised acute lymphoblastic lymphoma, breast cancer and acute myeloid leukaemia. Cortical strips were cultured for 6 days and 2 pieces per patient were collected for follicle count (hematoxylin/eosin staining) every second day.

Participants/materials, setting, methods: Ovarian cortex was pulled mechanically and cut into small interconnecting strips. The strips were cultured individually in 24-well plates containing supplemented McCoy's 5a medium with bicarbonate for 6 days at 37°C, humidified air and 5% CO_2. The strips were fixed in 10% paraformaldehyde and embedded in paraffin for hematoxylin and eosin staining. Follicles were classified by 2 independent observer according to the Gougeon (1986) classification. Statistical analysis (Independent samples Kruskal-Wallis test) was performed (SPSS v23).

Main results and the role of chance: The mean count of the 2 observers showed a total of 2099.00 follicles, with a mean of 699.67+/-242.06 follicles per patient (N = 3) and a mean of 524.75+/-166.04 follicles per culture day (N = 4) in trans men and a total of 481.5 follicles, with a mean of 160.50 +/-92.00 follicles per patient (N = 3) and a mean of 120.38+/-56.47 follicles per culture day (N = 4) in oncological patients. The total number of follicles did not differ significantly over the different culture days (P = 0.230 and P = 0.682 in transgender and oncological patients respectively). In trans men, a progressive decrease in number of primordial follicles was seen with a significant difference between culture day 0 and 6, (P = 0.01). A statistically significant decrease in primordial follicles was not noted in the oncological group. In both groups a shift towards the more mature stages was visible, however no significant change per follicle class was seen. Unfortunately, in both groups, limited secondary follicle and no antral follicle growth was observed.

Limitations, reasons for caution: The heterogeneous cortical follicle distribution undoubtedly affects the interpretation of the results described in this study. Also, we were not able to include a culture of ovarian tissue obtained during caesarean section to compare our data with the published results of E. Telfer et al. (2008) in a similar setting.

Wider implications of the findings: This culture model supports the primordial follicle activation in cortex. However, the preantral follicle development is limited and a focus for further culture optimization. Also, follicle activation and the support of follicle growth might require subtle adaptations in tissue preparation or culture amongst different patient groups.

Trial registration number: UZ Ghent reference: 2012/780 – Belgian registration number: B670201215468.

P-523 Optimizing cryopreservation methods for immature mouse testicular cell suspensions

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Study question: In view of a future clinical application of spermatogonial stem cell (SSC) autotransplantation in patients with threatened fertility, what is the best method to cryopreserve testicular cell suspensions (TCS)?

Summary answer: Cryopreservation in DMEM supplemented with 1.5 M dimethyl-sulfoxide, 10% fetal calf serum and 60 µM of z-VAD(Oe)-FMK in vials with a freezing rate of 1°C/min was optimal.

What is known already: Slow freezing is the endorsed method to cryopreserve SSCs. In the calf, SSC cryopreservation has resulted in a post-thaw viability of 68 ± 3% permitting a repopulation efficiency of 43% after SSC transplantation (Izadyar et al.,2002). However, the best post-thaw viability of human TCSs obtained so far is only 55 ± 24% (Sà et al.,2012). The development of an optimal procedure is urgently needed in perspective of a future clinical application.

Study design, size, duration: In a first quick screening, we have studied the effect of (1) different freezing rates, (2) the addition of sugars, (3) different vessels and (4) the addition of an apoptotic inhibitor on the efficiency of TCS cryopreservation. After thawing, TCSs were transplanted to recipient mice for further functional assay. After selection of the optimal cryopreservation procedure, a second experiment was initiated to compare transplantation efficiency between the selected freezing protocol and fresh TCSs.

Participants/materials, setting, methods: Donor prepubertal SV129xC57BL green fluorescent protein-positive (GFP+) mice testes were digested to obtain TCSs, which were frozen under different conditions. The percentage of recovered viable cells was assessed immediately after digestion (fresh), cryopreservation and thawing. Thawed cells were transplanted to adult busulfan-treated recipient mouse testes to evaluate SSC functionality. The number of testis presenting donor-derived spermatogenesis and the number of donor-derived colonies per testis were recorded.

Main results and the role of chance: Multiple and single rate controlled slow freezing procedures did not differ significantly in terms of recovered viable cells (41 ± 37% and 38 ± 25%), thus we opted for the best time- and cost-efficient procedure. Adding a non-permeating cryoprotectant (sucrose) did not result in a higher recovery of viable cells (38 ± 20%). Cells frozen in vials recovered better than those frozen in straws (50 ± 18% vs 35 ± 13%; P = 0.046). The inclusion of a broad spectrum anti-apoptotic factor (z-VAD(Oe)-FMK) significantly increased the recovery of viable cells after thawing (64 ± 12% vs 48 ± 12%; P = 0.034). After thawing, samples from each condition were transplanted to recipient mice to assay for stem cell activity of frozen/thawed cells. Testes transplanted with cells frozen with the optimal protocol presented donor-derived spermatogenesis in 75% (3/4) of the transplanted testes. When comparing the optimal cryopreservation procedure with fresh TCSs, donor-derived spermatogenesis was completed in 75% (3/4) of the transplanted testis with a lower number of donor-derived SSC-colonies per testis (18 ± 3 versus 46 ± 23; P = 0.0334). No differences were found in terms of colony length.

Limitations, reasons for caution: Since human testicular tissue is scarce, the present protocol was first developed in the mouse model. As the paramount perspective of this study is the translation of this protocol to human testicular cell suspensions, supplementary research is required to develop xenofree cryopreservation media.

Wider implications of the findings: The development of an easy, reproducible and cost-effective cryopreservation method is a pivotal step in the future clinical application of SSC autotransplantation in view of fertility restoration in patients for whom sperm freezing is not an option (e.g prepubertal boys undergoing gonadotoxic treatments).

Trial registration number: not applicable.

P-524 Fertility preservation in women with malignancies: the accuracy of AFC collected randomly during the menstrual cycle in predicting the number of mature oocytes retrieved

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Study question: Is Antral Follicle Count collected at any time during the menstrual cycle (random AFC) reliable in predicting the number of collected mature oocytes?

Summary answer: Random AFC is as reliable as serum AMH in predicting ovarian responsiveness to treatment.

What is known already: In young women with cancer who are scheduled for chemotherapy, there is the need to provide prompt counseling on future fertility issues including a reliable estimate of the possible benefits of fertility preservation techniques. Unfortunately, hormonal assessments such as serum FSH or AMH take time to be available and FSH needs to be taken in a particular menstrual phase. AFC is conversely immediately available but its use is claimed to be reliable only if collected in the early follicular phase (day 3-5 of the cycle).

Study design, size, duration: Retrospective case series of young women with malignancies undergoing ovarian hyperstimulation aimed at oocytes cryopreservation between July 2014 to December 2016. Random AFC and serum AMH was systematically recorded prior to initiate the therapy. All women received a standardized "random start" regimen of ovarian hyperstimulation.

Participants/materials, setting, methods: Seventy-two women were ultimately included. The total number of retrieved mature oocytes was the primary outcome. A good response was defined as ≥ 10 retrieved mature oocytes. The predictive capacity of AFC was evaluated using receiver operating characteristic (ROC) curves.

Main results and the role of chance: Indication to oocytes cryopreservation was breast cancer in 42 women (58%), lymphoma in 20 (28%) and other malignancies in the remaining 10 (14%). The mean ± SD age and AFC of the selected women was 31.4 ± 5.6 years and 18.7 ± 10.7, respectively. The median (interquartile range) serum AMH was 3.2 (1.3 - 4.2) ng/ml. Thirty-four women (47%) were in the follicular phase, 32 (45%) in the luteal phase and the remaining six (8%) were assuming oral contraceptives. Three cycles were cancelled for poor response. The mean ± SD number of mature oocytes retrieved in the remaining 69 women was 11.2 ± 7.7. Thirty-five women collected ≥10 oocytes (49% of the whole cohort).

The *c*-statistics for the prediction of ≥10 mature oocytes using AFC and serum AMH were similar. Specifically, the area under the curve (AUC) was 0.76 (95%CI: 0.66-0.87; p<0.001) and 0.82 (95%CI: 0.72-0.92; p<0.001), respectively. Similarly, when considering the subgroup of women who were not assuming oral contraceptives and who were recruited after day 5 of the cycle (proper random start, n = 49), the AUC resulted 0.77 (95%CI: 0.64-0.89; p = 0.001) and 0.83 (95%CI: 0.72-0.95; p<0.001), respectively.

Limitations, reasons for caution: Even if the accuracy of serum AMH and AFC resulted similar, larger series are required to rule out mild but potentially clinically relevant differences.

Wider implications of the findings: Random AFC should be systematically performed prior to counsel women with malignancies. It is a simple assessment and can be obtained rapidly. Moreover, one may even question whether random AFC may become a standard also in women without malignancies who need ovarian reserve assessment.

Trial registration number: not applicable.

P-525 Induction of spermatogenesis by grafting neonatal mouse testicular tissue into epididymal fat of castrated adult mouse

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Study question: Is the epididymal fat an appropriate site for spermatogenesis induction of stored prepubertal testis tissue?

Summary answer: Post-meiotic markers expression on transcriptional and protein in grafted neonatal testis proofed successful spermatogenesis induction 8 weeks after implantation to castrated adult mouse epididymal fat.

What is known already: Chemo- and radiation treatments used for childhood cancer can cause irreversible infertility in many of cancer survivors. Testicular grafting of stored tissue prior to cancer treatment has the potential to become a method to preserve fertility in this people. However, spermatogenic arrest at the pachytene spermatocyte stage is the main remaining challenge that failed this process. It has been known that epididymal fat is necessary for spermatogenesis, therefore in this study; spermatogenesis development was evaluated after grafting of fresh and frozen-thawed neonatal mouse testicular tissue fragments to epididymal fat region of bilateraly castrated adult mouse.

Study design, size, duration: Six neonatal (3-5 days old) male mice as the donors and six adult (6-8 weeks old) male mice as the recipients were used. After bilateral castration of recipient's mice, four pieces of fresh or frozen-thawed neonatal testis tissue fragments (approximately 1 mm^3 each) were grafted into recipient epididymal fat. Eight weeks after implantation, grafted testicular tissue were evaluated.

Participants/materials, setting, methods: After scarifying of recipients mice and collecting of grafted testicular tissues, hematoxylin and eosin (H&E) staining was used to evaluate the morphology of seminiferous tubules and level of spermatogenesis. Real time PCR (PLZF, TEKT1 and TNP1) and immunohistochemistry staining (PLZF, SYCP3 and Acrosin Binding Protien) were used to assess the germ cell development in grafted neonatal mouse testis tissue. TUNEL assay was used for detecting DNA fragmentation and defining the apoptosis level of grafted tissue.

Main results and the role of chance: Vascular anastomoses were seen at the graft site. At the time of grafting, spermatogonial cells were the only germ cells present in the seminiferous tubules. However eight weeks after implantation a gradient of different types of germ cells from spermatogonia up to the elongated spermatid were seen. The meiotic (TEKT1) and post-meiotic (TNP1) genes were upregulated in both fresh and frozen grafted groups. Presence of SYCP3 and Acrosin Binding Protien positive cells also confirmed the meiosis and post meiotic progression in grafted seminiferous tubules. Expression of PLZF as an undifferentiated spermatogonial cell marker stayed stable post grafting. TUNEL assay showed no significant differences between control and experimental groups.

Limitations, reasons for caution: The functionality of induced elongated spermatids in grafted tissues needs to be investigated by Intra Cytoplasmic Sperm Injection (ICSI).

Wider implications of the findings: The previous studies showed the spermatogenesis arrest in meiosis process. Due to the appropriate hormonal and temperature conditions of epididymal fat, it seems immature testicular tissue grafting to epididymal fat may be a powerful site to support the spermatogenesis and may pave way for fertility preservation in prepubertal cancer patients.

Trial registration number: Not applicable.

P-526 Relationship between primordial follicle density, ovarian reserve tests and the number of oocytes matured in vitro in candidates for fertility preservation

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Study question: Is primordial follicle density within ovarian cortex associated with markers of the follicular status and the number of oocytes cryopreserved after *in vitro* maturation (IVM)?

Summary answer: Primordial follicle density is significantly correlated with antral follicle count (AFC), anti-Müllerian hormone (AMH) levels and the number of oocytes recovered and matured following IVM.

What is known already: Oocyte vitrification after IVM and ovarian tissue cryopreservation may constitute alternative options for fertility preservation (FP) when ovarian stimulation is unfeasible. Since both techniques are considered experimental, their combination might increase the overall success rate. AFC and AMH levels are commonly used to assess the number of growing follicles. In addition, these markers may be significantly correlated with the number of immature oocytes recovered from small antral follicles and the number of mature oocytes obtained following IVM. Few data is available on the relationship between markers of the follicular ovarian status and the primordial follicle density estimated from ovarian tissue.

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Study design, size, duration: From July 2013 to December 2016, we prospectively studied 67 patients, 18 to 39 years of age, and candidates for FP using ovarian tissue cryopreservation associated with oocyte vitrification following IVM.

Participants/materials, setting, methods: All women had both ovaries present, deprived of morphological abnormalities. Exclusion criteria were ovarian or pelvic disease and history of gonadotoxic treatment. Transvaginal AFC and serum AMH levels were systematically measured before FP. Cumulus-oocyte complexes (COC) were recovered under ultrasound guidance, and incubated for IVM. Ovarian tissue was laparoscopically harvested and cryopreserved. For each patient, at least one fresh sample of cortex was processed for pathological analysis, including primordial follicle density assessment.

Main results and the role of chance: Among the 67 included patients, 61 were diagnosed with breast cancer, 4 had lymphoma and 2 underwent the procedure for other indications. Overall, the mean age of the population was 30.7 ± 4.7 years. In addition, mean AFC and serum AMH levels were 14.8 ± 9.7 follicles and 2.6 ± 2.7 ng/mL, respectively. As expected, AFC and AMH levels were strongly correlated with the number of COC recovered (r=0.65 and 0.63 for AFC and AMH, respectively, p<0.0001), as well as with the number of mature oocytes obtained after IVM (r=0.56 and 0.51 for AFC and AMH, respectively, p<0.0001). Likewise, a significant correlation was found between primordial follicle density and both markers of the follicular ovarian status (r=0.38 and 0.49 for AFC and AMH, p<0.003 and <0.0001, respectively), but also with the number of COC and *in vitro* matured oocytes (r=0.37 and 0.32 for COC and mature oocytes, p<0.003 and 0.008, respectively).

Limitations, reasons for caution: This study was conducted in a limited number of patients. The possible heterogeneity of primordial follicle density within the ovarian cortex may limit the relevance of assessing this parameter on a single sample. The outcome of ovarian tissue transplantation endowed with low follicle density, even in young patients, remains unknown.

Wider implications of the findings: Our findings indicate that AFC and serum AMH levels may reflect not only the pool of growing follicles, but also the primordial follicle stockpile. Further analysis are required to confirm the relevance of performing IVM and ovarian tissue cryopreservation in young women with low values of AFC and/or AMH levels.

Trial registration number: N/A.

P-527 Role of SIRT1 in the adaptive response of mouse ovaries to radiotherapy (X ray radiations)

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Study question: Is SIRT1 involved in ovarian adaptive response to gonadotoxic damage induced by radiotherapy in adult, prepubertal and aged mice?

Summary answer: SIRT1 orchestrates the adaptive response of mouse ovaries against radiotherapy in adult but not in prepubertal and aged mice.

What is known already: Radiotherapy is one of the most effective anticancer therapies and causes damage in both cancer and surrounding healthy tissues via ionization, formation of reactive oxygen species (ROS) and subsequent impairment of DNA double-strand integrity and oxidative stress (OS) establishment. Ovaries are very sensitive to radiation-related damage, which may cause premature ovarian failure and menopause. SIRT1, a histone deacetylase involved in oocyte and ovarian adaptive response to OS and chemotherapy, is activated by changes in the redox state and promotes cell survival/apoptosis. Saffron-derived crocetin is an antioxidant molecule with anti-tumor effects that protects non-malignant tissues against chemotherapy-induced toxicity.

Study design, size, duration: Part I) CD1 female mice aged 3-4 weeks (n = 12, Prepubertal mice); 8-10 weeks (n = 12, Adult) or 48-52 weeks (n = 6, Aged) were exposed to 3 Gy total body irradiation.

Part II) Ovaries from postnatal day 4 (PND4) CD1 mice were cultured in MEM-alpha with 3 mg/ml BSA supplemented with or without 50 µM crocetin for 24 hrs. Then ovaries were exposed to 2 Gy and cultured for further 24 hrs in medium with or withouth crocetin.

Participants/materials, setting, methods: Prepubertal, adult and aged mice were sacrificed at 24 hr after radiotherapy. Ovaries were removed and processed for protein analysis. The relative abundance of SIRT1, HuR, and pFOXO3a was investigated by Western blotting (WB). To investigate potential beneficial effect of a natural antioxidant, crocetin, on damage induced by radiotherapy, PND4 ovaries were processed at 24 hr after the irradiation and the expression of SIRT1, HuR and PGC1-alpha was assessed by WB analysis.

Main results and the role of chance: In ovaries from adult mice exposed to radiotherapy, we observed an increase of SIRT1 and SIRT1 mRNA stabilizer, HuR, demonstrating an early involvement of SIRT1 signalling to counteract damage by radiations (One-way ANOVA and Student-Newman-Keuls Multiple comparison p<0.05). By contrast in prepubertal or aged ovaries we observed that SIRT1 has a different expression level according to the class of age and decreases after radiations.

FOXO3A is a factor involved in the maintenance of follicle quiescence. Its inactive form pFOXO3a was found to increase in both adult and prepubertal mice after radiations, indicating a wave of primordial follicle activation.

PND4 ovaries are endowed of primordial follicle as unique follicle population. In these ovaries SIRT1 and HuR decreased in response to radiations, as observed in prepubertal mice. Medium supplementation with crocetin decreased SIRT1 expression but increased HuR, demonstrating a partial response to damage. Furthermore, since mitochondria have a central role in the regulation of redox state, we investigated the effects of radiations on PGC1-alpha, marker of mitochondria biogenesis and function. Crocetin supplementation prevented the decreased expression of PCG1-alpha induced in PND4 ovaries by radiations.

Limitations, reasons for caution: This mouse model only offers information on short-term effects of radiation, and how these ovaries would look a few weeks later is still a question.

Wider implications of the findings: This study increases the knowledge on the adaptive response orchestrated by SIRT1 in response to gonadotoxicity induced by radiotherapy and highlights the beneficial effect of saffron-derived crocetin, an antioxidant molecule with anticancer properties. Radiosensitivity is associated with a reduced ability to activate an adaptive response mediated by SIRT1 and HuR.

Trial registration number: not applicable.

P-528 BRCA 1 / 2 gene mutations do not affect the capacity of cumulo-oocyte complexes to mature in vitro in breast cancer candidates for fertility preservation

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Study question: To compare the maturation rates of oocytes recovered from small antral follicles breast in cancer patients, carriers or not for BRCA 1/2 gene mutation.

Summary answer: BRCA 1/2 gene mutations do not affect the capacity of cumulo-oocyte complexes (COC) to mature in vitro in breast cancer candidates for fertility preservation.

What is known already: Mutation in BRCA1 and BRCA2 genes are associated with an increased risk for developing breast and ovarian cancer. Controversy exists about ovarian reserve and fertility in BRCA mutation carriers. Studies suggest that these patients may have low ovarian reserve and poor response to ovarian stimulation. The impaired ability of the mutated BRCA gene to repair double-strand breaks in DNA may prompt oocyte aging, apoptosis and meiotic errors. IVM of COC retrieved at germinal vesicle stage, followed by vitrification of metaphase 2 oocytes, has recently emerged as an

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option for young women seeking fertility preservation, when ovarian stimulation is unfeasible.

Study design, size, duration: We retrospectively analyzed a cohort of 115 breast cancer candidate for fertility preservation using IVM between January 2009 to December 2016.

Participants/materials, setting, methods: Inclusion criteria were: age 18 – 40 years; two ovary presents; no history of chemotherapy. Before immature oocyte retrieval, all follicles measuring 2-9 mm in diameter were precisely counted on both ovaries and serum anti-Müllerian hormone was measured irrespective of the phase of the cycle. Number of COC retrieved, maturation rate and number of metaphase 2 oocyte cryopreserved were compared according to BRCA mutation status.

Main results and the role of chance: Overall, BRCA mutation carriers (n = 28) and controls (n = 87) were comparable in terms of age (31.9 ± 3.4 vs. 31.7 ± 4.0 years, respectively, NS), BMI (22.5 ± 4.5 vs. 23.0 ± 4.8 Kg/m^2, respectively, NS) and ovarian reserve tests (antral follicle count: 20.0 ± 9.4 vs. 21.4 ± 9.8 follicles, NS; serum AMH levels: 3.0 ± 1.6 vs. 3.7 ± 2.1 ng/mL, NS, respectively). The number of COC retrieved did not differ significantly between BRCA gene mutation carriers (9.0 ± 6.1 vs. 10.1 ± 7.4 oocytes, NS). After similar in vitro maturation rates (68.7 ± 20.4 vs. 60.0 ± 17.0%, NS), the number of metaphase 2 oocytes cryopreserved was similar in BRCA mutation carriers and patients not mutated (5.1 ± 3.1 vs. 5.9 ± 4.9, NS, respectively).

Limitations, reasons for caution: The main limitation of our investigation remains the small sample size. In addition, although oocyte cryopreservation following IVM is a promising method of fertility preservation, especially when ovarian stimulation is unfeasible, the true competence of these oocytes remains unknown.

Wider implications of the findings: Although patients who carry the BRCA mutation are known to have an altered DNA repair mechanism, their oocyte capacity to mature in vitro remains intact. Given the low incidence of the mutation, multi-center studies on BRCA mutation carriers who utilize IVM are needed to corroborate these preliminary findings.

Trial registration number: N/A.

P-529 Glycol ethers and semen quality: a cross regional study among infertile men workers in Tunisia

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Study question: The aim of our study was to investigate the relation between male infertility and occupational exposures, particularly to glycol ethers.

Summary answer: For this reason, one priority of research in industrial countries is to identify these factors and to reduce the occurrence of infertility through preventive means.

What is known already: In industrial countries, 15% of couples of reproductive age have difficulty to conceive naturally. A male factor is involved in half of these couples, and the etiology remains unknown in 25% of male infertility cases. Because medical history cannot explain all male infertility cases, these observations may be linked to a growing impact of potential occupational and environmental factors.

Study design, size, duration: A cross-sectional descriptive study was conducted at the Department of Histology, Embryology and Genetics of the Medical University of Sfax (Tunisia) involving male patients who require genetic counselling as part of their infertile condition during the period between 2007 and 2008.

Participants/materials, setting, methods: 250 infertile men gave semen and blood samples. Data on work history, occupational and non-occupational exposure to chemicals were obtained through an interview. According to the job-exposure matrix (Matgéné and Sumex2 programs), men were classified as either occupationally exposed or non-exposed. A standard karyotyping and semen analysis were investigated. The statistical analysis and correlations were conducted using SPSS IBM 20 for Windows. The significance in the differences was evaluated by using Student and ANOVA tests.

Main results and the role of chance: Of 250 infertile men, 48 were exposed to Glycol ethers: 17 patients suffered from azoospermia, 11 from extreme oligoasthenoteratospermia (OAT) and 10 from severe and very severe OAT. The 10 remaining men had moderate OAT or had one or two altered semen parameters. Major chromosomal abnormalities were detected in men whereas 41 had a 46,XY karyotype.

We found significant associations between occupational exposure to Glycol ethers and semen impairment (p = 0,028) as well as karyotyping abnormalities (p = 0,001).

Limitations, reasons for caution: Our findings support the results of previous studies regarding the association between occupation and semen abnormalities, particularly for Glycol ethers.

But this retrospective estimation of occupation using the job exposure matrix to Glycol ethers was likely to be imprecise. Thus, conclusions derived from such studies should be interpreted with caution.

Wider implications of the findings: Further studies are necessary to evaluate the association between occupational exposures to other reprotoxic agents and impaired semen parameters. Preventive measures must be established and could be completed by the use of biomarkers to a better characterization of exposure to chemicals and their spermiotoxic effects.

Trial registration number: Not applied.

P-530 Human Umbilical Cord Perivascular Cells (HUCPVCs) maintain mesenchymal stromal cell characteristics and regenerative properties after chemotherapy exposure: A promising candidate for prevention of chemotherapy-induced gonadotoxicity

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Study question: Do first trimester (FTM) and term HUCPVCs resist chemotherapy-induced cytotoxicity, and maintain mesenchymal stromal characteristics including their ability to support regeneration through paracrine mechanisms?

Summary answer: Human umbilical cord perivascular cells exposed to gonadotoxic chemotherapeutic agents resist cytotoxicity, maintain mesenchymal stromal cell properties *in vitro*, and exhibit prolonged survival *in vivo*.

What is known already: Various sources of human MSCs (bone marrow, adipose tissue or umbilical cord derived) have shown remarkable restorative effects in rodent models of cytotoxic gonadal injury. However, it remains unclear whether MSCs could be delivered prior to gonadotoxic chemotherapy regimens as a means of fertility preservation. While BMSCs and aMSCs have been shown to be relatively resistant to the effect of chemotherapy, little is known about the effect of chemotherapy on HUCPVCs, a novel young source of MSCs with increased regenerative potential compared to older MSC sources.

Study design, size, duration: We treated FTM and term HUCPVC and BMSC cultures with moderate (150 µmol/L) and high (300 µmol/L) doses of cyclophosphamide (CTX) for 6 and 24 hours, and cultured them for up to 2 additional passages. Untreated cells served as controls. Outcomes measured were viability, proliferative capacity, mesenchymal cell lineage differentiation capacity, immunogenicity, and paracrine-associated gene expression. We assessed cell survival in Wistar rats (n = 6) treated with CTX following intra-ovarian injection of FTM HUCPVCs.

Participants/materials, setting, methods: Viability and MSC-associated markers were measured by flow cytometry. Commercial kits were used for osteogenic, chondrogenic and adipogenic differentiation *in vitro*. Immunogenicity was assessed by measuring LDH release in lymphocyte:MSC co-cultures. The expression of 107 paracrine-associated genes was assessed by targeted RNAseq (Ion Torrent). The survival of HUCPVCs in vivo was assessed

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qualitatively using human AluI FISH. All experiments were repeated at least 3 times. ANOVA was performed to determine statistical significance.

Main results and the role of chance: The viability of FTM and term HUCPVCs following cyclophosphamide treatment was comparable to that of BMSCs at each dose and each time point (78 and 79 vs 77%, 52 and 55 vs 59% after 150 μmol/L after 6 and 24 hour exposures respectively, and 74 and 77 vs 79%, and 55 and 63 vs 59% after 300 μmol/L for 6 and 24 hours respectively). FTM and Term HUCPVCs continued to express the MSC-associated markers, CD105, CD29 and CD44 after treatment and after 2 passages. Proliferative rates were comparable between the three treated cell types and controls. Following treatment with 150 and 300 μmol/L for 24 hours, FTM and term HUCPVCs maintained their ability to differentiate towards chondrogenic, adipogenic and osteogenic lineages, their low immunogenic properties, their expression of paracrine-associated genes that are known to mediate the ability of MSCs to promote angiogenesis and cell survival, as well as their immunomodulatory and anti-inflammatory properties. FTM HUCPVCs were detected in female rats 7 and 21 days after loading dose of CTX.

Limitations, reasons for caution: Although a main component of chemotherapeutic protocols, cyclophosphamide is typically used in combination with other cytotoxic agents. Additional studies are needed to evaluate the effect of combinations of cytotoxic agents on FTM and Term HUCPVCs, and to determine whether their regenerative capacity is maintained following combination chemotherapy exposure *in vivo*.

Wider implications of the findings: Our findings suggest that both FTM and term HUCPVCs are viable and maintain many of their key regenerative properties following chemotherapy exposure. This suggest that HUCPVCs, a potent source of MSCs for regenerative medicine, are promising cell candidates for fertility preservation strategies.

Trial registration number: not applicable.

P-531 follicular response and outcomes for women diagnosed with cancer having fertility preservation, comparison of random start protocol and early follicular phase stimulation

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Study question: is response to gonadotropin stimulation in a random start protocol comparable to early follicular phase stimulation for fertility preservation in women at risk of ovarian failure due to gonadotoxic treatment

Summary answer: response to gonadotropin for controlled ovarian stimulation is comparable between women stimulated randomly and those starting in the early follicular phase of the menstrual cycle

What is known already: the desire for fertility preservation is ever increasing as the long term survival in women with cancer continues to improve. As the prognosis depends on prompt and eraly initiation of cancer therapy, time available to consider fertilitypreservation is always limited, therfore, women wishing to undertke ovarian stimulation are often offered the short antagonist protocol. Response to ovarian stimulation in the early follicular phase is comparable to women having routine fertility treatment. Studies have shown that stimulation starting in the luteal phase may result in lower oocyte yield and higher gonadotropin usage, though this is not uniform across studies.

Study design, size, duration: A retrospective case control study comparing ovarian response to gonaotropin stimulation on the antagonist protocol started in early follicular phase or randomly at any time in the cycle in all women referred for fertility preservation between February 2003 and June 2016. A total of 138 electronic records were reviewed.

Participants/materials, setting, methods: The primary end point was the number of mature oocytes retrieved resulting in stored oocytes and/ or embryos for future use. Secondary outcome measures were duration of stimulation and total gonadotropin used and a composite of untoward adverse events including any delay in starting cancer therapy. We also assessed the outcomes of the cryopreserved gametes and/ or embryos. Multivariate logistic regression analysis was performed. $P < 0.05$ was considered statistically significant.

Main results and the role of chance: There were no statistically significant differences in baseline characteristics between women who started ovarian stimulation in the early follicular phase compared to those who started randomly at any time in the menstrual cycle. 103 women started stimulation in the early follicular phase whereas 24 started randomly, in addition, nine used the long agonist protocol and 2 had in-vitro maturation (IVM). The mean number of eggs retrieved in the early follicular phase group was 12 .0 (SD 8.1) and 12.9 (SD 7.9) in the random start group ($P = 0.6016$). It required 11.6 days (SD 2.1) for the early follicular start and 12.2 days (SD 3.5) for the random start group ($P = 0.2690$) using a total dose of 2543.4 iu (SD 1100) of gonadotropin and 2812.02 iu (SD 1707.7) respectively ($P = 0.3391$). There was no difference in duration from initial consultation for fertility preservation and the day of oocyte retrieval 33.5 days (SD 2.8) and 33.9 days (SD 4.3) respectively. Only one cycle was cancelled due to failed fertilisation, however, none of the patients needed hospitalisation for treatment of complications following the fertility procedures. 89 women had oocytes and/ or embryos still in storage and 15 had attempted a pregnancy, 11 were deceased.

Limitations, reasons for caution: The study groups together women with difference types of cancers who may not necessarily have a similar clinical approach to treatment.

Wider implications of the findings: Our study agrees with previuos work that ovarian stimulation can be started at any point in the menstrual cycle highlighting that follicullogenesis and recruitment maybe a continous process. There did not appear to be a difference in the number of mature oocytes or total gonadotorpin dose used as previously reported.

Trial registration number: not applicable.

P-532 Fertility preservation. Anatomopathological analysis of ovarian cortex metastasis in a large cohort of patient undergoing fertility preservation through ovarian cortex cryopreservation

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Study question: How frequent is to find metastasis of malignant cells in the ovarian cortex of oncological patients undergoing fertility preservation by means of ovarian cortex cryopreservation?

Summary answer: Detection of malignant cells is a rare event although it cannot be excluded a priori. Hematological malignancies are the condition at higher risk

What is known already: The increasing survival rates and success of oncological treatments make FP procedures a key step in the holistic management of the oncologic patient. Ovarian cortex cryopreservation is the preferred technique in prepubertal girls since they cannot undergo ovarian stimulation for egg retrieval. Nevertheless, the risk of contamination of the ovarian cortex with malignant cells can be a limiting factor for this techniques to be applied. Different cases have been reported in the literature mainly related to hematological conditions and sarcoma.

Study design, size, duration: The aim of the present study was to describe the pathological findings of the ovarian biopsies of our patients undergoing ovarian cortex cryopreservation for medical reasons. A small biopsy of 2 × 2 mm is always sent for pathological analysis at the moment of ovarian cortex retrieval. Expert pathologists do the examination of such biopsy to assess the presence of malignant cells. A second biopsy was taken from the cryopreserved-thawed tissue and subsequently analyzed at the moment of reimplantation.

Participants/materials, setting, methods: A cohort of 735 patients underwent ovarian cortex cryopreservation in our fertility preservation

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program between 2005 and 2015. All the biopsies were paraffin-embedded and sections were made every 4 microns. All sections were stained with hematoxilin-eosin and also using immunohistochemistry techniques depending on the underlying condition motivating the fertility preservation. A subset of 8 pediatric patients with leukemia also under went minimal residual disease (MRD) detection by RT-PCR.

Main results and the role of chance: Breast cancer was the most prevalent condition in our series (n = 618 -60.3%-), followed by Hodgkin lymphoma (145 -14.2%-), non-Hodgkin lymphoma (61 -6.0%-), gynecological conditions (44 -4.3%-), sarcoma (16 -1.6%-), leukemia (12 -1.2%), autoimmune diseases (8 -0.8%-) and other solid tumors (120 -11.7%-). Malignant cells were only detected in three patients (0.4%) during the pathological analysis of the biopsies: lymphoma malignant cells were found in the ovarian cortex of one patient aged 18 y.o. with Burkitt lymphoma and in another patient aged 33 y.o. with diffuse large B-cell lymphoma. Ovarian adenocarcinoma cells were also found in one breast cancer patient aged 36 y.o. who turned-out to be a BRCA-2 mutation carrier. Among the 8 pediatric patients with leukemia undergoing RT-PCR for detection of MRD, three of them were positive (all acute lymphoblastic leukemia).

No malignant cells were detected in the second biopsy of the 44 patients undergoing ovarian transplantation of the cryopreserved tissue.

Limitations, reasons for caution: Technologies such as RT-PCR or flow cytometry could improve the detection rate of malignant cells in the ovary. The small proportion of tissue retrieved in a biopsy, can imply a false-negative rate that can result in an underestimation of the presence of malignant cells.

Wider implications of the findings: Pathological examination of the ovarian cortex must always be done in order to exclude the presence of malignant cells, even if the prevalence of such event is very low.

Trial registration number: N/A.

P-533 Oocyte numbers in relation to age and specific malignant and non-malignant diseases – analysis of >1.000 ovarian stimulation cycles performed for fertility preservation

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Study question: Is the number of collected oocytes after ovarian stimulation for fertility preservation purposes age and disease dependent?

Summary answer: Oocyte numbers are negatively correlated with age but are similar in different malignant diseases with lower oocyte yield in stimulation for non-malignant diseases.

What is known already: Ovarian stimulation to freeze fertilized and non-fertilized oocytes is still the most efficient procedure to preserve fertility. The number of collected oocytes in the fertility preservation programme remained equal over the years, indicating that the procedure is already very efficient. However, identification of prognostic factors for high or low oocyte yield depending on the underlying diseases is helpful in the patient consultation setting and may help to adjust stimulation dosages to increase the individual oocyte yield.

Study design, size, duration: A retrospective analysis was performed of 1,068 patients (18-40y) undergoing ovarian stimulation for fertility preservation purposes before gonadotoxic therapies due to underlying malignant as well as benign diseases in 75 centers in Germany, Austria and Switzerland. Stimulation cycles were documented in the fertility preservation network FertiPROTEKT from 1/2007-3/2016.

Participants/materials, setting, methods: From 1,200 stimulations 16 women were excluded due to age (1.3%), 6 due to missing data (0.5%), 10 due to cancelled stimulations (0.8%) and 100 due to incomplete data sets (8.3%). The number of oocytes and gonadotropin dosage were analysed according to age (≤25, 26-30, 31-35, 36-40y) and disease (992 (92.9%) malignant; 76 (7.1%) non-malignant). Statistical significance was analyzed using the analysis of covariance (Ancova), age adjusted.

Main results and the role of chance: Women with malignant diseases were on average 29.5+/-5.6 years old while women with benign disease were significantly younger with 26.5+/-4.9 yrs (p<0.0001). Despite this fact women with malignant diseases had a significantly higher oocyte yield with 12.8+/-8.4 compared to women with a non-malignant disease with 9.7+/-6.6 oocytes (p<0.0006). As known from general ovarian stimulation protocols, the number of retrieved oocytes was age dependant in women with malignant disease with an average of 15.2+/-8.8 oocytes in women <26y and 9.7.+/-7.8 in women 36-40y (p< 0.002). Within the disease categories however the oocyte yield was similar with only a non-significant higher oocyte yield in cerebral carcinomas and other malignancies and lower number in gynaecological malignancies.

Limitations, reasons for caution: The analysis is based on the number of oocytes but not on pregnancy rates. Data on fertilisation rates are missing.

Wider implications of the findings: Our data indicate which diseases result in higher and lower number of collected oocytes. Therefore, ovarian stimulation protocols and gonadotropin dosages can be adapted to increase efficacy of ovarian stimulations and therefore to improve the success rate of this fertility preservation procedure.

Trial registration number: Not applicable

POSTER VIEWING SESSION
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P-534 Morphokinetic analysis of human cleavage-stage embryos as related to the cell-size stage specificity

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Study question: Are there differences in morphokinetics between cell-size stage-specific (SS) and cell-size not-stage-specific (NSS) human cleavage-stage embryos?

Summary answer: Human cell-size stage-specific (SS) embryos show slower developmental cleavages towards the 3-cell, 5-cell, 6-cell and 7-cell stage in comparison to cell-size not-stage-specific (NSS) cleavage-stage embryos.

What is known already: Static morphological assessment of human cleavage-stage embryos revealed that a substantial number of cleavage-stage embryos have an incorrect relation between cell size and embryo developmental stage. The incorrect relation was substantially higher in day 3 embryos as compared to day 2 embryos. Furthermore their in vitro developmental potential to the blastocyst stage is reduced (Muyshond et al, 2016). Culture of human embryos in time-lapse incubators (TLI), in contrast to standard incubators (SI), allows to observe embryo developmental morphokinetics and can define the precise timings of embryo development in relation to insemination time.

Study design, size, duration: This study is an observational, retrospective analysis of time-lapse monitored embryos between March 2013 and September 2016. In annotated ICSI embryos (n = 257), cell-stage specificity was determined on day 2 (D2) and on day 3 (D3). Embryos having a correct relation between the cell size and the developmental stage were determined SS and if this correlation was not correct NSS. All embryos were cultured in sequential culture media formulations (Cook Medical) until day 5 (D5).

Participants/materials, setting, methods: Four embryo categories were identified according to the static morphological SS/NSS score of the embryos on D2 and D3. Group 1: SS embryos on D2 and D3; group 2: SS embryos on D2 but that developed to NSS embryos on D3; group 3: NSS on D2 and SS on D3; group 4: NSS on D2 and D3.

Statistical analysis was performed by Fisher´s exact-test and linear regression analysis (mixed models) on log-transformed morphokinetic timings (SPSSv23)(p≤0.05).

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Main results and the role of chance: Of the 257 annotated embryos, 62.6% (161/257) were SS embryos on D2 and 37.4% (96/257) were NSS embryos. On D2, t5, the time from insemination to the 5-cell stage, was significantly different in the SS group (Median (M)=51.3 h) as compared to the NSS group (M=47.2 h) ($p = 0.001$). On day 2 there were no statistical differences for t2, t3 and t4.

In the 161 D2 SS embryos 69.6% developed further to SS embryos on D3 (group 1) and 30.4% developed further to NSS embryos (group 2). In the 96 D2 NSS embryos 17.7% of the embryos developed further to SS embryos on day 3 (group 3) and 82.3% developed further to NSS embryos (group 4). On D3, time-lapse data was analyzed between the 4 groups. Significant differences in embryo developmental timings were found at t3, t5, t6 and t7 in group 1 (Mt3 = 38.2 h, Mt5 = 52.3 h, Mt6 = 55.3 h and Mt7 = 57.6 h) as compared to group 2 (Mt3 = 36.2 h, Mt5 = 48.9 h, Mt6 = 51.6 h and Mt7 = 53.9 h). More specifically, timings were always faster in group 2. There were no significant differences in embryo morphokinetics at t2, t4 and t8 in group 1 as compared to group 2. When analyzing the time-lapse images, we also observed reversed cleavage during embryo development.

Limitations, reasons for caution: This was a retrospective analysis and the annotations and the scoring of the relation between cell size and embryo developmental stage was done by several embryologists. For this study, SS and NSS embryos were identified only by a static morphological observation on D2 and D3.

Wider implications of the findings: Our study reveals differences between SS and NSS embryos in developmental morphokinetics in the asynchronous embryo cleavages which might explain a possible cause for cell-size not-stage specificity. We also confirm embryonic reversed cleavage with the potential to repair incorrect relations between cell size and cell stage.

Trial registration number: EC/2017/0086.

P-535 Real time pH monitoring shows important information about embryo culture conditions

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Study question: Is spot checking pH once a month or once a week sufficient to understand incubator performance?

Summary answer: No, several incubator conditions are investigated with pH monitoring. Some incubator conditions can result in pHs that are outside the desired levels for embryo development.

What is known already: The pH of media is important to control because embryos are unable to regulate internal pH and pH plays an important role in embryo metabolic activity. Spot checking practices in embryo culture are typically done after only one day of media equilibration in an incubator. Embryos are cultured with media in incubators for more than one day. It is important to understand the pH at times longer than one day and in various incubator conditions that can occur in the laboratory normally.

Study design, size, duration: This is a limited size study evaluating the effect of culture environment changes on the pH of media used for embryo culture. Three different types of incubators are evaluated.

Participants/materials, setting, methods: SAFE Sens TrakStation and TrakPods are placed in various incubator types including benchtop and a cabinet with thermal conductivity CO2 sensor and a cabinet with infrared CO2 sensor. pH monitoring is performed using an IVF media. Various external stimuli are applied to the incubator environment. The resulting pH measurements from those impacts are recorded and trended. pH values are also spot checked against a blood gas analyzer at various points in time.

Main results and the role of chance: Sufficient time for the initial equilibration of media in a CO2 environment is critical to achieve the desired pH. In many cases, at least 24 hours is needed for pH to reach steady state levels. The CO2 adsorption into media is slow due to the use of an oil overlay in normal culturing conditions.

Benchtop incubators do a very good job of maintaining pH during a 7 day period of time. Lid openings and temperature fluctuations in benchtop incubators do have an impact on the pH monitored.

Cabinet style incubators have some pH variability due to fluctuations in CO2 levels. Those incubators which use thermal conductivity CO2 sensors to maintain CO2 levels show much greater pH changes when humidity changes and when temperature changes. Door openings cause pH changes in the media.

Several figures showing pH profiles will be presented for full understanding of the impacts determined.

Limitations, reasons for caution: The technology looks only at a surrogate media sample that is similar to the media that embryos are cultured in during normal practice. There were no embryos cultured in these tests to show embryo development outcomes.

Wider implications of the findings: These studies educate us about fundamental mechanisms during embryo culture. The culturing environment is well known to be an important variable in embryo development. The impact on pH from these incubator variables studied can now be understood and improvements to culture environments can be made by any lab.

Trial registration number: NA

POSTER VIEWING SESSION
PARAMEDICAL - NURSING

P-536 Known, anonymous or known-anonymous oocyte donation: decision making in parents and prospective parents

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Study question: Why do heterosexual couples opt for known, anonymous or known-anonymous oocyte donation and how do parents experience this decision post-treatment?

Summary answer: Irrespective of the type of oocyte donation, being able to experience a pregnancy and being responsible for the education of the child was highly valued.

What is known already: The literature suggests that the genetic link between donor and recipient woman and clear boundaries and roles are important motivations to choose a donor. Couples are also led by their intention to maintain secret about or disclose the mode of conception to the child.

The strength of this study, compared to previous research, is the in-depth exploration of three types of oocyte donation preferences. Further to that, the data were not gathered during a counselling session but as a part of a research project by interviewers working independently from the fertility clinic where the participants were recruited.

Study design, size, duration: For this study, 19 heterosexual couples were recruited at the Department of Reproductive Medicine of a University Hospital. They consented for an in-depth, semi-structured couple interview. One couple was interviewed separately, from two couples only the woman participated. The interviews were conducted between June 2013 and May 2014. Data were analysed through step-by-step inductive thematic analysis. Approval of the clinic's Ethics Committee was obtained.

Participants/materials, setting, methods: 6 couples became parents after known sister-to-sister oocyte donation, 6 couples opted for known-anonymous donation (= the couple introduced an oocyte donor and in return they received oocytes from an anonymous donor) and 2 couples opted for anonymous donation. We also included prospective parents: 4 couples were pregnant after or awaiting treatment with known-anonymous donation. One additional couple opted for mirror donation (= the male partner donated sperm to reduce the waiting time for oocytes).

Main results and the role of chance: Known oocyte donation was mostly performed because the donor suggested this as an option. For the couples it

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was important to know where the genes came from. They were glad that the mother had a genetic link with the child through the donor (sister-to-sister oocyte donation) but the presence of this genetic link was also challenging for some (e.g. when the child resembled the donor).

Known-anonymous donation was mostly performed to avoid the waiting list for anonymous donation. The latter was the only option left when the couple had no available donor to perform known-anonymous donation. The benefits of either anonymous or known-anonymous donation were the protection of the position of the mother and of the family as a whole. Known donation was not an option because it was unclear how the relationship between the couple and the donor would evolve, because of feelings of obligation towards the donor afterwards and because they feared that known donation would be confusing for the child. For most couples, a genetic link between parent and (future) child was of no importance.

Limitations, reasons for caution: Although different types of oocyte donation were studied, sample sizes in subgroups were sometimes very small. It should be taken into account that known donation between sisters is different from known donation between non-relatives because of the presence of an indirect genetic link between mother and child in the former.

Wider implications of the findings: In countries where different types of oocyte donation are permitted, insight into the couple's motivations is an important basis for the counselling sessions. The counsellor can support the couples in their decision by discussing the consequences of each type of donation, even if they already made up their mind beforehand.

Trial registration number: Not applicable.

P-537 Easy-to-use software interface for family medical tree construction

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Study question: To develop family medical tree construction software for genetic counseling

Summary answer: We have developed software that can be used to construct a family medical tree.

What is known already: A family medical tree is a method for tracing the way genes are passed through families. However, no easy-to-use software tool for this purpose exists as of yet.

Study design, size, duration: Development of family medical tree construction software for genetic counseling

Participants/materials, setting, methods: The Pedigree-Builder-System (PBS) consists of two components: the PBS database and PBS tree viewer. The PBS database stores the recorded content of genetic counseling, including family and personal medical history. The PBS tree viewer visually constructs the family medical tree based on the information in the PBS database. The PBS database and PBS tree viewer were developed using FileMaker and Excel Visual Basic for Application (VBA), respectively. The tree construction algorithm was written using VBA.

Main results and the role of chance: People with a family history of a genetic disease can be concerned they have inherited it and/or that they could pass it on to their children. Genetic counseling provides information about genetic conditions and birth defects. Genetic counselors can use the family medical tree to understand the significance of genetic disorders in the context of personal and familial situations. Thus, we have developed software that can be used to construct a family medical tree. This system provides an easy-to-use, graphic interface that can be used by genetic counselors and other genetics professionals.

Limitations, reasons for caution: None

Wider implications of the findings: In recent years, the use of assisted reproductive technology (ART) has increased dramatically worldwide. The genetic causes of infertility are varied and include chromosomal abnormalities, single-gene disorders and phenotypes with multifactorial inheritance. Using our system, genetic counselors and other genetics professionals can visualize hereditary patterns efficiently.

Trial registration number: N.A.

P-538 Education on age related fertility decrease in women: who, what, when?

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Study question: How should education on age-related fertility decrease (ARFD) in women be provided to new generations for preventing future infertility problems?

Summary answer: Education on ARFD should be provided to young women and men by healthcare professionals and using online materials that encompass a personal risk evaluation.

What is known already: Ageing is against fertility in both men and women, mainly due to the diminution of gamete's quantity and quality with the passing of the years. Woman's age over 35 is the main risk factor for suffering from infertility, but the general public is not sufficiently aware of the age related fertility decrease in women. Moreover, there is a general overconfidence in the success of assisted reproductive technologies at any age. The aim of this study is to compile quantitative and qualitative studies focused on ARFD knowledge and the interventions performed to increase this knowledge and direct further education.

Study design, size, duration: Systematic narrative review of scientific studies published between January 2000-January 2017 in English, Spanish, French and Portuguese, with no country restriction. The search was conducted in 4 databases. We evaluated ARFD knowledge worldwide, highlighting those studies describing interventions aimed to increase it. ARFD knowledge was conveyed through questionnaires, interviews, focus groups and RCTs. Papers focused on contraceptives use, sexual behavior, STIs, cancer and consequences of aging on pregnancy and perinatal outcomes, were excluded.

Participants/materials, setting, methods: The initial search gave 1204 studies; 78/1204 were finally included; 34/78 asked for ARFD knowledge; 14/68 were interventional studies and 4/14 had an RCT design. Geographical distribution was: Europe (32, 41%), North America (24, 30.8%), Asia (10, 12.8%), Australia (8, 10.3%), other countries (2, 2.9%); international (2 2.9%). Gender distribution was: men and women (38, 48.7%), only women (47.4%), only men (3, 3.8%). Twenty-five (32.1%) were performed in university students; 5 (6.4%) in healthcare professionals.

Main results and the role of chance: ARFD knowledge was evaluated by the correct identification of the most fertile age for a woman (20-25 years) and the ages when a slight (25-29 years) and marked (35) fertility decrease occurs. The range of correct answers within the studies varies between 16% and 89.4% for the most fertile age, between 6% and 62.4% for the slight decrease and between 5.6% and 56.9% for the marked decrease. More than half of participants were usually able to identify the most fertile age for a woman, but less than half recognized the slight and marked decreases. In general, worse answers were obtained from people with lower education and from men. Tailored interventions aimed to increase fertility knowledge included printed and online materials, slide presentations and personal risk evaluation. In general, the interventions evaluated were effective in the short-term, but not maintained in the long term, nor resulted in a change in childbearing attitudes. This could be due to a lack of perceived susceptibility to suffer infertility in young people or a perception of lack of choice among the oldest women.

Limitations, reasons for caution: The interventions to measure and increase ARFD knowledge evaluated in this work had a setting of scientific research (original articles published in scientific journals). Surveys performed at other levels and the effect of information provided outside of educational interventions have not been investigated.

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Wider implications of the findings: ARFD knowledge could be increased through targeted campaigns to educate the general population about infertility risks and fertility protection, especially in younger people and enhancing personal risk evaluation. Collateral effects that ARFD information may cause (e.g. anxiety) may also be prevented by targeting interventions to younger people than currently done.

Trial registration number: NA.

P-539 What factors influence the timing of infertility treatment for a second child?

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Study question: What factors affect a woman's decision to begin fertility treatment for a second child after the birth of the first?

Summary answer: Second fertility treatment was delayed if the cause of the first infertility was unknown, and if the first birth was at a young age.

What is known already: Ninety percent of couples want a second child, according to surveys in Japan. However, previous studies suggest that the fertility rate decreases when the second pregnancy is not started within 3 years after the first birth. The pregnancy rate also diminishes in women aged over 40, even for those who have given birth previously. Therefore, the interval between the first and second conceptions is considered important for a second pregnancy.

Study design, size, duration: This was a qualitative, cross-sectional, paper-based questionnaire study. The respondents were 246 women planning to have a second child after having received infertility treatment before the first pregnancy. The study was conducted in Hanabusa Women's clinic in Kobe, Japan, from July 2015 to January 2016.

Participants/materials, setting, methods: The questionnaire included the woman's age, first child's age, number of years from the first birth to the start of the second fertility treatment, previous experience of infertility treatment or ART, and the cause of infertility at the first conception. The questionnaire was given to patients after they provided informed consent. The women were assured that they would experience no invasion of privacy and no disadvantages if they did not wish to respond.

Main results and the role of chance: The response rate was 81.5%. The average age of respondents was 36.7 years. The average age of the first child was 1.8 years and the most frequent age was between 1 and 2 years old. The average interval between the first birth and the start of the second fertility treatment was 1.6 years and the most frequent interval was 1 to 2 years (45.1%). Older women tended to start the second fertility treatment earlier than younger women: those aged under 29 waited an average of 1.9 years, whereas women over 40 waited only 1.1 years. The causes of infertility at the first child included fallopian tube abnormality, male factors, uterus factors, and unknown factors. The most frequent cause of infertility in women who started second fertility activity within three years after the first birth was fallopian tube abnormality (27.5%), whereas in women who waited longer than three years it was unknown cause (26.1%).

Limitations, reasons for caution: The study was based on a questionnaire with a limited sample size. Future studies should increase the number of patients recruited from multiple facilities to confirm this preliminary conception. Clinical outcomes should also be examined.

Wider implications of the findings: Unknown cause of infertility at first conception and having a first baby at a young age delay the start of second fertility treatment. Analyzing these factors may encourage women to start fertility treatment for the second child early as possible.

Trial registration number: N/A

P-540 Nursing protocol for the risk identification and prevention of Ovarian Hyperstimulation Syndrome (OHSS) in patients undergoing IVF treatment

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Study question: Can the nursing protocol identify the risk of ovarian hyperstimulation syndrome (OHSS) for women undergoing controlled ovarian stimulation (COS) for an *in vitro* Fertilization (IVF) cycle?

Summary answer: The OHSS nursing protocol propose to identify the risks and monitor signs of OHSS to prevent occurrence, signaling clinicians and allowing safer treatment protocols.

What is known already: OHSS is an iatrogenic and serious complication of COS, with estimated prevalence of 20%-33% in mild, 3%-6% moderate and 0.5%-5% severe. The prevalence might even be higher in women presenting risk factors. OHSS is clinically characterized by abdominal tenderness and swelling due to multi-follicular growth and increased ovarian volume. If the condition progresses to severe OHSS, the augmented vascular permeability results in a shift of fluid to the extravascular space and several haemodynamic changes take place, leading to severe morbidity. Early recognition of risk factors and identification of OHSS mild signs can help clinicians tailor treatment regimens and prevent occurrence.

Study design, size, duration: This study aim to propose an OHSS nursing protocol (OHSS-NP) to recognize risk factors for OHSS and monitor patients during COS in order to identify mild sign of this condition as early as possible, and prevent the occurrence of this syndrome. This tool will be initially applied for 30 patients undergoing COS to validation. After any necessary adjustment, the OHSS-NP will be implemented for all patients undergoing COS in the IVF clinic.

Participants/materials, setting, methods: The OHSS-NP construction was founded on evidence based medicine. A team composed by reproductive medicine nurses reviewed the literature to stablish all risk factors, signs and symptoms of OHSS. Based of those findings, the nursing team created the OHSS-NP to identify risks and monitor patients undergoing COS during IVF treatment. The tool was reviewed by reproductive medicine specialists with recognized experience in OHSS management.

Main results and the role of chance: The OHSS-NP will be set into two steps. Step-1 intend to identify the OHSS risk based on patient's history, infertility factors, antral follicle count, anti-mullerian hormone measurement, age, body mass index and others conditions associated. It will be applied before the COS starting and patients will be classified as high, moderated or low risk. The step-1 outcomes will guide the initial dose of gonadotrophin for COS aiming to decrease the risk for patient develop OHSS. The step-2 will be placed in the moment of fourth ultrasound during the COS, before the trigger, and it will include the monitoring of OHSS signs. This step will evaluate the number of follicles with 12-14 mm in diameter, serum estradiol measurement and any clinical sign reported by the patient. If the patient present risk or any sign of OHSS, the treatment protocol can be adapted in order to prevent occurrence or evolution. Also, nursing care post-operatory protocol will be applied to contact patients regularly and monitor symptoms. The efficacy of the OHSS-NCI will be evaluated by comparing OHSS rates occurred during 2016 (mild: 5.6%, moderated: 4.9%, severe: 2.1%) with the rates observed after the implementation of this instrument in a population with same clinical characteristics.

Limitations, reasons for caution: This is a study proposing a tool for risk identification and monitoring patients under COS aiming early identification of OHSS, allowing a tailor treatment regimens and avoid it progression and more severe complications. However, this tool was not implemented yet and it efficacy will be possible in the future.

Wider implications of the findings: The nursing care is essential in IVF treatment. Nurses have a closer relationship with the patient and monitoring strict protocols. Our OHSS-NCI can help the early identification of OHSS and

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prevents it progression and complications, by quick treatment tailor, reducing the incidence of moderate and severe forms of the syndrome.

Trial registration number: not applied

POSTER VIEWING SESSION
PSYCHOLOGY AND COUNSELLING

P-541 Mental health profile of infertile women seeking ART in China

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Study question: What is the mental health profile of infertile women and implications on the utilization of assisted reproductive technology in China?

Summary answer: 10.0% exhibited moderate to severe levels of depression, while 27.6% experienced significant marital stress. These outcomes were related to previous pregnancy experiences and childbearing expectations.

What is known already: Studies have shown that women in who sought infertility treatment had an impaired psychological well-being. Under the One Child Policy, infertile women in China experienced further deterioration in mental health following failed treatment. Since 2016, the revised Two-Child policy allow any married couples to have two children, however, the impact of policy change on the mental health status of infertile individuals remain unknown.

Study design, size, duration: This was a cross-sectional study. Female patients aged 20-45 years old attending outpatient clinics for non-malignant infertility problems were approached. 1142 completed the questionnaire. The study spanned two years.

Participants/materials, setting, methods: Participants were recruited from 7 outpatient clinics for reproductive health in Mainland China. Their average age was 32.0 (SD=5.2) and married for 6.1 years (SD=5.1). The majority of them had received tertiary education (80.1%) and engaged in full-time employment at the time of study. 52% had prior ART experience. Measurements include General Health Questionnaire 9, Kensus Marital Scale, Traditional Childbearing Belief Scale and Childbearing Importance Index.

Main results and the role of chance: The mean scores of GHQ-9 and KMS were 4.4 and 17.8, respectively. Slightly more than 50% of women experienced minimal depression, 32.9% with mild depression, and 10.0% having moderate to severe depression. Marital stress was significant in 27.6% of couples. Greater age (wives and husbands) and longer marriage were related to more marital stress. Confidence in reaching the ideal number of children by a desired age, subscription to traditional childbearing beliefs and greater perceived importance of childbearing were related to better marital adjustment. While longer treatment years and previous induced abortion were associated with poorer marital adjustment, spontaneous abortion and stillbirth history was however related to greater marital satisfaction but also depression. The status of infertility was related to greater depression, but concurrent childlessness was associated with better marital adjustment. While the presence of female cause was associated with greater depression, presence of male cause was however related to less depression. Attribution of childlessness to the development of personal interest was associated with less depression, but economic reasons was related to more depression and marital stress. Health reasons were related to greater depression but better marital adjustment. Statistical significance was indicated by $P < .05$.

Limitations, reasons for caution: The current study included only female patients of an outpatient obstetric and gynecology clinic, which may render the results less representative of a general female population of China. Depression was measured by a self-report instrument rather than a clinical interview.

Wider implications of the findings: Results highlights the mental health and relational implications of childlessness, infertility and ART treatment among women in the Mainland China. The perceived intentionality of childlessness, confidence in achieving ideal parity and the ability to turn reproductive loss to a relational lesson appears to determine the mental health and relational outcomes.

Trial registration number: Nil.

P-542 Myself, my family and the donor; the experiences and views of adult donor conceived persons

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Study question: How do adult donor conceived persons view and experience themselves, their family and the donor

Summary answer: Adult donor conceived persons view themselves as positively distinctive, view their families positively and see the donor as significant but not 'dad".

What is known already: Many donor conceived persons desire information and possible contact with their donor and genetic and half-siblings and research has shown generally positive outcomes when contact with the donor has taken place. There is some emerging research that describes how adult donor conceived persons make sense of their family building history and in particular the importance and significance of genetic and non-genetic relationships. Little is known about how this impacts on their view of family however.

Study design, size, duration: A convenience sample of 21 adult donor conceived persons who were born in New Zealand participated in the study which involved in-depth semi-structured interviews. The qualitative design enabled the experiences, meanings and perspectives of the participants to be ascertained. Interviews were conducted over a 5 month (February-July) period in 2016.

Participants/materials, setting, methods: 15 females and 6 males aged between 19 and 46 (mean age of 30) who were born in New Zealand participated in the study. All but one were born into heterosexual led dual parent families. Thirteen of the interviews were Skype video calls, one was a Skype audio call and seven were phone calls. Interviews were recorded, transcribed and thematic analysis conducted.

Main results and the role of chance: Three main themes emerged regarding the view of donor conception.1) positive distinctiveness, 2) gratitude to the donor, 2) appreciation of family. Family was defined by love, connectedness and shared experience. The main theme that emerged regarding the role of the donor in relation to the family was one of dual importance but distinctiveness e.g. dad and donor were seen as very different, donor and half-siblings were special, important and 'like family but different" because the bond is based on genes and not emotion. A second less commonly described theme emerged in which interest in or seeking information or contact with the donor was seen as a sign of disloyalty or ungratefulness to their parents. At the time of the interview half had attempted or made contact with the donor with three having met him. Reasons for seeking information/contact were curiosity about shared traits, medical and cultural history. A sense of uneasiness was a common theme amongst those who had little information and had not made contact with the donor and this sometimes leads to seeing the desire for information as an obsession.

The role of chance in these results is minimal.

Limitations, reasons for caution: The study was based on a convenience sample and is not likely to represent all donor conceived adults. The New Zealand culture and policy regarding donor conception will have impacted on participants experiences. As with all qualitative research the ability to generalise from the findings should be treated with caution

Wider implications of the findings: The findings have the potential to reassure parents concerning the positive way in which adult donor conceived persons are likely to see themselves, their families and the donor. Counsellors

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will be able to use these findings as part of preparing would be parent/s for family building using donor conception.

Trial registration number: This was not a clinical trial.

P-543 Decision control preference of Chinese infertile women

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Study question: This study was the first of its kind in China, to assess decision control preferences (DCPs) of Chinese infertile females and the influential factors.

Summary answer: Majority of Chinese infertile women had high and medium DCPs. Age, education, living area and severity of infertility were factors associated with DCPs.

What is known already: Shared decision making is one of the key issues in patient-centered care. DCPs is the quantity of control patients prefer to have over their medical decisions. Control Preference Scale by Ende J is a widely used instrument to measure patients' DCPs. DCPs can be defined as high (patients want to make all decisions on their own), medium (patients share decisions-making with their doctors) and low (patients leave all decisions to their doctors). Low DCPs among the general population estimate to be 13% to 44%. Factors as age, gender, education and health status might affect DCPs.

Study design, size, duration: 270 infertile females seeking assisted reproductive techniques (ART) procedures because of tubal occlusion were asked and 237 women were agreed to participate in the study (corresponding rate 87.78%). Some tubal occlusion women complicated with endometriosis, low ovarian reserve, premature ovarian failure (POF) or polycystic ovary syndrome (PCOS). Those illiterate patients and women with male factors infertility partners were excluded from the study. Prospective survey was performed during Oct, 2014 to April, 2016

Participants/materials, setting, methods: A cohort of 237 infertile female patients were recruited to participate in the study. A modified decision control preferences was used to assess the degree of control the infertile women prefer over their ART decisions. The original DCPs were modified to create a trilevel variable: patient-led (original option 1 or 2, high DCPs), shared decision making (medium DPCs, option 3) and physician-led (low DCPs, option 4 or 5)

Main results and the role of chance: Of the 237 patients,161 (67.9%) had tubal occlusion without other abnormalities, 68 (28.7%) were Minorities, 149 (62.9%) had college education or up, 154 (65%) living in city and 99 (41.8%) had previous medical decision-making experience as surgical operation, laparoscopy or ART. 46.8% (n = 111) preferred to share decision making (medium DCPs) while 23.6% (n = 56) preferred patient-led (high DCPs) and 29.5% (n = 70) physician-led (low DCPs). The mean age of patients with low,medium and high DCPs was 37 ± 5.1, 30 ± 3.4 and 29 ± 3.6,respectively. Of the 70 patients with low DCPs, 52 (59.72%) were more than 36 years old, 58 (82.9%) living in rural area, 60 (85.7%) with low education ,46 (65.7%) had no previous decision experience and 59 (84.3%) had abnormalities other than tubal occlusion. Of the 56 patients with high DCPs, 42(75%) were younger than 30 years old, 49 (87.5%) with college and up education ,53 (94.6%) living in city. Age (r=-0.610), education (r=0.574), severity of infertility(r=-0.653) and living in city (r=0.610) were correlated to DCPs (all p = 0.000)

Limitations, reasons for caution: Though the patients of our Hospital coming all over the country, the sampling limited to our hospital might affect the generalizability. The cross-sectional design of the study might not clarify the causality

Wider implications of the findings: Older age, low education,living in rural area and severity of infertility were demonstrated to play important roles in low DCPs of Chinese infertile women. More attention need to be paid to those females to help them improving their self-advocacy and thus improve the quality of patient-centered infertility care

Trial registration number: none.

P-544 The impact of psychological counselling on dyadic outcomes for infertile couples: a common fate analysis

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Study question: Do dyadic quality of life, marital satisfaction and need for parenthood differ for couples in infertility treatment who do or do not receive psychological counselling?

Summary answer: Couples who received psychosocial counselling had a higher level of dyadic quality of life and a lower level of dyadic need for parenthood.

What is known already: An important issue in delivering care to infertile couples is to improve treatment compliance and overall patient wellbeing during treatment. Tailored psychological interventions have been developed specifically for infertile patients to deal with the stressful experience of Assisted Reproduction Treatment (ART) and also to optimize the treatment experience.

Previously published reviews examined the efficacy of psychological interventions for infertile patients, obtaining different results. Unfortunately, most evaluations of couples' counselling focuses on outcomes or changes in the individual members of the couple, but do not focus on the couple-as-a-whole as the outcome variable.

Study design, size, duration: A longitudinal study, with a quasi-experimental design, was conducted on 34 infertile couples that received, and 34 matched (propensity scores) infertile couples that did not receive, psychosocial counselling during their infertility treatment, from February 2013 to November 2014.

The Common Fate Model (CFM) was used to examine dyadic changes in quality of life, need for parenthood and marital satisfaction. The CFM assesses whether the dyad has changed as a result of counselling.

Participants/materials, setting, methods: Patients undergoing ART at the ANDROS Day Surgery Clinic, Reproductive Medicine Unit in Palermo (Italy), filled in questionnaires on Quality of Life (QoL -Fertility Quality of Life Questionnaire), Need for Parenthood (NP - 'Need for Parenthood" subscale of the Fertility Problem Inventory) and Marital Satisfaction (MS - ENRICH Marital Inventory) at two points during infertility treatment (before treatment and on the day of IUI or Embryo-Transfer).

Main results and the role of chance: Both the male (T1=.76, P < .001; T2=.75, P < .001) and female (.62, P < .001; T2=.60, P < .001) partner's QoL had significant loadings on dyadic QoL at times 1 and 2. Time 1 dyadic QoL was significantly related to time 2 dyadic QoL (T1 = 1.05, P < .001). Couples receiving counselling had higher dyadic QoL at time 2 compared to couples not receiving counselling (0.14, p=.04).

Both the male (T1 = .78, p < .001; T2 = .80, p < .001) and female (.75, P < .001; T2=.80, P < .001) partner's NP had significant loadings on dyadic NP at time 1 and 2. Time 1 dyadic NP was significantly related to time 2 dyadic NP (0.93, P < .001). Couples receiving counselling had lower dyadic stress due to NP at time 2 compared to couples not receiving counselling (-0.17, p=.04).

Finally, both the male (T1=.86, P < .001; T2=.75, P < .001) and female (.81, P < .001; T2=.84, P < .001) partner's MS had significant loadings on dyadic MS at time 1 and 2. Time 1 dyadic MS was significantly related to time 2 dyadic MS (T1 = 0.83, P < .001). Contrary to our hypothesis, couples receiving counselling did not have higher dyadic MS at time 2 compared to couples not receiving counselling (0.03, p=.74).

Limitations, reasons for caution: The study has several limitations. Firstly, the low number of couples analyzed. Secondly, the couples receiving or not receiving counselling were not randomly assigned. Finally, the couples' counselling was brief; with longer-term, more intensive counselling there may have been more counselling-related changes in the dyadic effects.

Wider implications of the findings: The CFM allows researchers to examine how the dyad-as-a-whole responds to counselling, highlighting relational

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dynamics. The findings provide support for continued research into the interpersonal and intrapersonal dynamics that can change as a result of couple counselling, especially when a stressful situation such as infertility and ART has been experienced.

Trial registration number: Not necessary.

P-545 What is the reproductive health of former egg donors a decade after their donation?

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Study question: What is the long-term reproductive health of oocytes donors a decade after donation?

Summary answer: The long-term reproductive health of former oocyte donor does not differ from the expectations for their age group.

What is known already: The short term physical and psychological outcomes of oocyte donors have been studied extensively and are well established. However, there are very few reports studying the long term reproductive outcomes of former oocyte donors. Additionally, most such studies are both small in number of women included, and focused mostly on the psychological aspect of the donation. As thousands of oocyte donations are performed worldwide each year, there is an urgent need to analyze the long-term impact of this technique on donors' reproductive health. This study evaluates the reproductive health of former oocyte donors a decade after their donation.

Study design, size, duration: Structured anonymous telephone survey, consisting of 12 questions covering several aspects of reproductive health, such as number of pregnancies and outcomes, gynecological disorders after donation, menopause symptoms and contraceptive use. The survey was performed by 3 trained reproductive nurses between June and November 2016. A total of 141 former oocyte donors were contacted by phone and 121 agreed to participate in the survey (Response rate: 85.8%).

Participants/materials, setting, methods: Former donors were contacted chronologically (oldest donations first); inclusion criteria were to be ≥40 y.o. to encompass most of their reproductive life and speaking Spanish. Most participants were Spanish nationals (99.2%), and were 41.6 ± 1.9 y.o. at the time of interview (range: 40-49); they were 32.2 ± 2.2 y.o. at the time of their first donation (range: 26-35); 5 to 16 years had elapsed on average since the donations, which had been on average 2.5 ± 1.8 for each donor.

Main results and the role of chance: At the time of interview, 84 former donors (69%) had children and 64 (53%) were already mothers at the time of their donation (number of children 1.1 ± 0.9; range: 0-3). In the decade following oocyte donation, 95 women (78.5%) reported having used hormonal contraceptive methods and most (81%) attended annual well-woman exams. In the years following the donation, 22 women (18%) reported losing a pregnancy, either spontaneously (9%) or by medical intervention (9%). Of the 39 pregnancies resulting in live births, 6 complications were reported by 5 donors: 3 preterm births at week 35-36, 1 minor bleeding with amniotic fluid loss, and 1 minor preeclampsia. Currently, 2 of 121 (1.6%) former donors reported being in menopause (ages 41 and 45). Twenty-three women (19%) reported gynecological events in the years since donating: the most frequent being myomas (7), ovarian cysts (4) and endometriosis (2). Five women (4.1%) had experienced infertility, and 2 of them considered this a direct result of their donation. These 5 women were younger than 40 at the time of infertility. At the time of interview, 1 of them had achieved a pregnancy. One woman reported being told by her physician that her myomas were caused by the donation.

Limitations, reasons for caution: A limitation of the current study is the inclusion only of donors who spoke Spanish, which might have limited the diversity of the population. However, this ensured a high level of understanding with the nurses contacting the participants, ultimately adding to the quality of the study.

Wider implications of the findings: This study represents one of the largest so far on the long-term reproductive health of former oocyte donors, and the only one following them into the post reproductive years. Former donors present low incidence of gynecological/fertility problems. Long term reproductive health seems adequate and no specific alteration could be evidenced.

Trial registration number: NA.

P-546 Why undertaking parenting alone? Single gay men's experiences and decisions of having a child by surrogacy

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Study question: Which factors contribute to gay men's decision of having a child by surrogacy as a sole parent?

Summary answer: Factors are: right time, having 'worked through" their own internalised homophobia, being tired of waiting for the 'right" relationship, desire for a child, social support.

What is known already: As it is only recently that single people, whether they are heterosexual, lesbian or gay, have been accepted by clinics as candidates for using assisted reproduction to become parents, no studies have yet examined single gay men's decisions of having a child by surrogacy. Earlier studies on single mothers through donor insemination may inform us partly, showing that relevant factors to 'go it alone" are financial security, the right time in their career, the sense that time is running out, the feeling that they have sufficient social support, and the desire of being a mother.

Study design, size, duration: Twelve Italian single gay fathers (aged 41–55 years) who conceived their children (aged 1–4 years) by gestational surrogacy in California (n = 8), Canada (n = 3), and Thailand (n = 1) participated. Participants were recruited from an association of gay parents (n = 6), Facebook groups of single parents (n = 3), and snowballing (n = 3) between November 2016–January 2017. Three aspects were discussed: decision and timing of becoming parents through surrogacy; satisfaction with their reproductive choice; reactions of family and friends.

Participants/materials, setting, methods: In-depth semi-structured interviews were conducted in family homes (n = 8) or over Skype (n = 4) by a research psychologist trained in the study techniques. The interviews lasted approximately 60–90 minutes, were audio recorded with the participants' consent and transcribed verbatim. Data were analysed through inductive thematic analysis with the aid of the software package, MAXQDA and a continuous auditing process by the co-authors resulted in themes that were grounded in the data.

Main results and the role of chance: The decision to have a child by surrogacy as a sole parent was prompted by five main factors: timing felt right, in terms of career and financial security, as well as of fear of getting older; sense that they had 'worked through" their internalised homophobia; being tired of waiting for the 'right" relationship; desire of raising a child on their own; feeling of sufficient social support. Although all of the men were happy with their decision to go it alone, over half of them (n = 8) would have preferred to have a child within the context of a relationship. When directly asked, the majority (n = 10) stated that they had support by their family and/or part-time nanny as much as was needed, with only 2 participants wishing more support. From the start 3 and 7 participants received supportive reactions to their reproductive choice from their families of origin and from friends, respectively. The remaining reported that initial reactions were mixed, with families (n = 9) and friends (n = 5) having questions or feeling confused about both surrogacy and single parenthood, but then embracing the child when he or she finally arrived.

Limitations, reasons for caution: This study was limited due to the small sample size. Furthermore, there is a risk of selection bias, with fathers who had a negative surrogacy experience less likely to participate in research, although diverse recruitment strategies were used.

Wider implications of the findings: As prior to this study we didn't know the reproductive experience of single gay men using surrogacy, this study highlighted the complexity of factors affecting their decisions to become fathers in this way. Findings are of relevance to clinics and professionals supporting intended single fathers before, during and after treatment.

Trial registration number: Not applicable.

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P-547 A Randomized Controlled Trial of the Impact of Music Therapy on Pain and Stress Reduction During Transvaginal Ultrasound-guided Oocyte Retrieval

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Study question: Does music therapy reduce pain and stress scores during transvaginal ultrasound-guided oocyte retrieval (TUGOR)?

Summary answer: Both group of women had similar stress score but women in the music group experienced significantly less pain score.

What is known already: A recent randomized controlled trial demonstrated that music therapy reduced anxiety in patients undergoing embryo transfer. However, it is not known if music therapy may help in women undergoing TUGOR.

Study design, size, duration: A randomized controlled open label study of 180 women (music group n = 60, headphone only group n = 60, no intervention group, n = 60) undergoing TUGOR over a 12 months period in an IVF unit of a university based teaching hospital.

Participants/materials, setting, methods: Women undergoing TUGOR were recruited and randomized into 3 groups (music group, headphone only group and no intervention group). Patient's psychological status was systematically assessed through various instruments including visual analog scale of pain (VAS-P), state-trait anxiety inventories (STAI), Beck depression inventory (BDI) and general health questionnaire (GHQ). Salivary alpha amylase (sAA) was examined before and after TUGOR.

Main results and the role of chance: There was no difference in mean (± SD) psychological scores between 3 groups in (STAI, music group: 54.8 ± 9.4, headphone only group: 56.9 ± 10.3, no intervention group: 54.9 ± 10.2; BDI, music group: 6.6 ± 6.2, headphone only group: 6.8 ± 6.4, no intervention group: 8.1 ± 8.2; GHQ, music group: 2.3 ± 2.5, headphone only group: 2.8 ± 2.8, no intervention group: 2.5 ± 2.6). There was also no difference in the change of mean (± SD) sAA level (U/ml) between 3 groups (music group: 4.5 ± 0.7, headphone only group: 4.7 ± 0.6, no intervention group: 4.6 ± 0.7). However, the mean (± SD) vaginal pain scores in women receiving music therapy (19.3 ± 22.4) was significantly (P< 0.034) lower than those receiving headphone only (29.4 ± 26.9) and no intervention (30.6 ± 28.2).

Limitations, reasons for caution: Due to the nature of the study, double blinding was not possible.

Wider implications of the findings: This is the first randomized controlled trial to demonstrate that music therapy significantly reduced the perception of pain during TUGOR. Music therapy, being simple and inexpensive, is now routinely offered in our unit during TUGOR.

Trial registration number: This study was registered at Centre for Clinical Research And Biostatistics (Primary Registry and Trial Identifying Number: ChiCTR-TRC-11001657).

P-548 Resilience and Psychological stress after more than two IVF cycles failure

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Study question: Does higher Resilience levels in women help to cope with or overcome deterioration of emotional wellbeing after IVF treatment failure?

Summary answer: After more than two IVF cycles failure psychological stress levels were similar in women with high and normal/low Resilience.

What is known already: Previous cross-sectional studies on the course of emotional response to fertility problems have revealed curvilinear relationships between length of treatment and emotional distress with a positive relationship between distress and the number of unsuccessful treatment cycles. Resilience it is a measure of how individuals cope with, overcome, or become positively strengthened by changes and challenge. Furthermore, complex relationships between depressive symptoms and resilience exist. It has not been study yet if infertile women who display higher resilience may adapt better to emotional distress after IVF cycles failure.

Study design, size, duration: A prospective longitudinal, observational study on 202 couples during IVF-ICSI cycle was carried out between January of 2015 and December 2016 at the Asturias Central University Hospital (Oviedo, Spain) and In Vitro Fertilization Center of Asturias (CEFIVA). The research protocol of the study was reviewed and approved by the Asturias Ethical Committee, Oviedo, Spain.

Participants/materials, setting, methods: Ninety two couple were in first IVF cycle, 68 in second IVF cycle and 38 in third IVF cycle. Before starting the controlled ovarian hyperstimulation with a GnRH antagonist protocol, patients were asked to fill out an itemized general questionnaire (personal and partner sociodemographic data), the Connor Resilience Scale, the Center for Epidemiologic Studies Depression Scale (CESD-10) and the 10 items Cohen Stress Scale.

Main results and the role of chance: Women who showed higher resilience levels according to Connor Resilience Scale showed lower mean psychological stress levels (11.3 ± 6.1 SD).than women with normal/low resilience (16.3 ± 5.9 SD) p <0.001. After three IVF cycles failure no significant changes in psychological stress levels were detected in women with normal/low resilience levels (14.9 ± 6.2 SD vs 17.5 ± 5.5 SD) p= ns, but in women with higher resilience levels a significant increase in psychological stress levels were detected (9.6 ± 5.1 SD vs 15.3 ± 6.4 SD) p = 0.005. No changes in CESD-10 were detected in both arms of the study

Limitations, reasons for caution: The short length of the follow-up period is a study limitation due to long-term psychological consequences. Moreover, there was a high rate of non-respondents and this might have introduced a reporting bias, since it could be that women who experienced more symptoms were less likely to fill in questionnaires

Wider implications of the findings: Women who showed higher resilience levels deal with psychological stress associated to IVF cycles during their first cycle, but after three IVF cycles failure have high psychological stress levels, similar to women with normal/low resilience levels. Higher resilience levels did not protect from psychological stress associated to IVF failure.

Trial registration number: N/A.

P-549 "I don't believe in statistics": Why do women keep choosing ineffective fertility treatments?

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Study question: Why do women over age 43 choose to pursue ART treatments using their own eggs, while the chances for success are extremely low?

Summary answer: Despite being informed, most advanced age ART patients have extremely unrealistic expectations of ART and actually ignore their estimated prognosis when making decision regarding treatments.

What is known already: Delivery rate per IVF cycle at age 43-5 is 5% at the most, and cumulative delivery rates are estimated at 15%. Patients often make decisions based on preconceptions, ignoring established medical knowledge. However, whether this happens in the context of ART is unknown.

Study design, size, duration: An anonymous questionnaire was distributed to patients during the years 2015-6. The participants filled it out on their own,

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responding to closed-ended questions, such as – what their success rates are according to their physicians and to themselves, and open ended questions, such as whether they had any personal characteristics that increased their fertility chances.

Participants/materials, setting, methods: Women having fertility treatments at age 43-5 using their own oocytes were asked to fill an anonymous questionnaire. Setting was seven fertility clinics. The recruiter asked the women to fill out a questionnaire, and made it clear that participation would have no influence on their care and that their attending physician would not be informed of their responses. Participants were 72 women, scheduled for ART treatments using their own oocytes at mean age 43.8 ± 0.7.

Main results and the role of chance: 60 (83%) respondents had previous treatments before participation in the study. Participants estimated delivery rates in the next treatment cycle at 48 ± 32%, and cumulative delivery rates after all treatments they will undergo at 62 ± 34%. Only 15% reported they received no information from their providers regarding estimated success rates, while 73% reported they were given verbal and /or numerical/quantitative information. 41 (59%) women described how their providers estimated their chances. Of those, 24 (58%) said they had been given a very low estimation of success in a clear way, either numerically (1-5% delivery rate) or verbally ("very low chances" etc.). 14 (34%) had been given verbal description which may be interpreted as less pessimistic ("low chances", "possible chance"). Only three women (7%) claimed they had been given optimistic delivery prospects. When asked "had you known your estimated chance for delivery is lower than a given value, would you avoid having treatment in the first place?" 80% responded they would have attempted treatments regardless. 65% of respondents would like their providers to inform them about estimated success rates even when those are very low. In contrast, 30% would like to receive such information only if they ask directly about it.

Limitations, reasons for caution: This study was performed in Israel where fertility treatments are fully reimbursed until age 45, without capping their number. Findings may have been different where patients pay fully for ART, or where caps exist, which send a message of how many IVF treatments are enough.

Wider implications of the findings: While advanced age was chosen as an inclusion criterion, findings may be applicable to other subgroups of ART patients facing ineffective treatments. Complex psychological factors seem to affect decision making of ART patients facing a poor prognosis. These findings may contribute to developing strategies aimed for better decision making.

Trial registration number: not applicable.

P-550 Relationship between psychological stress and assisted reproduction outcomes in Tunisian infertile women

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Study question: Is there any association between anxiety and assisted reproduction outcomes in Tunisian women undergoing IVF treatment?

Summary answer: Anxiety as assessed by cortisol level is correlated to assisted reproduction outcomes in Tunisian infertile women.

What is known already: The powerlessness to conceive children and the process of assisted reproduction technologies leads to a high level of anxiety. The impact of psychological stress on assisted reproduction outcomes is still a matter of controversy. The few available data on this topic especially in developping countries such as Tunisia don't seem to be sufficient to make conclusions concerning the possible benefits of stress management in infertile couples. The present study forms at the best of our knowledge the first one which was carried out in Tunisia to assess in which extent may stress influence reproductive outcome in couples seeking infertility treatment.

Study design, size, duration: This was a prospective study on women undergoing their in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at the Reproductive Medicine Unit at Farhat Hached University Hospital (Sousse, Tunisia).

Participants/materials, setting, methods: We recruited 64 infertile women. All patients who had an existing psychological problem were excluded. Participants were asked to fill out the Beck anxiety inventory (21 items) on the day of oocyte retrieval. Three blood samples were collected to assess free cortisol by radioimmunoassay (RIA) at three timepoints during the IVF treatment cycle (T1: before commencing IVF/ICSI treatment; T2: on the day of oocyte retrieval and T3: on the day of embryo transfer).

Main results and the role of chance: As clinical pregnancy is the ultimate goal in assisted reproduction, the most important finding in our study was that cortisol level on the day of embryo transfer was negatively correlated with clinical pregnancy rate ($p = 0.039$; $r = -0.309$). All patients that succeed to achieve pregnancy had an easy embryo transfer.

Cortisol level at T1 was negatively correlated with both fertilization and segmentation rates ($p = 0.02$; $r = -0.309$ and $p = 0.05$; $r = -0.257$). Patients with a long past of infertility have the highest cortisol levels on the day of oocyte retrieval (T2) ($p = 0.044$; $r = 0.273$). A positive correlation was also found between the follicle stimulating hormone level and cortisol value at T2 ($p = 0.015$; $r = 0.326$). Interestingly, there were no correlations between Beck score and neither cortisol level nor IVF/ICSI outcomes.

Limitations, reasons for caution: Patient groups included in the study were heterogeneous: women starting ART treatment for the first time and those who underwent repeated ART treatments.

Wider implications of the findings: This study is the first to evaluate the impact of anxiety on ART outcomes in Tunisian women. Our results show that stress management is of great importance and should be part of treatment protocol.

Trial registration number: This study was approved by the ethics commetee of Farhat Hached University teaching hospital (IRB00008931).

P-551 Reliability of FertiQoL in assessing fertility related quality of life in the Indian population

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Study question: Is FeriQOL reliable in estimating quality of life in Indian sub fertile couples and to assess the impact of infertility on quality of life in Indian sub fertile couples

Summary answer: The FertiQol instrument has demonstrated high overall reliability in the Indian population, though some of the subscales had poor internal consistency.

What is known already: Fertility adversely affects the quality of life in multiple domains and FertiQOL has been reliable in assessing the same in many population across the globe.

Study design, size, duration: The study was a cross sectional study and was conducted on 166 infertile couples. The data collection for the study was conducted over a period of 18 months from April 2015 to September 2016.

Participants/materials, setting, methods: The participants were infertile couples seeking treatment from a speciality reproductive centre in metropolitan city of Bangalore, India, selected by convenient sampling. Informed written consent was obtained and confidentiality of the participants was maintained. FertiQoL, a fertility specific quality of life assessment instrument, was used. Internal consistency of each item of the questionnaire was assessed by Cronbach's alpha (CA). Mean and standard deviation of each subscale score and the overall score was presented as recommended.

Main results and the role of chance: Majority of the women were between 26 to 35 years of age. More than 50% of the husbands were aged between 31 to 35 years. In majority of the subjects (65.7%) the duration of infertility was between 1 to 5 years. PCOS was the most common cause of infertility, seen in 50 (30.1%) couples, which was followed by unexplained (24.7%) and male factor (12.7%). Among the study population, majority (71.1%) of the women had primary infertility.

Among the 6 subscales of FertiQoL questionnaire, emotional and mind body components had shown highest internal consistency (CA of 0.75 and 0.79

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respectively). Social and tolerability components have shown moderate internal consistency (CA of 0.54 and 0.67 respectively). The components which have shown poor reliability were relational and environment subscales (CA of 0.38 and 0.37 respectively). When grouped together into core and treatment components the items have shown good internal consistency (CA of 0.86 and 0.62). The overall internal consistency of the entire instrument was 0.87. The mean subscale scores indicted that relational, environmental and social aspects of quality of life to be worse than emotional, mind body and tolerability aspects.

Limitations, reasons for caution: Generalizability of study findings is limited, considering the single centre and predominantly urban nature of study population. The effect of various sociodemographic and fertility related parameters on reliability could not be evaluated, due to limited sample size.

Wider implications of the findings: Will help in drawing the attention of clinicians towards importance of addressing the quality of life related aspect of infertility, through appropriate interventions. This in turn may help the subfertile couple to cope better.

Trial registration number: Not applicable.

P-552 Experiences of and wishes on guidance of donor-conceived offspring

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Study question: How do donor-conceived offspring experience guidance and what are their wishes for guidance?

Summary answer: Donor-conceived offspring wished that their parents would have received specialist guidance before donor sperm treatment, appreciated peer-support and the availability of specialist guidance for themselves.

What is known already: Most studies on donor-conceived offspring focus on psychological well-being and family relationships. These studies have shown that secrets in families about the genetic origin of the child can harm the parent-child relationship and as a consequence early disclosure of donor conception is recommended. Whether donor-conceived offspring wish to have guidance to cope with their questions and feelings related to being a donor-child is not known.

Study design, size, duration: We performed a qualitative in-depth interview study from October 2015 until February 2016 with 24 Dutch donor-conceived offspring. They were recruited by two network organisations where donor-conceived offspring can be matched with half siblings and the donor and who facilitate peer contact for donor-conceived offspring. Recruitment was also done through snowball sampling.

Participants/materials, setting, methods: We held semi-structured in-depth interviews with male and female donor-conceived offspring (M_{age} = 26.9, range 17-41) born within father-mother (n = 11), two-mother (n = 7), or single mother families (n = 6). The interviews were fully transcribed and analysed using the constant comparative method.

Main results and the role of chance: All donor-conceived offspring would have appreciated openness from their parents about being a donor-child and assistance in getting access to trustful information about characteristics and identifying information of their donor. Most donor conceived offspring had visited peer meetings and had exchanged feelings and experiences with other donor-conceived offspring. Most of them wished that their parents would have received adequate specialist guidance before donor sperm treatment to prepare them to talk openly with their child about donor conception. All wished to know where to find specialist counsellors for guidance when needed.

Limitations, reasons for caution: Recruitment was done through networks where donor-conceived offspring can be matched with half siblings and the donor and who facilitate peer contact, which may have led to selection bias. This may have been reinforced -by default- by the absence of donor-conceived offspring who are not informed about their genetic origins.

Wider implications of the findings: Specialist guidance should be available for all intended parents to share donor conception more easily with their partner, with people around them and with their future child. Furthermore it is highly recommended that peer-support and specialist guidance will be available and provide easy access for all donor-conceived offspring.

Trial registration number: Not applicable. Ethical clearance by Medical Ethical Committee of the Academic Medical Centre of the University of Amsterdam (NL53349.018.15).

P-553 Using or refusing 'social oocyte freezing': A qualitative analysis

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Study question: Goal of this qualitative study was the analysis of multiple reasons of men and women for using or refusing 'social oocyte freezing'.

Summary answer: Participants reported a wide spectrum of attitudes toward 'social oocyte freezing', with distinct misjudgment regarding fertility decline and men being much more critical than women.

What is known already: 'Social oocyte freezing', the human oocyte cryopreservation for non-medical purposes offers healthy women a medical procedure to postpone having children for 'social' reasons, like career intentions or lack of a partner. Since 'social oocyte freezing' is a relatively new and originally for cancer patient designed reproductive procedure, it stirs up many ethical and social debates. In the last years, there has been a growing research investigating intentions and fertility knowledge of potential freezers and non-freezers. However, qualitative studies exploring and analyzing 'social' reasons thoroughly, particular for men, are still missing.

Study design, size, duration: The qualitative study was part of a quantitative online study, approved by the Ethics Committee of the Heidelberg Medical Faculty. The study took place from April to June 2015 with a total of N = 643, thereof N = 144 participants in the qualitative part.

Participants/materials, setting, methods: The qualitative study was part of a quantitative online study, approved by the Ethics Committee of the Heidelberg Medical Faculty. The study took place from April to June 2015 with a total of N = 643, thereof N = 144 participants in the qualitative part.

Main results and the role of chance: In total, N = 85 women (Ø age: 36.1; 15,4% of total female sample) and N = 59 men (Ø age: 38.8; 65,6% of total male sample) noted 197 reasons for and against cryopreservation for non-medical purposes. Men and women showed distinct differences in their attitudes towards 'social oocyte freezing', with men having a much more negative attitude than women. Whereas women wrote 107 free-text reasons, among these 85 reasons in favor and 22 reasons against this technique; men reported 90 reasons, amongst 27 reasons in favor and 63 reasons against 'social oocyte freezing'. Qualitative analysis revealed that having no partner was the most mentioned reason by women, followed by fertility decline (in retrospective view) and early onset of menopause. Only few women indicated career issues and no woman reported reproductive autonomy or coverage - contrary to men, for whom those were the most mentioned reasons. Statements against human oocyte cryopreservation contained particularly ethical factors like being 'evolutionary nonsensical' and 'technically dangerous' for both sexes. Women noted additionally age factors, while men pointed out exploitation, a wrong societal development and indeterminate medical consequences.

Limitations, reasons for caution: Generalization of findings is limited due to the nature of online surveys and a possible motivational bias regarding the willingness to answer additional items.

Wider implications of the findings: The major differences between men and women reasons for and against 'social oocyte freezing', have to be investigated further to clarify specific needs necessary for future counseling. Furthermore, several statements indicated huge information deficits about reproductive techniques in the society, especially on fertility decline, that need to be dissolved.

Trial registration number: None.

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P-554 Six years of experience with DNA-linking donor-conceived offspring and sperm donors

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Study question: Aim of this study was to describe expectations and experiences of donor-conceived adults (DCO) and donors who registered in a DNA-database to search for genetic relatives.

Summary answer: Donor-conceived offspring feel they have the right to know their genetic relatives. Donors waive their anonymity in order to know more about their offspring.

What is known already: DCO are in search for information about or contact with those to whom they are genetically related through donor conception. This also applies to previously anonymous donors, they have a desire to know more about the results of their donations. Fiom is an independent knowledge center for questions related to people's descent and provides assistance with searching for genetic relatives in and outside the Netherlands. In 2010 Fiom established together with the Canisius-Wilhelmina Hospital, a DNA-database for DCO and sperm donors who have anonymously donated prior to 2004 (since 2004 DCO have the legal right to know their donor's identity).

Study design, size, duration: Qualitative data were collected during a six-year period in which support was provided to DCO and donors seeking genetic relatives. In addition, a questionnaire-based survey was used to generate information about DCO and sperm donors who enrolled in the voluntary DNA-database (Fiom KID-DNA Databank) between May 2015 and December 2016.

Participants/materials, setting, methods: Up to December 2016, 747 DCO and 316 donors have registered in the DNA-database. For 154 people, a family relationship was determined with at least one other person. These were 119 DCO and 35 donors involved in successful DNA-linking. Quantitative data were available for 213 DCO and 85 sperm donors. They answered a questionnaire with items regarding their demographic background, motives and expectations.

Main results and the role of chance: The mean age of DCO was 29 years and 71% of them were women. A third of the DCO were informed over their origins in early childhood (< 6 years), whereas 43% was informed at the age of 17 of older. DCO register themselves because they are curious and find that they have a right to know who they are descended from. They want information about their donor and seek recognition. Some DCO indicate that they experience a loss, others are just looking for medical information. Two third of the DCO are supported by their parents in their search for genetic relatives. The mean age of the donor is 63 years and 62% of them have done more than 10 donations. Their registration was supported by 83% of their partners and just more than one third of the children in the donors' family was informed about the donation and were supportive. Donors agree with DCO that they have the right to ask questions about them and they are interested in information about or contact/meeting with DCO. On a scale of 1 until 10 about the importance of a DNA-link between DCO and donor, DCO reported 7,8 and donors reported 6,4.

Limitations, reasons for caution: Although ending of donor anonymity in 2004 has increased openness around donor conception in the Netherlands, it is important to recognize that these findings only account for DCO who are informed about their conception and are actively searching for their genetic relatives. This may limit the generalization of the results.

Wider implications of the findings: This study provides useful information about DCO and donors who want to be found by genetic relatives. Because DCO and donor increasingly start a search for each other, these findings hold important implications for practice and policy.

Trial registration number: Not applicable.

P-555 Marital satisfaction and social support in infertile women with and without polycystic ovary syndrome seeking assisted reproduction treatment

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Study question: Is there any difference in marital satisfaction or social support between infertile women with and without polycystic ovary syndrome seeking assisted reproduction treatment?

Summary answer: The results showed that there is no significant difference between marital satisfaction and social support among infertile women with or without polycystic ovary syndrome ($P > 0.05$).

What is known already: Polycystic ovary syndrome has been found to have impaired sexual function, irregular mensturations, hirsutism, acnes, and obesity. Previous studies showed Polycystic ovary syndrome reduces sexual satisfaction in women which is a component of marital satisfaction. Social support is often referred to as peoples' receiving of social and mental helps in critical life conditions. Due to the importance of marital satisfaction in the overall satisfaction of the person and family and its impacts on the society's health, it is necessary to investigate the effects of this syndrome on marital satisfaction and social support in infertile women.

Study design, size, duration: A cross-sectional survey was conducted on 300 (150 PCO and 150 non-PCO) infertile women referred to the Royan institute, a referral infertility clinic in Tehran, the capital of Iran, between 2015 and 2016.

Participants/materials, setting, methods: The study was conducted in Royan Institute, a referral clinic in Tehran capital of Iran. Participants completed three questionnaires: The demographic questionnaire, the marital satisfaction Scale developed by Enrich, and Zeman et al.'s and Perceived Social Support Scale (MSPSS).

Main results and the role of chance: Data analysis was carried out by Uman-Wietni tests and dependent t-test. The results showed that there is no significant difference between marital satisfaction and social support among infertile women with or without polycystic ovary syndrome ($P > 0.05$). According to the results, it can be said that although polycystic ovary syndrome may have negative effect on women in different aspects, it cannot reduce social support and marital satisfaction in infertile women. Moreover, after adjusting for the association among response variables in multivariate model, and enter demographic variable such as age, occupation, education, causes of infertility and BMI in the model, the same results were found.

Limitations, reasons for caution: Because this study was cross-sectional, no conclusions on causality were inferred. We relied on patients coming to only one center, which is a referral clinic for infertility. So, the sample may net be a good representative of infertile women in Iran.

Wider implications of the findings: It seems that infertility has big negative effects on the women and psychological impacts of PCO is overwhelmed by infertility.

Trial registration number: none.

P-556 Fertility quality of life for patients undergoing IVF depends on infertility diagnosis

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Study question: Does type of infertility diagnosis influence the fertility quality of life (FertiQoL) of couples undergoing fertility treatments?

Summary answer: Endometriosis and anovulation have negative effect on FertiQoL compared to other infertility diagnosis. Women's age, and duration of infertility also have negative impact on FertiQoL

What is known already: The association between infertility and substantial personal distress is well established. Infertility treatments may worsen the stress of the infertile couple and decrease their quality of life. Only a few studies have tried to evaluate whether types of infertility diagnosis have an influence on fertility quality of life. Women with PCOS were found to have lower FertiQoL compared to couples with unexplained infertility, whereas endometriosis was not found to have negative impact on FertiQoL compared to patients without endometriosis.

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Study design, size, duration: Questionnaires were distributed to patients attending a public academic reproductive center between February 2015 and April 2016. All couples of infertility, who attended the clinic, were invited to complete questionnaires on quality of life. Questionnaires included socio-demography and the validated Fertility Quality of Life (FertiQoL). Overall, 1006 patients completed the questionnaires, 630 women and 376 male partners.

Participants/materials, setting, methods: Patients reported their infertility diagnosis in this survey and FertiQoL was evaluated according to infertility diagnosis. The male questionnaires were divided between male and non-male factor diagnosis. In the women's group comparison of FertiQoL according to the fertility diagnosis was calculated. Comparison between study groups was performed using t-test and Chi-square test. We performed logistic regression analysis to evaluate additional factors which may influence FertiQoL including age, parity, infertility duration, socioeconomic status and education.

Main results and the role of chance: Ninety-three (24.7%) males reported male factor as their infertility diagnosis, whereas 283 others (75.3%) reported infertility due to non-male related cause. Among females patients, self-reported infertility diagnosis were male factor in 114 cases (18.1%), anovulation in 120 (19.0%), tubal factor in 41 (6.5%), diminished ovarian reserve in 44 (7.0%), unexplained infertility in 200 (31.7%), endometriosis in 15 (2.4%), combined female and male infertility in 35 (5.6%), other reasons in 35 (5.6%) and unaware of the diagnosis in 26 (4.1%). Demographic characteristics of males with different fertility factors including age, parity, duration of infertility and education were comparable. However, age and duration of infertility among females with different infertility factors were different. The total FertiQoL scores of male patients with male or non-male related fertility diagnosis were comparable. Low core scores were found among women with endometriosis ($p = 0.045$ CI 95% 0.2, 18.5), and those with anovulation had lower treatment score ($p = 0.047$ CI95% 0.05, 8.8). Logistic regression analysis (ANOVA) revealed that age, infertility duration and education level significantly affect female's FertiQoL score. After adjusting for those factors, low FertiQoL scores among women with endometriosis and anovulation remained.

Limitations, reasons for caution: Not all patients agreed to participate. Not all males completed the questionnaire, most likely due to low number accompanying their spouses to the clinic. Also male patient may feel uncomfortable reporting on their FertiQoL. In addition, fertility diagnosis was based on the patient self-reporting that may lead to inaccurate diagnosis.

Wider implications of the findings: This is the largest study evaluating the impact of infertility diagnosis on fertility quality of life for patients undergoing fertility treatments. Male factor infertility does not seem to affect male's FertiQoL. Females with endometriosis and anovulation have significantly reduced FertiQoL and may need additional psychological support during the treatment.

Trial registration number: None.

P-557 The effect of mind body-based interventions on anxiety in infertile women undergoing IVF: a systematic review

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Study question: Do mind body-based interventions improve anxiety state in infertile women undergoing IVF?

Summary answer: Mind/body based interventions improve anxiety-state measured by the State-trait Anxiety Inventory (STAI) in women undergoing IVF treatment.

What is known already: Infertility and its treatments can be emotionally and physically challenging. Anxiety has a negative impact quality of life during treatments and leads to treatment discontinuation.

The goal of mind-body based interventions is to achieve a state of alert, focused relaxation by deliberately paying attention to thoughts and sensations without judgment allowing stress reduction in numerous physical and psychological conditions. This promising approach has been developed and used in infertile patients. Different dedicated programs have been developed for infertile patients including discussion, breathing exercises, yoga positions, relaxation and meditation.

Study design, size, duration: This study is a systematic review. A comprehensive and systematic search of literature was conducted in October 2016. We identified records by electronic searches in the following databases: PubMed, PsychINFO, EMBASE, the Cochrane Library. When conducting the searches we combined the keywords representing the two primary concepts infertility and mind body based interventions.

Participants/materials, setting, methods: Studies were considered eligible if they 1) reported data on infertile participants, 2) evaluated the effect of a mind-body intervention (mindfulness, mindfulness stress reduction programs (MBSR), yoga), 3) included baseline and post intervention measures of anxiety by the State-trait Anxiety Inventory (STAI) or another anxiety questionnaire, 4) used a quantitative search approach.

Main results and the role of chance: In total 8 publications met the eligibility criteria. One was excluded since only the congress abstract was available without access to full data. There were two uncontrolled studies with a pre and post intervention evaluation of state anxiety, 3 non-randomized controlled studies and 2 randomized controlled trials assessing pre and post intervention state anxiety levels in the intervention and control groups. The timing of the intervention in regard to the infertility treatment differed between studies. The 2 uncontrolled studies reported a significant reduction in STAI-state scores before and after the completion of the intervention. The 5 controlled studies all reported a significant improvement in anxiety state in the intervention group in comparison to the control group. We calculated the effect size for mean differences of groups with unequal sample sizes with a pre-post-control (PPC) design. The intervention had a small effect on anxiety state in 4 studies (Effect size d of 0.19, 0.23, 0.29, 0.34, respectively) and a large effect (Effect size d of 1.52) in one study.

Limitations, reasons for caution: Only two studies had a randomized controlled design and all studies except one included women only. The timing of the intervention differed between studies; it is therefore not possible to evaluate the optimal timing of the mind-body intervention during IVF treatments.

Wider implications of the findings: Mind body based interventions, based on relaxation, breathing and meditation, could be a useful approach in anxiety reduction in women undergoing IVF treatments. Future studies should focus on the timing of these interventions during IVF treatments.

Trial registration number: not applicable.

P-558 Long-term effects of the Mindfulness Based Program for Infertility (MBPI): A seven-year follow-up study

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Study question: Are there long-term term effects of the Mindfulness Based Program for Infertility (MBPI) regarding emotion regulation processes such as mindfulness skills and experiential avoidance and in depression and anxiety symptoms?

Summary answer: In the seven-year period after the MBPI there were sustained benefits in emotion regulation processes as well as in psychopathological symptoms of depression and anxiety.

What is known already: Mindfulness based interventions have been broadly applied and proved effective in several health conditions (e.g., chronic pain, cancer, endometriosis, anxiety disorders, depression). Specifically in infertility, the MBPI showed to be effective in improving mindfulness skills and infertility self-efficacy and in reducing depressive symptoms, shame, entrapment and defeat feelings.

Study design, size, duration: This was a longitudinal study encompassing four assessment moments: pre-MBPI (T1), post-MBPI (T2), six-months follow-up (T3) and seven-year follow-up (T4). Fifty-five women completed the MBPI and were recruited through a website announcement posted at the national patients association. Data collection took place between October 2009 and December 2016.

Participants/materials, setting, methods: Fifty-five infertile women completed the MBPI. All participants filled in a paper-pencil set of self-report

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instruments for the assessment of depressive symptoms, anxiety symptoms, experiential avoidance and mindfulness competencies pre, post and six-months after the program completion. Concerning the seven-year assessment, participants were contacted by email and completed the self-report measures online. For T4 there was a response rate of 73% (n = 40). Analyses were conducted through repeated measures ANOVAs.

Main results and the role of chance: Significant direct effects of time with medium effect sizes were found in mindfulness skills ($F = 7.16$; $p < .001$; $\eta^2p = .24$), experiential avoidance ($F = 10.75$; $p < .001$; $\eta^2p = .22$), depressive symptoms ($F = 11.68$; $p < .001$; $\eta^2p = .23$), and anxiety symptoms ($F = 7.31$; $p < .001$; $\eta^2p = .16$). Mean comparisons showed that benefits achieved after the MBPI completion were maintained for the seven-year follow-up. Mean scores in experiential avoidance showed to be increasingly lower over time and in mindfulness skills increasingly higher. As for depressive symptoms there was a significant decrease from T1 (M = 10.75; SD = 7.27) to T2 (M = 5.93; SD = 4.18) and from T1 (M = 10.75; SD = 7.27) to T3 (M = 4.45; SD = 4.63) but not to T4 (M = 7.35; SD = 7.26). Concerning anxiety symptoms there was a significant decrease from T1 (M = 47.53; SD = 13.28) to T3 (M = 40.00; SD = 10.82) and to T4 (M = 39.07; SD = 11.39).

Limitations, reasons for caution: Further research is needed to replicate or even expand these findings through a randomized controlled trial. Participants in the control group showed a dropout rate of 50% at six-month assessment and were not included at seven-year follow up. Therefore time comparisons were only examined for participants who completed the MBPI.

Wider implications of the findings: The current study highlights sustained benefits of MBPI seven years after the intervention on mindfulness skills and experiential avoidance. Moreover therapeutic gains were also maintained in terms of depression and anxiety symptoms, contributing to emotional well-being. The MBPI benefits seem to persist over a long period of time.

Trial registration number: ClinicalTrials.gov Identifier - NCT03012412.

P-559 Communication modalities between centers and patients should decrease stress and costs by limiting errors: results of a large survey

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Study question: The study aimed to examine patients' perception, comprehension and execution of physician's instructions for a first *in vitro* Fertilization (IVF) cycle.

Summary answer: The understanding of patients, dialogue between physician and patients, and means of transmission for instructions could lead to fear of errors and some misinterpretation.

What is known already: Even though the clinical pregnancy rate varies from about 29-33% after assisted reproductive techniques in Europe, a significant proportion of couples discontinue fertility care before achieving a pregnancy. The pregnancy rate following an injection error is estimated at only 7%. Burden of the treatment, errors in administration of treatment, lack of adherence and success, psychological stress, fear of adverse events, lack of patient-centered care, difficulty to contact assisted reproductive technology (ART) services play a great role in influencing the decision to discontinue treatment.

Study design, size, duration: A non-interventional, descriptive, longitudinal study was conducted from August 2013 to Octobre 2015 in ART centres located in France where patients could have a first IVF attempt.

A total of 39 ART centres participated in the enrollment of 502 patients.

Participants/materials, setting, methods: Women having a first IVF attempt for ART, and accustomed to use applications were recruited. Treatment-related instructions on duration, prescriptions, laboratory tests required were received by the patient from physicians, nurses, mid-wives or other healthcare providers through a smartphone application (Fertilisim). Questionnaires including visual analogue scales (VAS) were completed by patients and physicians before the first cycle (Visit [V] 1), on the day of oocytes retrieval (V2), and before the second cycle (V3).

Main results and the role of chance: A total of 488 patients (mean age: 32 years) were eligible before the start of the first IVF cycle.

According to the patients' opinion (N = 305), deviations to injection schedules (doubtful injection dose: 13.6%, and error on injection dose: 1%) were documented.

However, discrepancies about modalities of treatment administration were reported in 89.9% and 92.1% of patients at V1 and V3, respectively, mainly due to transcription error in dose or excess in gonadotrophins.

Compliance to treatment rated by physicians was considered good in 94.3% of patients, but extra hormonal tests and pelvic scans were performed by 38.3% and 30.5%, respectively.

Despite high rates of discrepancies, understanding of injections schedule (86.2), exams schedule (84.9), instruction transmission means (86.1) assessed through a VAS were considered satisfactory by patients at V1. Similarly, understanding of words used and answers given by the medical team were considered high (86.4 and 90.4, respectively).

Overall, 95.2% of patients (N = 305) reported to be satisfied to very satisfied with the mean of transmission for instructions during treatment at V2. Instructions sent were assessed as totally or rather understandable by most patients (94.4%). A total of 69.9% of physicians (N = 76) reported that the smartphone application allowed a better traceability.

Limitations, reasons for caution: This open and observational study could potentially affect physician and patient behaviors. This survey could be limited by a selection bias, as the sample of participating physicians was not randomly selected, leading to a possible lack of representativeness. Moreover, the reported findings regarding questions asking patients' opinion were subjective.

Wider implications of the findings: Unlike well-studied IVF laboratory potential errors, errors during treatment are underestimated as their impact on success rate. Areas for improvement may include the development of an IVF Smartphone application. This stress-limiting solution enables to send treatment instructions using a secure process, improve monitoring and should reduce costs induced by errors.

Trial registration number: The study was supported by MSD France, a Subsidiary of Merck & Co., Inc, MSD (France).

Trial Registry number

The trial registry number was MK#308-00.

P-560 Implantation rate remains stable when patient anxiety is reduced after embryo transfer

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Study question: Is it possible to actively reduce a patient's anxiety levels following embryo transfer, and in this way improve the success rate of fertility treatment?

Summary answer: Active management of the patient from the embryo transfer until the pregnancy test reduces patient levels of anxiety, but doesn't improve the embryo implantation rate.

What is known already: The level of anxiety felt by patients undergoing any type of fertility treatment is high, and it increases in the 10 days between embryo transfer and the pregnancy test. In recent years patients and professionals in the field have shown a growing interest in therapies to reduce anxiety between embryo transfer and the pregnancy test, for example using acupuncture or psychotherapy to try to improve the patient's experience at the stage of the treatment where the degree of anxiety reaches its maximum.

Study design, size, duration: Between June 2015 and November 2016, 431 patients on IVF treatment were recruited to a prospective trial, to evaluate their degree of anxiety on the days between embryo transfer and pregnancy test. They were randomised to one of 3 groups: 'ON', each patient used a Babypod™ device that emits music via the vagina twice daily for 20 minutes; 'OFF', double blind allocation of a Babypod™ that didn't play music; 'NO', control group without Babypod ™.

Participants/materials, setting, methods: We evaluated the patient's degree of anxiety on day +1 (D+1) after transfer with an intial test that scored

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from 0 up to 5. The day before of the pregnancy test, on day +9 (D+9), all patients repeated the same test to be able to compare the degree of anxiety.

We compared the results of the 3 groups for: reduction in the degree of anxiety, patient pregnancy rate and miscarriage rate.

Main results and the role of chance: Of the 431 patients recruited, 165 were randomly assigned to the ON group, wearing music with Babypod™, 145 to the OFF group, wearing the same device without music, and 121 to the control group, that didn't use any device. A homogenicity study was completed within the 3 groups and no significant differences were found between the groups with regard to all the IVF treatment parametres.

There were no significant differences between the 3 groups regarding the pregnancy rate per transfer (65% ON, 60% OFF, 64% NO), nor the miscarriage rate (12% ON, 16% OFF, 14% NO).

In the ON group, feedback from the patients showed however a 78% reduction in the anxiety felt by patients, between D+1 and D+9. In the OFF group 80% of patients also noted lower levels of anxiety. Furthermore, there were significant differences in anxiety levels between groups that had undergone active anxiety management by means of the Babypod™ when compared with the NO group that didn't use the musical device (11%, $p = 0.0012$)

The fact that there was an ON and an OFF group, both in themselves active management using the Babypod™, allows us to verify that it was not the musical stimulus itself that modified the success rates.

Limitations, reasons for caution: The main limiting factor of the study is that only the level of anxiety related to the treatment undertaken was assessed. No other possible extrinsic factors that could have modified the result of the studies were evaluated.

Wider implications of the findings: Active management signficantly reduces the degree of anxiety felt by the patient following embryo transfer. This reduction in anxiety does not influence the embryo implantation rate. However, we shall continue investigating alternative methods of active management because they do significantly improve the patient's experience of the treatment process.

Trial registration number: Not applicable.

P-561 Patient experience of IVF, watching their embryos develop in real time and online

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Study question: What is the emotional response of patients to a program that allows them to view the evolution of their embryos online and in real time?

Summary answer: Watching their embryos develop lowers patient anxiety levels, and gives them a better understanding of what is happening to their embryos.

What is known already: The laboratory phase of the IVF cycle is a time of anxiety for patients, principally because they do not know what is happening to their embryos. The Embryoscope™ time lapse technology allows embryologists to make continuous embryo observations without removing them from the incubator. This provides precise information about embryo development that is of great therapeutic and diagnostic value. We have designed a software program that allows patients undergoing IVF to follow the evolution of their own embryos from their computer or mobile telephones in order to help them establish the first emotional link with their future child.

Study design, size, duration: Between January 2012 to December 2016, 387 patients who had chosen to do IVF with additional use of the Embryoscope™ incubator were randomly selected to access in real time the images of their developing embryos. They were asked to complete a short questionnaire about this experience, in particular their emotional response to the images and degree of anxiety. We also asked them to select what they thought was the embryo with the best quality.

Participants/materials, setting, methods: On the day of their egg collection and ICSI fertilisation, an e-mail was sent to patients with a user name and password to allow access to an encrypted website where they could see their embryos in the time-lapse incubator. The patients were then able to follow embryo development in real time from their computers or mobile devices until the embryo transfer. On the same web page they were asked to complete the study questionnaire.

Main results and the role of chance: A 100% of patients considered watching their embryo development to be a positive experience that allowed them to feel involved in the laboratory process; of these patients, 88% considered it a highly positive experience and kept watching, and 12% decided to stop watching out of fear or respect; this latter group were worried about detecting non-evolutive embryos. 91% of patients said they felt calmer watching the embryo development.

We evaluated how many times per day patients would connect to watch their embyos: 23% of them connected only once, 27% between 2-4 times, and 43% 5 or more times every day (average 3,8 connections / day).

We asked patients if they felt they could select the embryo of best quality: 23% did not answer, 26% answered no, and 51% of the patients selected the best embryo from their point of view. 74% of these patients chose correctly the embryo that was finally used for transfer or vitrification.

Limitations, reasons for caution: The main limiting factor in this study is that it's a descrpitive trial based on an on-line questionnaire.

Wider implications of the findings: Giving patients direct access to the development of their embryos in the laboratory is a unique experience and reduces the anxiety often felt by patients at this stage in the treatment process. It boosts the bonding process as the parents watch the embryos make their first steps in life.

Trial registration number: Not applicable.

P-562 Psychopathological status, previous fertility treatment results and treatment adherence in men of infertile couples

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Study question: Does the psychopathological status relate to previous treatment results and treatment adherence in men of infertile couples?

Summary answer: Men of infertile couples with failed treatment presented higher scores of Depression, and those that did not continue the treatment had higher scores of Hostility

What is known already: There are few studies that describe psychological and psychosocial symptoms of men in infertile couples. Most studies worldwide are about women's experiences and psychopathology, and what happens to men when facing an infertility diagnosis and treatment remains unknown.

As far as we know there is no evidence that describes Hostility in men of infertile couples.

Study design, size, duration: Prospective, relational study. First stage of a wider research. During two months, March-May 2016, 51 men of 51 new infertile couples from the CER Medical Institute from Buenos Aires, Argentina completed a questionnaire.

Participants/materials, setting, methods: Fifty-one men from infertile couples (Age:$\bar{x}$ 39.48 +/-7.42 years) agreed to complete the Symptom Checklist-90-R (SCL-90-R)(Derogatis, 1994), a self-report questionnaire, in the waiting room before their first appointment at the fertility clinic. The SCL-90-R is a standardized instrument that assesses a wide range of current psychological symptoms and emotional distress divided in 9 dimensions and 3 global indexes.

Non-parametric analysis was made with Mann–Whitney *U* test, with the SPSS 20. Statistical significance = $p < 0.05$.

Main results and the role of chance: Data obtained from medical records: 58.8% previous failed treatment, 15.68 % male factor; 45.09% female factor; 31.37% combined, 7.84% unexplained diagnosis.

The sample obtained higher scores in the following SCL-90-R dimensions: Somatization ($\bar{x}$48.22 +/- 10.24), Depression ($\bar{x}$45.20 +/- 8.60) and Obsessive- compulsive ($\bar{x}$ 45.06 +/- 10.9). The lower score was in Phobic Anxiety ($\bar{x}$ 40.25 +/- 12.35).

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Men that did not continue with the fertility treatment obtained statistically significant higher scores in Hostility than men who continued (45.10 versus 40.25 x̄ respectively, p = 0.035). They also presented higher scores in the Index of Positive Symptoms than men who continued with the fertility treatment (44.67 versus 39 x̄ respectively, p = 0.038).

Men with previous failed treatment had higher scores of Depression than those for whom it was the first consultation (7.5 versus 41.9 x̄ respectively, p = 0.034).

Limitations, reasons for caution: It was a small sample and limited in time so results should be generalized with caution.

Wider implications of the findings: We found new evidence that suggests that high levels of Hostility might relate to treatment abandonment. This could be a first approach to study male psychological predictors of treatment adherence to develop specific interventions

Trial registration number: N/A.

P-563 A double-edged sword: the attitudes of men and women towards fertility education

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Study question: What are the attitudes of men and women towards fertility education?

Summary answer: Men and women speak positively about fertility education and believe it should be accessible at a time when fertility is relevant to the individual.

What is known already: Research shows there are considerable gaps in fertility knowledge. Recent efforts to increase fertility knowledge through public education strategies have been unsuccessful in promoting lasting change in fertility knowledge levels and fertility intentions (e.g., timing of pregnancy). Lack of change could be due to unfavourable attitudes and unmet preferences towards fertility education, calling for more research on views of fertility education.

Study design, size, duration: Individual qualitative interviews in the 5th year of a mixed methods longitudinal study concerned with planning for parenthood with university students and staff in a large urban centre. At study entry participants were childless, not pregnant and wanted children in the future. The present assessment took place with participants that were still childless. Participants were randomly assigned to receive control or fertility information. Interviews lasted on average 45 minutes, and were recorded and transcribed verbatim.

Participants/materials, setting, methods: Participants in the longitudinal study were invited to the interview study. 37 expressed an interest and 18 attended an interview (17 women, 1 man). On average those attending were 29.68 (SD: 3.62) years old. The main area of questioning was their opinion about fertility education. Participants completed the Cardiff Fertility Knowledge Scale (CFKS, Bunting et al., 2013). Qualitative data was analyzed using a thematic analysis. Differences according to knowledge level and intervention group were examined.

Main results and the role of chance: The average knowledge score was 70.9% correct (SD: 13.9, range: 38.5-92.3). Regardless of knowledge level or intervention group, respondents expressed positive attitudes towards fertility education, with descriptions of its usefulness and its importance in promoting informed decision-making about parenthood options and its timing. Key themes included attitudes about preferred timing of exposure to fertility education (i.e., when it is relevant, such as being on "the cusp of readiness" to conceive, during sex education in school, or during contraceptive visit to doctor). Preferred sources of information were doctors, popular media/television and online sources. Participants highlighted the necessity of information being provided by a reputable source. Preferred content included information on age-related fertility decline, lifestyle factors with risks to fertility, parenthood options (e.g., adoption, third party reproduction), and types of assisted reproduction. Respondents highlighted the "double edged sword" of fertility education citing its importance along with the concern that it would increase people's anxiety about being able to have children and encourage people to have children before they were ready. Of note, participants with lower knowledge levels reported a lack of access to fertility education prior to the study.

Limitations, reasons for caution: This is a qualitative study on a self-selected university sample of men and women of reproductive age having agreed to participate in a study of parenthood planning. The results may not be generalizable to the larger population. Future examination with larger, more representative samples is needed.

Wider implications of the findings: Educational efforts should take the target audience's attitudes and preferences into account and should be easily accessible and appropriate for a lay audience. Future research needs to determine how to reach audiences with lower levels of knowledge and how to provide education without increasing the pressure to have children.

Trial registration number: Not applicable.

P-564 Fertility-related knowledge in clusters of German women and men determined by fertility intentions and cultural milieus

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Study question: What differences do clusters of women and men based on fertility intentions and cultural values show concerning fertility-related knowledge?

Summary answer: Self-oriented persons before family planning had the most realistic fertility-related knowledge. Especially, self-oriented participants close to the end of reproductive lifespan gave pessimistic answers.

What is known already: Studies revealed generally poor fertility-related knowledge in young people, especially in university students who tend to underestimate the age-related fertility decline and overestimate the chance of successful IVF-treatments. Satisfactory knowledge was found in females, graduates, well-paid employees and those from developed countries. In actual family planners, more realistic knowledge was also correlated with absence of consultation with a fertility expert. A combination of fertility plans and social positions and values may reflect other important connections between beliefs and preconceptions and fertility facts.

Study design, size, duration: An online survey was conducted between April and June 2015 and was approved by the Ethics Committee of the Heidelberg University Hospital.

Participants/materials, setting, methods: 643 participants living in Germany answered all the questions encompassing fertility-related knowledge, fertility intentions, basic values in terms of Delta-Milieus (defined by a combination of social classes from lower to upper class and values from traditional to self-orientation) and sociodemographic data. With hierarchical cluster analysis, different clusters were identified on the basis of fertility intentions and social milieus. Differences between the clusters in sociodemographics and fertility knowledge were described with chi-squares and analyses of variance.

Main results and the role of chance: Four clusters could be found: Cluster 1 before family planning (N = 166) consisted of more self-oriented milieus with a general fertility intention in the future. Cluster 2 close to the end of reproductive lifespan (N = 242) contained also respondents from self-oriented milieus with an actual (62%) or without (38%) wish for a child. In this group, 48% struggled with fertility problems ($chi^2 = 60.83$, $p<0.001$), the average age was the highest of all four clusters (39.11 ± 10.57, F=54.18, p<0.001). The rate of university degree was the highest in the self-oriented clusters (81% and 74%, $chi^2 = 74.98$, $p<0.001$). Cluster 3 with traditional participants (N = 62) came from conservative and middle class milieus. Cluster 4 (N = 89) contained respondents from mainly self-managed milieus. In the two latter groups, there was a variety of family planning from general and actual to no fertility intentions.

81% of all participants gave correct answers to only 1-3 from the 7 questions connected to fertility-related knowledge.

Members of Cluster 1 had more correct answers regarding fertility-related questions than those of Cluster 4 (F=3.13, p<0.05), and less pessimistic (negative over-/ or underestimation) answers than persons from the Cluster 2 (F=7.51, p<0.001).

Limitations, reasons for caution: Respondents represented about the three-quartered of the original groups of Delta-Milieus. Experiencing fertility problems plays a role to be more pessimistic in rating the success of pregnancy

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naturally or per IVF, but the numbers of pessimistic answers were also salience among participants without fertility problems.

Wider implications of the findings: Acquiring proper fertility knowledge is shaped by several factors, as well as by which social and basic value-cluster a person belongs to. Therefore, fertility education programs should target on women and men with different cultural background and parenting attitudes.

Trial registration number: -.

P-565 Single or double embryo transfer? Decision-making process in patients participating in an oocyte donation programme

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Study question: Does the information provided to oocyte recipients on the consequences of multiple pregnancy influence the decision on the number of embryos to be transferred?

Summary answer: After being informed about treatment success rates and the consequences of multiple pregnancy, a significant number of recipients changed their preference to single embryo transfer.

What is known already: In IVF/ICSI programs, it has been demonstrated that, after receiving the information about the similar cumulative live birth rate after single embryo transfer (SET) and double embryo transfer (DET) and the obstetric and perinatal risks of multiple pregnancy, a significant number of patients opt for SET. Up to date, no comparable studies have been published in oocyte recipients. Although similar results are expected, this group includes a significant proportion of women of advanced age, thus involving a distinctive medical, psychological and social context which can affect their expectations and preferences.

Study design, size, duration: Longitudinal, prospective study of 38 cycles. Recipients' preferences on the number of embryos to be transferred and the relevance for the decision-making process that they attribute to certain factors were analysed through an anonymous questionnaire. Recipients completed the questionnaire at two distinct times of the cycle: before receiving the information about success rates and the possible risks of DET and after counselling was provided. A 6 months study period was expected.

Participants/materials, setting, methods: Patients undergoing their first oocyte donation cycle expressed their preference on SET or DET pre and post-counselling. Preference changes were analysed by McNemar's test. According to the relevance for their decision, recipients scored from 1 (less relevant) to 10 (more relevant) different factors: partner's opinion, age, previous ART, temporary urgency, desire for singleton/twins, desire for healthy pregnancy, probability of pregnancy, mother/child risks, cost and medical recommendation. The mean scores of pre and post-questionnaires were compared.

Main results and the role of chance: Before counselling, from 38 recipients that completed the questionnaire, 20 preferred DET, 11 preferred SET and 7 were undecided. From the 20 recipients who initially preferred DET, 10 (50%) maintained their preference after counselling, 8 (40%) changed their decision to SET and 2 (10%) changed to undecided. The McNemar's test showed the intervention had a significant impact on the decision ($p<0.05$). For each factor analysed in the questionnaire, the mean score's difference (MSD) between pre and post-questionnaires showed the influence of counselling, with a negative MSD meaning an increasing importance of the specific factor. In recipients who changed their preference from DET to SET, the counselling had an impact on the following factors: mother risks, previous ART, probability of pregnancy and temporary urgency. While the relevance attributed to mother risks increased (MSD=-3.13), the previous ART (MSD=3.00), the probability of pregnancy (MSD=2.38) and the temporary urgency (MSD=2.25) became less important. Conversely, in the recipients who maintained their DET preference, the scores barely changed between pre and post-counselling questionnaires.

Limitations, reasons for caution: Although the sample size is sufficient to answer whether counselling influence the oocyte recipients' decision on the number of embryos to be transferred, more cases would be needed for an in-depth analysis of the specific factors that affect this change in the decision.

Wider implications of the findings: This study provides valuable information on the decision-making process on the number of embryos to be transferred in oocyte recipients. It is especially useful to reinforce and improve the information strategy for this kind of patients, in order to help them make a more reasoned decision.

Trial registration number: In progress.

P-566 Expecting the best but experiencing the worst: The effect of dispositional optimism on psychological adjustment after IVF treatment failure

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Study question: What is the effect of dispositional optimism on depression and anxiety after IVF?

Summary answer: After a failed IVF cycle, patients high in dispositional optimism experience similar levels of depression and anxiety as those who had successful cycles.

What is known already: While IVF provides the opportunity for pregnancy for couples who otherwise would not have been able to conceive, it still fails more often that it succeeds. Few longitudinal studies have addressed the psychological consequences of failed IVF. In particular, we know little about psychological factors that might increase or decrease the negative effects. Dispositional optimism, defined as the tendency to expect the future to turn out well, is a robust predictor of positive mental and physical health. However, little research attention has addressed what happens when optimistic people experience objectively negative outcomes (such as a failed IVF cycle).

Study design, size, duration: A prospective longitudinal cohort study over an 18 month period was employed. Women were recruited from five fertility practices. Women completed interviews and questionnaires at baseline (shortly after their initial fertility consultation) and 4, 10, and 18 months later. This report concerns the 201 women who initiated their first IVF cycle during the study period.

Participants/materials, setting, methods: Dispositional optimism (Life Orientation Test, Revised; LOT-R), depression (Center for Epidemiological Study of Depression scale; CESD; > 15 is at risk), and anxiety (State Trait Anxiety Inventory; STAI; > 39 is at risk) were measured at baseline. CESD and STAI were repeated at follow-ups. IVF outcome was categorized as "success" (pregnancy) or "failure" (incomplete, not pregnant, or miscarriage). For this report, post-IVF depression and anxiety scores were taken from the first follow-up after the IVF.

Main results and the role of chance: Mean baseline CESD was 10.96 (SD=9.02) and mean post-IVF CESD was 14.73 (SD=10.50). Mean baseline STAI was 40.06 (SD=11.54) and mean post-IVF STAI was 42.05 (SD=13.54). The first IVF cycle was successful for 28% of the women. Regression models were used to assess study questions, with post-IVF CESD and post-IVF STAI scores entered as the outcome variables, respectively. Age, years trying to conceive, ethnicity, educational level, and baseline CESD and STAI (respectively) were entered as covariates; LOT-R, IVF outcome, and their interaction were entered as explanatory variables. Regarding post-IVF depression, after accounting for covariates, IVF outcome had a significant effect on CESD, such that women who had failed cycles had higher CESD scores compared to those with successful cycles ($B = -3.89$; $p < .05$). There was also a significant interaction effect ($B = 0.58$, $p < .05$), such that women with failed cycles who were high in LOT-R experienced similar CESD levels to women with successful cycles. The results followed an identical pattern for post-IVF anxiety, with the model showing a significant main effect for IVF outcome ($B = -5.98$; $p < .01$), as well as a significant interaction effect ($B = 0.84$; $p < .05$.).

Limitations, reasons for caution: This study was not a randomized trial, nor did we attempt to manipulate any variables. Therefore, we cannot provide proof regarding a causal relationship of any variables. Also, we do not know if these findings extend to fertility treatments other than IVF or to those who experience ongoing failure.

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Wider implications of the findings: The expectation of positive outcomes does not put women at risk for poor psychological adjustment when IVF fails; instead it seems to protect them from elevated distress. The ability to imagine positive outcomes in the future could be a modifiable cognitive target for preventive mental health interventions for IVF patients.

Trial registration number: NA.

P-567 A systematic review and narrative report of the profound psycho-socio-cultural consequences of the growing burden of infertility in developing countries

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Study question: Does the growing incidence of infertility have a detrimental psycho-socio-cultural impact on the well-being of individuals particularly in developing countries?

Summary answer: Citizens of developing countries distinctively face negative consequences of infertility like social stigma, lack of economic security and taboos against adoption to a greater degree.

What is known already: Infertility has a negative impact on the psycho-socio-cultural being of individuals both in developed and developing countries. The degree of availability of reproductive technology plays a major role in shaping perceptions of and responses to infertility thereby distinguishing between the psycho-socio-cultural impact in developed and developing nations. Infertility is treated as a medical condition with psychological consequence rather than a socially-constructed reality. In developing countries, bearing children is considered necessary for marital completion. Childlessness is associated with poorer adjustment and greater intra-personal vulnerability. Intrinsic religiosity, sexual satisfaction and familial support are associated with better coping and adjustment.

Study design, size, duration: Online databases were searched for articles published from January 1990 till October 2016 regarding the psycho-socio-cultural effects of infertility in consultation with a search methodologist using the desired key-words. In the case of double publications, the latest study was included. Studies published only as abstracts and those from other parts of the world apart from developing countries were excluded. Relevant journals and conference abstracts that were not covered in the databases were also hand searched.

Participants/materials, setting, methods: There were 120 studies on the psycho-socio-cultural impact of infertility that met the inclusion/ exclusion criteria. It was not possible to pool the data due to heterogeneity in the study design and methods, therefore, a systematic review and narrative report was compiled. After a primary screen of all titles and abstracts retrieved, the full texts of all potentially eligible studies were retrieved. Study investigators were contacted if clarification was needed for study eligibility.

Main results and the role of chance: Children are considered as indicators of wealth and prosperity in a community Parenthood is highly emphasized in developing societies as children are required for care and maintenance of older parents. In childless couples, the status, respect and authority are compromised. Features like lack of economic security and care in old age, social stigma, concerns based on ideology and religious beliefs, hindrance in adoption and ethical concerns are unique to the societies of developing countries which face the negative consequences of infertility to a greater degree. The infertile couples are treated as outcasts and lack equal opportunities in social and religious functions. The psychological effects manifest in form of anxiety, depression, feelings of blame and guilt when the couples feel that the reality of life without children is too much to handle. Social consequences range from stigmatization, alienation and strained social interactions. Partners and families may be abusing the women as a result of childlessness. Culturally, taboos against adoption, donor gametes, embryo freezing impede the treatment. All these features put together shape the consequences of infertility. Hence, infertility is a poorly controlled chronic stressor especially in the developing countries where the poor cannot access reproductive techniques.

Limitations, reasons for caution: Prospective case-control studies would overcome the retrospective nature of our study. Heterogeneity in the design and methods of the selected studies making it difficult to pool the data is another limitation.

Wider implications of the findings: There is a dire need for addressing the infertility crisis by facilitation and prioritization of treatment and bringing about a radical change in the attitude to the problem of infertility in developing countries. Efforts should be tailored towards empowering women especially to handle domestic abuse

Trial registration number: not applicable.

P-568 The effect of introducing Reproductive Life Plan-based counselling during men's sexual health visits: a randomized controlled trial

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Study question: Can reproductive life plan-based counselling during a sexual health visit increase men's fertility awareness?

Summary answer: Three months after the counselling session, fertility awareness was moderately increased.

What is known already: Several studies indicate that many men have limited fertility awareness and are in need of preconception health counselling. Still, preconception counselling directed towards men is unavailble in several European countries. The Reproductive Life Plan (RLP) is a tool that was developed in the US to encourage people in reproductive age to reflect on their reproductive intentions and find strategies to reach their goals. No studies have as far as we know evaluated the use of RLP during health consultations with men.

Study design, size, duration: A randomized controlled trial took place in Sweden October 2014-February 2016. In total 229 men were recruited to the study and randomized; 118 to intervention (IG) and 111 to control group (CG). The randomization was blind to the participant. All participants received standard care. The intervention group also received brief information about preconception health guided by the RLP-tool. The health visit lasted about 15 minutes. Follow-up was made by telephone after three months.

Participants/materials, setting, methods: Recruitment took place at two sexual health clinics located in two major cities. Most men attended the clinics at drop-in hours for STI-testing. Fertility awareness was measured by a questionnaire, before the intervention and at follow-up. The participants were also asked about their impression of the intervention. To evaluate the effectiveness of the intervention on fertility awareness, knowledge scores were calculated and the difference between baseline and follow-up was analyzed by Paired samples t-test.

Main results and the role of chance: The baseline measurements include 201 men (28 men dropped out/had to be excluded after randomization). Mean age was 28 years. Twenty-one men were fathers already, and 71% wanted to have (more) children in the future. There was no difference in background characteristics between IG and CG. The follow-up sample consisted of 78 men from IG and 82 men from CG. The total score measuring fertility awareness, which was based on 6 questions (maximum 12 points), increased from 4.6 ± 2.1 to 5.5 ± 2.2 points ($p = 0.004$) in IG. There was no improvement in the CG (4.6 ± 1.9 to 4.7 ± 2.3, $p = 0.693$). The average number of lifestyle factors affecting male fertility mentioned by the participants increased from 3.6 ± 1.9 to 4.4 ± 1.6 ($p < 0.001$) in IG. There was no improvement in CG (3.4 ± 1.7 to 3.5 ± 1.5). The intervention had now effect on at what age men wanted to become fathers. In IG, 76% had a fairly or very positive experience of the counselling and 76% had received new information. Almost all men (95%) agreed that it is important to educate men about these matters.

Limitations, reasons for caution: The generalizability is limited by the fact that recruitment took place in urban areas and the sample consisted of men who already proved some health awareness by attending an STI-clinic. Long waits at the clinics could explain why some men dropped out before the intervention took place.

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Wider implications of the findings: The effectiveness of the intervention is comparable to a similar study with female university students (Stern et al, 2015).

The counselling was positively received and led to new thoughts about fertility and reproductive health. Discussing RLP during health visits could thus be a gateway to increased fertility awareness.

Trial registration number: ClinicalTrials.gov Identifier: NCT02736214.

P-569 A re-examination of the factor structure of Traditional Chinese FERTIQOL with subfertile women undergoing ART

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Study question: What is the factor structure of the Traditional Chinese FertiQOL for Chinese women undergoing ART?

Summary answer: Five factors were revealed for the 24 core items of the Traditional Chinese FertiQOL, whereas a 2-factor structure was recovered for the 10 treatment items.

What is known already: FertiQOL has been widely used to measure quality of life in cases of subfertility. Validation studies have been conducted with Dutch, Korean and Taiwanese samples. However, the factor structure of the core and optional treatment items have yet to be scrutinized. A re-examination of the factor structure of FertiQOL will help reveal how Chinese subfertile women understand and construe the impacts of subfertility on their quality of life.

Study design, size, duration: This was a cross-sectional study. 477 Chinese female subfertile patients were approached and 246 completed the questionnaire (response rate: 51.6%). Their average age was 37.2 (SD = 3.4), married for 7.3 years (SD = 3.8) and diagnosed with subfertility for 4.0 years (SD = 2.5). Majority of them had prior experience with ART (66.3%), with a mean ART treatment history of 3.2 years. The study spanned two years.

Participants/materials, setting, methods: Participants were recruited from fertility clinic of a university-affiliated hospital in Hong Kong. Those who have given consent to participate were asked to complete a set of questionnaire including their demographics, reproductive history, ART treatment history and fertility related quality of life (FertiQoL).

Main results and the role of chance: A confirmatory factor analysis (CFA) was conducted to re-examine the fit of the 4-factor structure of the core items (Boivin et al., 2011) on the local dataset. The model fit was insufficient [chi-sq = 590.927, df = 246, p = .000. CFI = .882, RMSEA = .075]. The core items were then submitted to a principle component analysis with promax rotation. A 5-factor solution was recovered with 61.4% total variance explained [F1: "Emotion-physical impact" (items 1, 2, 3, 7, 8, 9, 16, 18, 23, 24); F2: "Social alienation" (items 10, 12, 13, 15, 17, 22); F3: "Couple coping efficacy" (items 19, 20); F4: "Satisfaction with relationships" (items 5, 6, 11, 21); F5: "General coping efficacy" (items 4, 14). Another CFA was run for the 2-factor structure (tolerability and environment) of the 10 treatment items, and an adequate model fit was found [chi-sq = 85.169, df = 34, p = .000, CFI = .939, RMSEA = .078]. Significant correlations with anxiety and depression scores supported the convergent validity of the core and treatment subscales (ps <.05). Results of sensitivity analyses suggest that older age and previous pregnancy was related to higher scores on tolerability, environment, emotion-physical impact and social alienation.

Limitations, reasons for caution: The 2 subscales with the lowest eigenvalues (satisfaction with relationships, general coping efficacy) in the core FertiQOL had low Cronbach alphas. The resultant factor solution requires verification with future studies. Self-selection bias and self-report nature of the survey is a concern.

Wider implications of the findings: Our alternative factor structure revealed how Chinese sub-fertile women construe their fertility related quality of life. While the emotional and physical impacts were merged into a single factor, the presence of four social-relational factors demonstrate a nuanced categorization of the social-relational impacts of subfertility.

Trial registration number: not applicable.

P-570 Does illness cognition mediates the adverse impact of traditional childbearing beliefs on mental health? - Directions for psychosocial interventions for women undergoing assisted reproduction technologies

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Study question: Does illness cognition mediate the adverse impact of traditional childbearing beliefs on anxiety and depression among Chinese women undergoing IVF?

Summary answer: Illness cognition (helplessness, acceptance, perceived benefits) significantly mediated the effect of childbearing beliefs on anxiety and depression among Chinese women undergoing IVF.

What is known already: The emphasis on honouring the family through procreation has contributed to the plight of sub-fertile couples and stigmatization of fertility treatment in many cultures. According to the stress and coping model of Lazarus and Folkman, appraisals of the stressor determine the outcomes of the stressful event. However, such theoretical framework has seldom been applied to examining the coping processes of sub-fertile women. This study fills the knowledge gap by exploring the mediation effects of patient's appraisal of their sub-fertility (i.e., illness cognition) on the adverse mental health impacts of adhering to traditional childbearing ideals among Chinese women.

Study design, size, duration: This was a longitudinal questionnaire study. Questionnaire data was collected at three time-points: baseline (T0), six weeks after (T1) and 3 months after (T2) with 151 infertile female patients. The study spanned two years.

Participants/materials, setting, methods: Participants were recruited from a fertility clinic in Hong Kong. On average, they were 37.2 years old (SD=3.3), married for 7.2 years (SD=3.7) and diagnosed with subfertility for 3.8 years (SD=2.3). At the time of study, majority had experience with ART (66.3%) and a 3.2 years mean treatment history. Measurements include Chinese Childbearing Belief Scale; Illness Cognition Questionnaire and Hospital Anxiety Depression Scale.

Main results and the role of chance: The mediation effects were explored by path analysis with 5000 bootstrapped samples using the PROCESS macro on SPSS 19.0. The summary score of the childbearing belief scale was entered as the independent variable. Illness cognitions (helplessness, acceptance, and perceived benefits) were entered as mediating variables. The dependent variables were the anxiety and depression subscales of HADS. Six models were conducted. Results show that helplessness and acceptance completely mediated the effect of childbearing beliefs on anxiety as well as depression, as the indirect effects were found to be significant (ps<.05) whereas the direct effects were not (ps > .05). The mediating effects of perceived benefits on anxiety and depression were significant but incomplete, with significant indirect and direct effects (ps<.05).

Limitations, reasons for caution: Self-selection bias at recruitment and self-reporting nature of the survey remains a concern.

Wider implications of the findings: Our findings highlight the importance of acknowledging the mental health impact of cultural ideals on adjustment to infertility. Our results also call for future psychosocial support to empower patients through reducing their sense of helplessness and capitalizing on their ability of acceptance and benefit-finding in the tormenting treatment journey.

Trial registration number: Nil.

P-571 Intrapersonal and interpersonal predictors of fertility-related distress - constructing holistic interventions for women undergoing ART treatments

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Study question: Do psychospiritual and cultural factors predict subfertility related stress during ART treatments? How will such findings inform frontline practices in fertility counselling and psychosocial interventions?

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Summary answer: Psychospiritual and cultural factors provided significant incremental predictability to subfertility stress beyond clinical factors among women receiving ART treatments

What is known already: ART treatments have long been understood as a stressful process for subfertile couples. For women in particular, not only does the clinical treatment physically demanding, the process is also known to inflict poor mental health outcomes, including depression and anxiety. Subfertility is a medical condition embedded with strong socio-cultural implications. Not being able to extend the family bloodline through reproduction is traditionally viewed as a biological, social, and moral failure in many cultures. In this light, this study examined the psychospiritual and cultural factors related to subfertility related stress among a group of Chinese women receiving ART.

Study design, size, duration: This was a cross-sectional study. 536 female subfertile patients who were going to undergo ART treatment were approached. 271 completed the questionnaire (response rate: 51.6%). The study spanned two years.

Participants/materials, setting, methods: Participants were recruited from a fertility clinic at a university hospital in Hong Kong. Their average age was 36.5 (SD = 3.2), married for 6.7 years (SD = 3.2) and received diagnosed for 3.8 years (SD = 2.4). Majority(68.3%) had prior ART experience (mean treatment history of 3.4 years).

Measurements include Fertility Problem Inventory (FPI), Holistic Well-being Scale (WHS), Traditional Childbearing Belief Scale (TCBS) and Childbearing Importance Index (CII).

Main results and the role of chance: A step-wise multiple linear regression was conducted with global FPI as the criterion variable. Statistical significance was indicated by $p < .05$. In Step 1, clinical characteristics including age, frequency of previous treatment, subfertility year, and causes of subfertility (unexplained, number of female causes, number of male causes) were entered. The 7 subscale scores of HWS were entered in Step 2. Lastly, child-bearing attitude was added to Step 3. Results showed that all three steps contributed significant incremental explanatory power to subfertility stress (Step 1: r2 change = .079, $p = .008$; Step 2: r2 change = .362, $p = .000$; Step 3: r2 change = .101, $p = .000$). In Step 1, younger age, unexplained cause, and female causes were significantly associated with greater subfertility stress. In Step 2, only younger age and female causes remained as significant clinical characteristics. Psychospiritual attributes including emotional vulnerability, spiritual disorientation, non-attachment, and spiritual self-care were significant predictors. Except for younger age, all the significant predictors of Step 2 preserved their statistical significance at Step 3. Childbearing attitudes added significant explanatory power after controlling for psychospiritual and clinical characteristics.

Limitations, reasons for caution: Self-selection bias and self-reporting nature of the survey remained a serious concern. The current study followed an ecumenical perspective in measuring spirituality. Future studies may explore how religious activities and affiliation impact subfertility stress.

Wider implications of the findings: Our findings showed psychospiritual and cultural factors offer significant incremental explanatory power on subfertility related stress beyond established clinical attributes. Support services should assist clients to draw on their psychospiritual strength and cope with cultural pressure imposed on their physical condition. An intervention model addressing multidimensional well-being will be discussed.

Trial registration number: Nil.

P-572 The importance of genetic parenthood for infertile men and women

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Study question: Do men and women turning to a fertility clinic prefer genetic over non-genetic parenthood and if so, what are their motivations?

Summary answer: Nearly all infertile men and women prefer genetic parenthood. The majority indicated that for nearly all motivations for becoming a parent, genetic parenthood was important.

What is known already: Clinicians assume that all infertile couples prefer genetic parenthood and, therefore, treatments with donor gametes are considered a last resource option. The exact strength of the preference of infertile men and women for genetic over non-genetic parenthood remains unclear, as do their precise motivations. Previous studies identified 30 motivations for genetic parenthood, and 51 motivations for parenthood for which a genetic link between the parents and their child is not strictly necessary, but might be deemed required. The relative importance of all these motivations has yet to be examined.

Study design, size, duration: A questionnaire was developed based on literature review, assessed by professionals with different backgrounds and pilot tested among patients. A postal cross-sectional survey disseminated the coded questionnaire among both the female and male partner of 201 heterosexual infertile couples after their first consultation at one of two Belgian fertility clinics. The study was carried out between October 2015 and May 2016

Participants/materials, setting, methods: The survey addressed: (i) the overall preference for genetic parenthood for themselves and for their partner, (ii) the importance of 30 motivations for genetic parenthood, (iii) the importance of 51 motivations for parenthood and whether genetic parenthood was, according to the respondent, necessary to attain these. To simplify presentation of the results, all 81 motivations were grouped into reliable categories of motivations using psychometric analyses.

Main results and the role of chance: A total of 104 women and 91 men completed the survey (overall response rate: 49%). Almost all respondents (98%) favoured genetic over non-genetic parenthood for both their partner and themselves. One third of the respondents stated they only wanted to parent a genetic child. Achieving genetic parenthood for their partner was considered significantly more important than achieving genetic parenthood for themselves. In univariate analysis, being male and not university educated were significantly associated with finding genetic parenthood important; in multivariate only educational level remained significant. The 30 motivations for becoming a genetic parent clustered into eleven categories of which *'to experience a natural process'* was deemed most important. The 51 motivations for becoming a parent for which a genetic link between the parent and child is not strictly necessary clustered into fourteen categories of which *'to contribute to a child's well-being'* and *'to experience the love of a child'* were most important. For 40 out of 51 of these motivations for parenthood, the majority of respondents indicated that being the genetic parent was needed to attain them.

Limitations, reasons for caution: We included couples that visited the fertility clinic for the first time, and the preference for genetic parenthood over non-genetic parenthood and motivations therefore might change during the course of treatment. Moreover, what prospective parents expect to be important for their future well-being might not really define parents' well-being.

Wider implications of the findings: The presumed preference of couples for genetic parenthood was confirmed. Resistance against using donor gametes is more likely among lower educated individuals. Researching whether non-genetic parents actually achieved their 51 motivations for parenthood for which a genetic link is not strictly necessary, could be a basis for developing patient information.

Trial registration number: Not applicable.

P-573 Online guidance through ART: design, development and qualitative evaluation of 'myFertiCare'

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Study question: Is it feasible to develop an online application to support couples undergoing Intracytoplasmic Sperm Injection with surgically retrieved sperm during their treatment trajectory?

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Summary answer: A multi-faceted online application, called myFertiCare, has been designed, developed and qualitatively evaluated. It provides personalized and interactive features, tailored to patient- and professional preferences.

What is known already: The infertile patient population, mostly consisting of young, highly educated couples, wishes both partners to participate actively in their diagnosis and treatment process and values use of the Internet for health-related purposes. We hypothesized that an online application can be a suitable instrument to support couples during their full treatment trajectory. By providing personalized information and offering the opportunity of communication we aim to decrease infertility-specific anxiety and stress, increase knowledge, enhance patient-centred care and thus improve patient well-being.

Study design, size, duration: A qualitative study to identify patients' needs regarding E-health by means of a literature review and semi-structured in-depth interviews with 11 couples. Based on this, a functional design for the application was developed in collaboration with medical and technical professionals. Pilot-testing took place through 9 think aloud sessions in which patients executed tasks in myFertiCare, whereafter the functional design has evolved. Finally, later this year myFertiCare will be quantitatively evaluated in a larger population.

Participants/materials, setting, methods: The study started in 2014 in the Radboud university medical center in Nijmegen, the Netherlands. Eligible for participation were couples undergoing PESA/TESE-ICSI in this period. Consulted were professionals from both the urology and reproductive medicine department. For technical aspects of the design, both internal and external technical specialists with experience in E-health were involved. Currently, myFertiCare counts more than hundred unique users.

Main results and the role of chance: A multi-faceted online application, called myFertiCare, has been developed, which can be accessed using a computer, tablet or smartphone. The application is tailored to patient- and professional preferences based on qualitative research. It contains personalized features like the visualized treatment trajectory of each individual, which forms the base of the application. This provides insight into scheduled and yet unscheduled future appointments, accompanied by information tailored to the specific phase of the treatment trajectory. Furthermore, couples are provided access to their diagnostic results and to a personal checklist to help them comply with the prescribed regimens before being eligible for treatment. The application also provides interactive features, namely a forum where couples can interact with peers, which is joined by care providers. It is also possible to target private questions to an individual care provider.

During the pilot phase, myFertiCare has been qualitatively evaluated and based on these results the functional design has evolved. The login method was simplified to improve accessibility. Furthermore, improvements mainly consisted of small textual changes. Later, a quantitative evaluation will be performed according to an established framework focused on human, organization and technology factors to conclude successful implementation.

Limitations, reasons for caution: Although myFertiCare has been thoroughly designed and successfully pilot-tested, the quantitative evaluation will indicate if the application is successfully implemented. Limitations of the study include the single center design and the relatively small subgroup of infertile patients, namely couples undergoing PESA/TESE-ICSI.

Wider implications of the findings: By providing personalized and interactive features, we speculate that myFertiCare will enhance patients' participation with their treatment. This way, it offers the opportunity to improve patient-centeredness in fertility care. After successful implementation for couples undergoing PESA/TESE-ICSI, the concept can probably be extended to all infertile patients and other fertility clinics.

Trial registration number: not applicable.

P-574 Impact of Assisted Reproductive Technologies (ART) on sexual behaviors in infertile couples

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Study question: How and when does ART impact sexual life of infertile couples?

Summary answer: The prevalence of sexuality impairment is relatively high in infertile couples, both in men and women, the most critical period being ovarian stimulation.

What is known already: Infertility diagnosis is often experienced as a major stressor by couples, exposing them to a high risk of impaired sexual function and subsequently degraded quality of life.

Data about the sexuality of infertile couples undergoing ART are sparse, most studies being conducted in limited samples and using heterogeneous methods. Moreover, the most critical period for couples, as well as the respective impact of ART on women and men have not been clearly identified. As the use of ART increases worldwide, it is essential to explore the sexual outcomes associated with fertility treatments in order to improve medical support and counselling.

Study design, size, duration: This multicentric cross-sectional study was conducted in 31 French ART centers over a 7-month period in 2009. A multidisciplinary panel of specialists (psychologists, gynecologists, midwives) selected relevant items previously reported in the literature in order to develop a new questionnaire specifically designed to evaluate sexual behaviours in couples undergoing ART cycles. A total of 1442 questionnaires were distributed to both members of 721 infertile couples.

Participants/materials, setting, methods: The questionnaire was distributed to both members of heterosexual couples who recently experienced live birth after ART cycle (either IVF, IVF/ICSI or IUI). After oral explanation and consent obtainment, men and women were strongly asked to fill out the questionnaire separately. No demographic or medical selection criteria was applied. Questionnaires were then collected and sent for centralized statistical analysis.

Main results and the role of chance: Participation rate was high, with 647 women (89.7%) and 615 men (85.3%) filling out the questionnaire. Both members of the couple completed the questionnaire in 615 cases, leading to an overall participation rate of 85.3%. The majority of couples suffered from primary infertility (77% of women, 75% of men) and obtained live birth after IVF (or IVF-ICSI) cycle.

Patients were first asked to indicate how important they considered sexuality in their current lives. The majority of women and men reported that sexuality was quite important (45% in women, 48% in men). However, the proportion of men reporting that sexuality was very important was significantly higher than in women (29% vs 19%, $p < 0.0001$), whereas the proportion of women reporting that sexuality was moderately important or of little importance was higher than in men (30% vs 20%, $p = 0.0001$ and 5.5% vs 3%, $p = 0.04$). The overall proportion of couples reporting sexuality impairement was 72% in women and 54% in men. Most participants identified ART treatment, i.e. controlled ovarian hyperstimulation, as the most critical period (31% in men and women). No significant difference could be observed in men and women according to the etiology of infertility (male, female, mixed or idiopathic) and its duration.

Limitations, reasons for caution: First, we chose not to use a validated questionnaire such as Female Sexual function index or GRISS, as these questionnaires might not be absolutely relevant in our study. Second, our results should not be extrapolated to all infertile couples, as we only included couples who had a live birth.

Wider implications of the findings: We confirm the relatively high prevalence of sexuality impairement in infertile couples. ART treatment seems to be the most critical period for infertile couples' sexuality, while infertility type or duration does not seem to impact significantly sexuality.

Trial registration number: not applicable.

P-575 Mindfulness-based intervention for lifestyle modification and weight loss in infertile women: randomized controlled trial

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Study question: Does a mindfulness-based stress reduction program improve weight loss through lifestyle modification in infertile women?

Summary answer: Mindfulness-based intervention does not improve weight loss during a short term (12 weeks) lifestyle modification program.

What is known already: Weight loss is a first line therapeutic goal for infertile women who are overweight or obese. This is particularly important in the polycystic ovary syndrome, as weight loss may contribute significantly to reduction of circulating levels of insulin and androgens. However, compliance to lifestyle modification is usually poor. Strategies based on psychological support and complementary interventions have been developed in order to improve adherence and maintenance of a healthy lifestyle. In this study, we evaluated the short-term impact of a mindfulness-based stress reduction program on the body measurements of overweight/obese infertile women seeking weight loss through lifestyle modification.

Study design, size, duration: The participants were randomly assigned to 8 consecutive weekly sessions of mindfulness-based intervention (n = 51) or no intervention (n = 49). All participants from both groups received a customized dietary plan and instructions to perform regular physical exercise. Body measurements were performed at baseline and 12 weeks later. The primary outcome was body weight. Secondary outcomes were waist circumference, hip circumference, and quality of life (QOL).

Participants/materials, setting, methods: The participants were infertile women aged 18-48 years (median 37 years) with body mass index > 25 Kg/m^2 (median 31 Kg/m^2) attending a reproductive endocrinology unit of a teaching hospital between January 2015 and March 2016. Body measures were obtained by trained personnel, blinded to the patient intervention group. QOL was assessed by the Psychological General Well-Being Index (PGWBI) questionnaire.

Main results and the role of chance: There was a mean reduction of 1.8 kg in the intervention group (p = 0.002, paired Wilcoxon's test) and mean reduction of 1.0 kg in the control group (p = 0.022). There was an average reduction of 1.5 cm of waist circumference in the intervention group (p = 0.007) and 0.8 cm in the control group (p = 0.633). Hip circumference shortened 2.0 cm in the intervention group (p = 0.002) and 0.3 cm in the control group (p = 0.100). The intervention group also showed a significant improvement in QOL (23 percent gain in PGWBI total score, p = 0.001), which was not observed in the control group (0.9 percent variation, p = 0.829).

Limitations, reasons for caution: This was a short term program, therefore the lack of benefit of mindfulness intervention on weight loss compared to no intervention may only reflect the limited follow-up. This is reinforced by the fact that only the intervention group had a significant (although modest) reduction of waist and hip circumferences.

Wider implications of the findings: Although we did not observe an impact on body weight, this study shows that mindfulness-based intervention favors waist and hip circumference reduction and improves the patient QOL. An extension study should evaluate if these benefits last longer and eventually contribute to weight loss.

Trial registration number: RBR-7by76r

P-576 The effectiveness of mindfulness-based stress reduction program on infertility stress, psychiatric symptoms and quality of infertile women's life

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Study question: What is the effectiveness of mindfulness-based stress reduction program on infertility stress, psychiatric symptoms and quality of infertile women's life?

Summary answer: Intervention of mindfulness-based stress reduction decreases the Infertility stress, the psychological symptoms & increases quality of life in infertile women.

What is known already: Studies that have examined the impact of psychological factors on infertility treatment, indicate that psychological factors not only in times have got far-reaching impact on the daily lives of the infertile patients, but they also affect treatment outcomes and in some cases they hinder the success of treatment.One of the variables that affect the response to treatment in patients with infertility treatment and should be considered is the stress of infertility.Infertility experience that some have called it Infertility crisis is associated with physical, economic, psychological and social stresses that affect all aspects of a person's life.

Study design, size, duration: The method of this study is quasi-experimental (pre-test and post- test) with a randomly assigned control group. The population is the entire women who referred to Royan Institute. In this study, sampling is targeted and the sample size is 150 persons that after pre-test evaluations were randomly assigned to two experimental groups and one control group that eventually the number of people in each group had 75 patients.

Participants/materials, setting, methods: 24 people of the total 75 patients in the experimental group were excluded due to lack of participation in the course of the study and a total of 51 for final data analysis in the group were tested. 49 people of 75 patients in the control group finally attended to complete the post-test and retained criteria for participation in the study.

Main results and the role of chance: *The results indicated that one can say with 95% confidence that overall score of concise syndrome and infertility stress (social concerns, worries about relationships, rejecting childless life, need for parenthood) in the two groups with controlling the effect of pre-test suggest that after the presence of infertile women in the mindfulness-based stress reduction sessions, the scores has had a significant decrease compared to the members of the control group. Also one can say with 95% confidence that the quality of infertility life score (quality of mind-body life, quality of relationship life, quality of social life and quality of life all in all) in the two groups with controlling the effect of pre-test suggest that after the presence of infertile women undergoing ICSI / IVF in mindfulness-based stress reduction sessions, the score of the members who participated in the experimental group has had a significant increase (005/0 P <, 007/33 = (1.94) F) compared with the control group.*

Limitations, reasons for caution: the incidence of personality conflicts in these couples, especially those who have experienced treatment failure is dramatic, that these factors also affect the treatment outcome.

Wider implications of the findings: the therapeutic components of mindfulness-based stress reduction techniques such as running the meditation, mindfulness techniques, increasing awareness and inner consciousness, increasing distress tolerance, identifying physical and emotional sensitivity, training to identify and replace dysfunctional and negative beliefs and thoughts and increasing health and inner peace.

Trial registration number: not applicable.

P-577 Psychosocial guidance of mothers of donor-children

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Study question: Are there associations between unmet needs for guidance of mothers of donor-children in father-mother, two-mother, and single-mother families and their parenting experiences and wellbeing?

Summary answer: Mothers in father-mother families have more unmet needs for guidance and the lack of guidance is associated with more parenting problems and less wellbeing.

What is known already: Previous studies showed that most mothers of donor-children have questions and needs in relation to donor conception (e.g., how to disclose, relationship with non-biological parent, influence of possible

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contact with donor). It is unclear whether mothers find appropriate guidance for these needs and whether there is a relation between unmet needs for guidance of mothers of donor-children and parenting experiences and mothers' and childrens' wellbeing.

Study design, size, duration: Cross-sectional study (online questionnaire) among 223 Dutch mothers of donor-children born since 2004, the year in which Dutch law prohibited donor sperm treatment with anonymous donor sperm. We recruited mothers between January 2016 and January 2017 both inside and outside the Dutch clinics.

Participants/materials, setting, methods: Mothers (N = 223; *M*age = 36.85, *SD*=4.63) in father-mother (*n* = 60), two-mother (*n* = 75) and single-mother (*n* = 82) families with a donor-child between 0-15 years old (*M*age=3.00, *SD*=2.29, range=0-15) filled out an online questionnaire. This questionnaire existed of validated instruments to measure unmet needs for guidance, parenting experiences (subscales: co-parenting satisfaction, parental burden, emotional involvement, parental concern), mother's wellbeing (subscales: psychosocial problems, partner relationship satisfaction), child's wellbeing (only for children aged 1,5 and older).

Main results and the role of chance: Mothers reported the highest unmet needs for guidance regarding the future contact between the donor and the donor-child and possible impact of this contact on the relationship with the non-biological parent. We found significant correlations between the mothers' unmet needs for guidance and family type, negative parenting experiences, co-parenting satisfaction, psychosocial problems of the mother, partner relationship satisfaction, and psychosocial problems of children aged 1,5-5. The more unmet needs for guidance related to being a parent of a donor-child mothers had, the less co-parenting satisfaction, the more parenting stress, the more concerns about their children by the mothers, the more psychosocial problems of the mother, the less partner relationship satisfaction and the more psychosocial problems for the child. Mothers in father-mother families had more unmet needs for guidance than those in two-mother families.

Limitations, reasons for caution: Causality can not be assessed, due to the cross-sectional design. Most mothers had children between 1,5 and 5 years old (0-1,5: *n* = 42, 1,5-5: *n* = 150, 6≥ *n* = 31). Therefore, caution is needed by generalizing these results to mothers of older and younger donor-children.

Wider implications of the findings: Guidance to help mothers cope with the uncertainties regarding possible future contact between their child and the donor should be made available.

Trial registration number: Not applicable. Ethical clearance by Medical Ethical Committee of the Academic Medical Centre of the University of Amsterdam (NL53349.018.15)

POSTER VIEWING SESSION
REPRODUCTIVE (EPI)GENETICS

P-578 The development and clinical evaluation of non-invasive chromosomal screening (NICS) assay

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Study question: This study involves the development and clinical evaluation of a biopsy-free and non-invasive preimplantation genetic screening (PGS) technique.

Summary answer: Both *in vitro* and clinical study demonstrated that the NICS assay was a reliable approach for chromosomal analysis of *in vitro* fertilized embryos.

What is known already: Conventional PGS involves the blastomere or trophectoderm biopsy from embryos at an early developmental stage. These procedures are invasive and technically challenging. Plus, the long-term effect of embryo biopsy has not been fully investigated. A prior study showed that the genetic materials from the blastocoele fluid could be an alternative source for PGS testing. In addition, genomic and mitochondrial DNA was detected in embryo culture medium, which suggested the possibility of using culture medium for preimplantation chromosomal screening.

Study design, size, duration: We developed a non-invasive chromosomal screening assay that used embryo culture medium for identifying chromosomal abnormalities of *in vitro* fertilized embryos. The technique was first validated by *in vitro* experiments followed by a clinical study.

Participants/materials, setting, methods: 16 couples undergoing *in vitro* fertilization (IVF) cycles were enrolled for the clinical study. 14 of them experienced idiopathic recurrent pregnancy loss or were diagnosed with chromosomal abnormalities including translocation, insertion, deletion, or duplication. Intracytoplasmic sperm injection (ICSI) was performed and embryos were screened for chromosomal abnormalities using the NICS assay before embryo transfer. Amniocentesis was performed at a later stage to confirm the karyotype of fetuses.

Main results and the role of chance: Among 16 couples, 14 of them had embryos passing the NICS assay. 1 or 2 embryos were selected to be transferred for each couple. A total of 20 embryos were transferred and 12 (60%) of them were successfully implanted. The pregnancy success rate of PGS using the NICS assay reached 78.6% (11/14). 5 pregnant participants received amniocentesis and all test results were normal. 5 babies were delivered and the cytogenetic analyses showed that the newborns were completely normal.

Limitations, reasons for caution: A larger scale clinical study may be helpful to further validate this technique.

Wider implications of the findings: This non-invasive and more simplified NICS assay may enable a wider application of PGS at IVF centers with different clinical settings.

Trial registration number: N/A.

P-579 Effects of high progesterone level on the day of human chorionic gonadotrophin administration in in-vitro fertilization cycles on epigenetic modification of endometrium in peri-implantation period

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Study question: Does high progesterone on the day of human chorionic gonadotrophin (hCG) administration in IVF cycles affect epigenetic modification of endometrium in the peri-implantation period?

Summary answer: DNA methylation, histone modification levels were increased in the endometrium of patients with high progesterone level on the day of hCG administration in IVF cycles.

What is known already: Epigenetic modification in the endometrium plays an important role in the regeneration, proliferation, angiogenesis and implantation, but the underlying mechanism is still not fully understood.

Study design, size, duration: A single-centre, retrospective cohort study between June 2013 and December 2013 included 40 infertile women, 20 women with normal progesterone and 20 women with high progesterone on the day of hCG administration after controlled ovarian hyperstimulation in an in vitro fertilization cycles.

Participants/materials, setting, methods: Endometrial tissues were collected 7 days after hCG administration in women with normal or high progesterone levels on the day of hCG administration. Immunohistochemical staining of DNA methylation (5-methylcytosine, 5-mC), histone methylation (H3K4me2/3, H3K9me2, H3K27me3) and histone acetylation (H3K4ac, H3K9ac) were performed.

Main results and the role of chance: In luminal epithelium, the expression of H3K9me2 in high progesterone group was significantly higher than that in normal progesterone group. In glandular epithelium, the expression of 5-mC,

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H3K9me2, H3K9ac in high progesterone group were significantly higher than that in normal progesterone group. In stroma, the expression of H3K27me3 in high progesterone group was significantly higher than that in normal progesterone group. 5-mC in glandular epithelium, H3k9me2 in glandular and luminal epithelium and H3K27me3 in stroma were significantly correlated with progesterone levels. H3k9me2 in glandular epithelium were significantly correlated with estrogen levels.

Limitations, reasons for caution: The expression was examined with immunohistochemical staining which was semi-quantitative. The sample size was not sufficiently large to permit in depth analysis of confounding variables.

Wider implications of the findings: Altered epigenetic modification status of the endometrium may dysregulate the expression of genes which then adversely affect endometrial receptivity in women with high progesterone on the day of hCG administration.

Trial registration number:

P-580 A novel mutation in the C10orf2 gene causes Perrault Syndrome with primary ovarian insufficiency

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Study question: To determine the genetic cause of a family with Perrault syndrome.

Summary answer: A novel mutation in the *C10orf2* gene was identified and resulted in functional defects of mitochondria.

What is known already: Primary ovarian insufficiency (POI) occurs in either isolated or syndromic forms. Perrault Syndrome is characterized by typical POI and sensorineural hearing loss. The causative mutations have been identified in five genes including *HSD17B4, HARS2, LARS2, CLPP and C10orf2*.

Study design, size, duration: Genetic and functional studies were performed to illustrate the etiology and pathogenesis of Perrault Syndrome.

Participants/materials, setting, methods: Whole exome sequencing was completed on five members of the Perrault syndrome family, and Sanger sequencing was used to confirm the results. The immortalized cell lines were generated by infecting lymphocytes from the family members with Epstein-Barr (EB) virus *in vitro*, and the subsequent experiments were carried out to measure ATP level, reactive oxygen species (ROS), mtDNA copy number and oxygen consumption rate.

Main results and the role of chance: A new homozygous mutation c.1388 G>A (p.R463Q) was identified in the helicase domain of the *C10orf2* gene, which encodes a hexameric DNA helicase in mitochondria and plays a critical role in mtDNA replication. The results of the functional studies *in vitro* showed that this homozygous alteration decreased the mtDNA copy number, ATP production, and especially the maximal respiration potential in the immortalized lymphocytes. However, the ROS level and mitochondrial ultrastructure remained unaffected.

Limitations, reasons for caution: The functional studies were carried out using immortalized lymphocytes but not oocytes or ovarian cells, which may not display direct impairment of the ovarian function.

Wider implications of the findings: Our study provided the evidence that the *C10orf2* gene mutation caused Perrault Syndrome with functional defects of mitochondria, suggesting the important role of mitochondria in ovarian function and POI pathogenesis.

Trial registration number: None.

P-581 Paternal parameters do not affect embryo ploidy: male age, semen quality, and PGS/NGS outcomes in oocyte-donors with normal karyotyping

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Study question: What is the correlation between paternal parameters and embryonic/individual euploid rates in oocyte-donors with normal karyotyping?

Summary answer: Paternal parameters do not affect euploid rate while oocyte quality was good, and donated-oocyte recipient with lower euploid rates do not have poor semen quality.

What is known already: The correlation between advanced male age and decreasing sperm quality has been indicated in the previous report, but the effect to embryonic chromosomal constitution remains unclear. Although increasing risk of sex chromosome aneuploidy was reported in the suboptimal sperm samples, no significant effect of paternal parameters to final reproductive outcomes in the oocyte-donor cycles was observed.

Study design, size, duration: A retrospective cohort study during 2015 to 2016, a total of 526 embryos screened by next-generation sequencing (NGS) from 131 oocyte-donor cycles were included.

Participants/materials, setting, methods: The association among male age, semen parameters (volume, concentration, percentage of progressive motility, total progressively motile sperm count, morphology), and embryo ploidies diagnosing by high-resolution NGS (aneuploid, mosaic, euploid) was analyzed in the oocyte-donor cycles (mean donor age: 25.6 years, all the donors went through chromosomal karyotyping). The initial semen state in patients with lower euploid rates than the average was compared with that of controls.

Main results and the role of chance: No significant relationship between male age and embryonic euploid rate was found: 57.0% in < 34 years, 57.3% in 35-40 years, 63.0% in 41-45 years, 55.9% in 46-50 years, 57.7% in >50 years; and the pure mosaicism rate (diploid/aneuploid mixture) did not decrease with increasing male age. With cutoffs according to WHO 2010 semen analysis guidelines, no differences in ploidy distribution were observed between the groups with normal and poor semen parameters. On the other hand, the means of each parameter showed no different among the aneuploid, mosaic, and euploid embryos. In patients with lower euploid rate than the average (< 58.2%), their paternal parameters displayed similar with those of controls (age: 42.7 years vs. 42.5 years; volume: 2.9 ml vs. 3.1 ml; concentration: 55.1×10^6 vs. 59.0×10^6; progressive motility: 22.4% vs. 25.9%; morphology: 1.4% vs. 1.4%; total progressively motile sperm count: 3598×10^4 vs. 4634×10^4). Moreover, no differences were found between the twice semen states in 11 patients taking the second oocyte-donor cycle due to poor euploid rate at the first cycle.

Limitations, reasons for caution: Half of cycles (47.1%) used frozen sperm samples which would be bias due to poorer viability after thawing. ICSI was mostly performed for both the patients with frozen-sperm and patients with poor semen parameters, and thus the outcomes could be masked.

Wider implications of the findings: The present study suggested that paternal parameters do not significantly affect embryonic chromosomal constitution in the oocyte-donors with normal karyotyping, indicating that good oocyte quality could withstand the adverse potential of poor sperm samples.

Trial registration number: Not applicable.

P-582 Preimplantation genetic diagnosis in Portuguese patients with Corino de Andrade disease

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Study question: Do patients with Corino de Andrade disease, either asymptomatic or after treatment, have normal reproductive capacity?

Summary answer: Female patients (clinical pregnancy) and transplanted patients (implantation, clinical pregnancy, live birth delivery and newborn) show worst clinical outcomes.

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What is known already: There are no large studies on clinical outcomes of patients with Corino de Andrade disease treated with preimplantation genetic diagnosis (PGD) for the prevention of the disease. Previous studies on the subject refer to the first 7 cases treated with PGD, where authors established the molecular diagnostic test, and a later a case report.

Study design, size, duration: Retrospective evaluation of 47 consecutive treatment cycles using PGD for the treatment of patients with Corino de Andrade disease (Portuguese patients presenting Transthyretin-related hereditary amyloidosis-ATTR). This study was conducted at the Centre for Reproductive Genetics A. Barros between 2000 and 2013 (13 years).

Participants/materials, setting, methods: The groups analysed were: total cases (28 patients, 47 cycles), cases with female ATTR (11 patients, 22 cycles), cases with male ATTR (17 patients, 25 cycles), asymptomatic cases (14 patients, 19 cycles), symptomatic cases (14 patients, 28 cycles), liver transplanted cases (10 patients, 22 cycles) and Tafamidis treated patients (4 patients, 6 cycles). Demographic, stimulation, embryological, clinical and newborn outcomes were evaluated for each group.

Main results and the role of chance: Comparisons between female (11 patients-P, 15 embryo transfer cycles-ETC) and male (17 P, 16ETC) patients revealed, in female patients, a significant higher dose of gonadotropins used, a lower number of high quality embryos (AB: **87%** vs 94%) and a lower clinical pregnancy rate (CP: **33%** vs 69%). No significant differences were found for the rates of fertilization (FR: 74% vs 76%), embryo cleavage (ECR: 98% vs 100%), blastocysts (BL: 42% vs 47%), implantation (IR: 29% vs 46%), live birth delivery (LBDR: 33% vs 63%) and newborn (NB: 8-53% vs 10-63%).

Comparisons between asymptomatic (14 P, 14ETC) and symptomatic (14 P, 17ETC) patients, revealed no significant differences for the rates of FR (78% vs 72%), ECR (98% vs 100%), AB embryos (91% vs 91%), blastocysts (44% vs 45%), IR (53% vs 33%), CP (64% vs 41%), LBDR (57% vs 41%) and NB (10-71% vs 8-47%).

Comparisons between transplanted (10 P, 14ETC) and Tafamidis taking (4 P, 3ETC) patients revealed no significant differences for the rates of FR (72% vs 77%), ECR (99% vs 100%), AB embryos (90% vs 96%) and blastocysts (43% vs 56%). Transplanted patients presented significant lower IR (**20%** vs 75%), CP (**29%** vs 100%), LBDR (**29%** vs 100%) and NB (5-**34%** vs 3-100%).

Limitations, reasons for caution: The major limiting factor is the low number of patients. However, CGR is one reference European center for performing PGD for patients with ATTR, and these are all cycles performed during this period of time.

Wider implications of the findings: Female patients presented a significant lower rate of high quality embryos and a significant lower clinical pregnancy rate, and a no significant lower rate of implantation and LBD. Transplanted patients presented a significant lower rate of implantation, clinical pregnancy, live birth delivery and newborn.

Trial registration number: None.

P-583 Comparison of blastocysts in PGD with GnRH antagonist and agonist protocol

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Study question: To investigate the differences of blastocyst formation rate and aneuploidy rate between PGD with GnRH antagonist and agonist protocol.

Summary answer: For women performed PGD, antagonist protocol show advantages in both blastocyst formation rate and aneuploidy rate albeit with less MII oocytes and fertilized eggs.

What is known already: Whether blastocyst formation rate and aneuploidy rate are different in GnRH antagonist and agonist protocol is in dispute while most studies show that decreased number of eggs and embryos result in decreased OHSS rate with uncompromised pregnancy rate in GnRH antagonist group.

Study design, size, duration: A before-after study in the same patient was conducted on 109 couples performed PGD from December 2012 to November 2016. PGD was performed to each patient for the reason of chromosomal aberrance. GnRH flexible antagonist protocol was applied to the patient if she failed the first cycle with agonist protocol or reversibly. The interval between the two cycles was limited to less than one year.

Participants/materials, setting, methods: The study was conducted at a Reproductive and Genetic Hospital. Blastocysts were scored according to the standard Gardner blastocyst grading system and laser assisted trophectoderm biopsy was performed on day 5 or 6 post oocytes retrieval. Comprehensive chromosome screening was performed with single-nucleotide polymorphisms or next generation sequencing. T-tests and chi-square test was used for statistical analysis.

Main results and the role of chance: Follicles with diameter≥14 mm (9.78 ± 4.09) and MII oocytes recovered(8.08 ± 4.64) of antagonist group are statistically less than those of agonist group (12.19 ± 4.33 and 9.67 ± 4.23, respectively) ($p < 0.01$). Fertilized eggs of antagonist group are also less than that of agonist group (6.4 ± 4.28 vs. 7.91 ± 4.49, $p<0.05$). But there are no statistical differences between the two groups with the number of blastomeres (5.83 ± 4.11, 6.65 ± 4.13, $p = 0.13$), blastocysts (2.74 ± 2.65, 2.25 ± 2.24, $p = 0.57$) and aneuploidy blastocysts (0.679 ± 0.99, 0.954 ± 1.33, $p = 0.21$). Nevertheless, number of high-quality blastocysts is higher in antagonist group than in agonist group (1.073 ± 1.66, 0.679 ± 1.33, $p<0.05$). There are also statistical differences between the two groups (antagonist vs. agonist) with blastocyst formation rate (42.4% vs. 32.6%, $p<0.01$) and high-quality blastocyst rate (39.5% vs. 26.6%, $p<0.01$). Aneuploidy rate (newly-occurred chromosome aberrance rate) is lower in antagonist group compared with agonist group (25.0%vs. 33.09%, $p<0.05$).

Limitations, reasons for caution: The study is limited for its before-after study in the same patient design and limited sample size.

Wider implications of the findings: Our results suggest that GnRH antagonist protocol show advantages in formation of high-quality and euploidy blastocysts in PGD.

Trial registration number: None.

P-584 High carrier frequency of GJB2 related non-syndromic sensorineural hearing loss and assisted reproductive technique

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Study question: What is the carrier frequency of autosomal recessive non-syndromic sensorineural hearing loss in the assisted reproductive technique population?

Summary answer: This study shows that 4.5% of analyzed individuals are carriers of mutations in the GJB2 gene related with non-syndromic sensorineural hearing loss.

What is known already: Hearing loss is the most frequent sensorineural impairment, affecting 5% of the world population. Congenital hearing loss occurs in approximately 1 in 1000 births. In 95% of cases, the parents of children with hearing loss have normal audition. Genetic causes account for up to 60% of hearing loss cases, both syndromic and non-syndromic. Non-syndromic sensorineural hearing loss is a heterogeneous genetic condition with different patterns of heritage; the autosomal recessive pattern (SNHLAR) accounts for 80% of the cases. More than 10% of SNHLAR cases are induced by GJB2 mutations.

Study design, size, duration: Retrospective cohort of 3,366 individuals attending to an ART clinic between March 2015 and November 2016. The study consisted of 2,380 women and 986 men. We also analyze 894 genetics match between oocyte donors and patients (747), oocyte donor and sperm donor (114) and infertile couples (33).

Participants/materials, setting, methods: An expanded carrier-screening blood test was performed (CarrierMap® by Recombine®), covering 2647 mutations implicated in 311 diseases. Briefly, DNA samples were prepared and

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purified following the QIAamp DNA Purification Protocol via QIAcube (QIAGEN). Samples were assayed using the Infinium iSelect HD Custom Genotyping BeadChip platform (Illumina). Regarding SNHLAR, the test screens for 30 mutations related with GJB2 gene, 2 related with LOXHD1 and 10 with MYO15A.

Main results and the role of chance: We found that 43% of our population has at least one genetic mutation (1458/3366). This percentage was similar in women (43% = 1012/2380) and in men (45% = 446/986). 43% of carriers for any mutations identified themselves as Europeans, 20% Mediterranean and the rest Latin American (15%), African (4%) and Asian (1.5%). Mutations in the GJB2 gene were the second most common ones among carriers, representing 10% (152/1458) of the total carriers, resulting in a carrier frequency of 1:22. This percentage was not statistically different between women (4% = 97/2380) and men (2% = 55/986; p>0.05). The vast majority of GJB2 mutation carriers were Europeans (54%) and Mediterranean (21%). No carriers were identified for mutations in the LOXHD1 and MYO15A genes. When considering the attempted genetic matching, 26 of 894 were of high reproductive risk (2,9%); of them 4/26 (15%) were related with GJB2 mutations.

Limitations, reasons for caution: The carrier frequency of SNHLAR is likely underestimated, because the test we applied screens for 30 specific mutations in GJB2, while more than 200 mutations have been described related with this gene alone.

Wider implications of the findings: The World Health Organization (WHO) considers hearing impairment a disability. Identification of couples where both partners are carriers will provide us with the opportunity to decrease their reproductive risk. Awareness programs directed towards reproductive aged people and healthcare professionals alike should be developed and implemented.

Trial registration number: NA.

P-585 MPS-based preconception carrier screening: development, verification and user experience

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Study question: Massive parallel sequencing (MPS) is a powerful tool in service of clinical genetics. What application can reproduction clinic find for such an instrument?

Summary answer: MPS-based preconception carrier screening reduces the risk of delivery a child with genetic disorder.

What is known already: The only mandatory genetic test for gamete donors in Russia is karyotyping. Therefore, personal and family history for genetic conditions of given donor remains unknown or vague. Some clinics offer carrier screening for common pathogenic variants of selected genetic disorders, leaving rare mutations undetected. Implementation of MPS-based carrier screening provides simultaneous deep analysis of numerous genes associated with inherited conditions at affordable turn-around time and in a cost-effective way. Obtained data allows genetic counselor to evaluate reproductive risks for referring couple and help them make informed decisions.

Study design, size, duration: NextGen21 genetic test includes 21 genes associated with most frequent, severe genetic disorders with no cure existing and/ or lifelong treatment. Target regions were selected based on genes' structure and function knowledge alongside with international guidelines and recommendations where applicable. Our database consist of over 16000 variants of established clinical significance, aggregated from various reliable databases and other sources. NextGen21 was verified by means of both human DNA standards (NIST, 1000 Genomes) and Sanger sequencing.

Participants/materials, setting, methods: Sample consisted of 1 NIST human standard DNA, 22 specimens from 1000 Genomes project (British population), 32 oocyte donors, 10 sperm donors and 6 patients. Carriers of X-linked and autosomal-dominant conditions with late-onset were excluded from donor programme. Patients underwent genetic counseling to establish if both partners were carriers for the same condition.

Main results and the role of chance: Overall 1096 unique genetic variants were detected, 14 of which were pathogenic/ likely pathogenic according to ACMG guidelines. Number of variants in one sample ranged from 139 to 259 with mean value of 192. Resulting rate 1 in 5,8 person being carrier at least of one genetic condition corresponds with estimated value 1 in 5, calculated from each disorder carrier frequency. 2 oocyte donors carrying X-linked disorders (Duchenne muscular dystrophy, hemophilia A) were excluded from donor programme. Surprisingly detected variants are single nucleotide polymorphisms while common type of genetic changes for given disorders are copy number variations (65-85% of DMD) and structural variations (around 55% of hemophilia A cases). During verification period NextGen21 was once used as a tool for detection of causative variant in 56 years old Ashkenazi Jewish woman with Hereditary Breast and Ovarian Cancer. Common pathogenic variant 6174delT in BRCA2 gene was found alongside with incidental findings of 2 pathogenic variants R241H and N409S in genes PAH and GBA respectively. Rare pathogenic variants found in genes CFTR, PAH, GBA, DMD and F8 emphasize advantages of test design based on structural and functional role of genetic regions over design targeting common mutations for given genetic disorder.

Limitations, reasons for caution: Major problem MPS-based carrier screening rises are variants of unknown clinical significance. Complexity of method requires appropriate pre- and post-testing genetic counseling. Patients should understand advantages, limitations and application NextGen21 has.

Wider implications of the findings: We plan to validate NextGen21 on samples with complex variants for genes traditionally challenging for MPS. Depending on validation outcomes we may change the list of genes screened by our test. We also consider adding several genes frequently requested by our patients.

Trial registration number: Not applicable.

P-586 INCREASED FIRST-TRIMESTER FALSE-POSITIVE ANEUPLOIDY SCREENING FOLLOWING ART

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Study question: Do assisted reproductive technologies (ART) modify the predictive value of first-trimester aneuploidy screening?

Summary answer: ART is associated with a higher false-positive aneuploidy screening rate for trisomy 21, 18 and 13 when compared to spontaneously pregnant women.

What is known already: Previous studies on the effect of ART on first trimester aneuploidy screening have shown conflicting results. Specifically, while ART does seem to significantly change the levels of trisomy 21 serum markers, the repercussion of these changes on the overall risk of false-positive screenings remains elusive. Furthermore, no study has previously assessed the effect of ART on the false-positive rate of combined trisomy 21,18 and 13 screening.

Study design, size, duration: Retrospective cohort analysis including 1128 women with a singleton pregnancy who performed their first-trimester combined aneuploidy screening for trisomy 21, 18 and 13 at our tertiary hospital between February 2013 and July 2016. We only included women with known final pregnancy and/or neonatal outcomes. Patients were divided according to whether they conceived spontaneously (n = 876) or following ART (n = 252; n = 152 IVF, n = 67 ICSI and n = 33 FET). Vanishing-twins were excluded from the analysis to minimize bias.

Participants/materials, setting, methods: All serum samples were processed under the same conditions (Elecsys®, Roche) and using the same combined risk calculation algorithm certified by the Fetal-Medicine-Foundation. This algorithm gives a combined adjusted-risk for trisomy 21, 18 and 13 accounting for: presence of aneuploidy markers diagnosed during the first-trimester ultrasound, serum levels of PAPP-A and βhCG, and other relevant female risk-factors (including age). Trisomy 21, 18 and 13 risks were considered elevated whenever above 1:300, 1:150 and 1:150, respectively.

Main results and the role of chance: Women performing ART group were significantly older (36,0 vs 32,3 years-old) and performed their first-trimester ultrasound sooner (at the gestational age of 85,0 vs 88,0 days) when compared

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to those who conceived spontaneously. Gestational-age-adjusted PAPP-A MoM levels were significantly lower in the hormonally-stimulated IVF/ICSI groups (median 0,7, 0,7 and 1,0 for conventional IVF, IVF-ICSI and spontaneous conceptions, respectively). Meanwhile, the MoM of βhCG and nuchal translucency did not vary significantly among the groups. Most importantly, ART pregnancies had a significantly higher chance of having a high-risk aneuploidy screening (11,2%, 11,9% and 5,6% for conventional IVF, IVF-ICSI and spontaneous conceptions, respectively) and also a higher risk of a false-positive result (9,9%, 11,9%, and 5,0% for conventional IVF, IVF-ICSI and spontaneous conceptions, respectively).

Limitations, reasons for caution: Our results cannot be extrapolated to older women or blastocyst transfers given the fact that our sample only included women under 40 years of age performing cleavage-stage embryo transfers. Furthermore, we cannot exclude the possibility that other cases of aneuploidy may have been missed amongst the women lost to follow-up.

Wider implications of the findings: These results could prompt the development of newer aneuploidy screening algorithms accounting for the type of conception, potentially reducing the number of unnecessary invasive diagnostic tests. Given the potential risk of miscarriage following these invasive diagnostic procedures, such algorithms could be beneficial for couples who conceived after ART.

Trial registration number: Not applicable.

P-587 Pre-implantation genetic testing does not decrease cumulative drop-out rate when compared to standard in-vitro fertilization in women with advanced maternal age

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Study question: Does performing pre-implantation genetic testing (PGT) influence the drop-out rate from in-vitro fertilization (IVF) treatment in women with advanced maternal age (AMA)?

Summary answer: Female age, but not performing PGT, significantly contributes to drop-out decision from further IVF treatment in women with AMA.

What is known already: Discontinuation from IVF treatment is frequent (17-70%) and contributes to compromised cumulative pregnancy rates in IVF. Pre-implantation genetic testing with single frozen euploid embryo transfer (SFEET) increases the live birth rate per embryo transfer and decreases the multiple pregnancy rate in AMA cases. Since poor prognosis and failure to achieve embryo transfer are among the main known contributors of drop-out from further IVF treatment, we aimed to evaluate whether IVF treatment coupled with PGT have any influence on drop-out rates compared to standard IVF in AMA patients.

Study design, size, duration: Longitudinal cohort comparison of pre-intervention (standard IVF; June 2013-October 2014; 198 couples) and post-intervention (PGT with SFEET; April 2015-June 2016; 203 couples) periods in AMA (≥38 yr-old) cases undergoing IVF, regardless of ovarian reserve. Exclusion criteria were azoospermia and no follicular growth to ovarian stimulation with no further treatment advised by the physician. Only AMA patients with their first cycle at our center during defined time periods were included.

Participants/materials, setting, methods: In both arms, all started stimulation cycles (with/without cycle cancellation) were counted as separate cycles. In the PGT arm, ovarian stimulation and first SFEET was counted as one cycle; all other frozen replacement cycles, in both arms, were counted as separate cycles. Not commencing a new treatment cycle 6 months after failing to achieve an ongoing pregnancy was defined as drop-out. The incidence and factors related to drop-out from three IVF cycles with/without-PGS were studied.

Main results and the role of chance: Female age (40.9 ± 2.2 vs. 40.7 ± 2.2, p = 0.506), antral follicle count (8.0 ± 5.3 vs. 7.7 ± 6.2, p = 0.518), number of previous IVF attempts (1.0 (0-3.0) vs. 1.0 (0-3.0), p = 0537), number of patients with previous childbirth (24.7% vs. 21.2%, p = 0.233) were comparable among the with-PGT or without-PGT arms, respectively. The drop-out rates following 1st failed IVF cycle were 62.8% vs. 70.2% in the with-PGT or without-PGT arms, respectively (p = 0.143). The respective figures for the 2nd cycle were 57.4% and 69.6% (p = 0.209). Cumulative drop-out rates for 3-planned cycle were not significantly different (69.7% and 77.3%, p = 0.090, respectively) with an overall rate of 73.8%. Cumulative ongoing pregnancy rate per patient was comparable between the two arms (20.7% vs. 16.2%, p = 0.369). However, ongoing pregnancy/live birth per embryo transfer was significantly higher in the PGT arm (42.2% vs. 16.4%, p < 0.001). As expected, multiple pregnancy per ongoing pregnancy was significantly less in the PGT arm [1/41 (2.4%) (mono-zygotic twin) vs. 5/33 (15.1%), p = 0.046]. When Cox-regression analysis was performed, only the female age was the significant contributor to drop-out (HR 1.07; 95% CI, 1.09–1.13). However, previous childbirth, antral follicle count, cycle cancellation, number of metaphase-2 oocytes, failure to achieve embryo transfer and performing PGT, were not significant contributors.

Limitations, reasons for caution: Retrospective study design is a limitation. These data reflect drop-out rates from a single private-based IVF center; hence, the results may not be extrapolated to drop-out rate from IVF treatment elsewhere. Rates might also be affected by type of payment, patient/insurance/government based, which cannot be addressed with the current series.

Wider implications of the findings: ~ 75% of AMA cases, not achieving an ongoing pregnancy, discontinue IVF program before completing 3 cycles. Performing PGT, does not reduce such drop-out rate. Female ageing is the only significant contributor with the hazard of discontinuation from further IVF treatment by 7% with female ageing of 1-yr.

Trial registration number: None.

P-588 Directly distinguish chromosome balanced rearrangement carrier embryos from truly normal embryos in 56 PGD cycles

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Study question: We established a clinical applicable approach to identify rearrangement breakpoint region linkage specific single nucleotide polymorphisms (SNPs) haplotype and to further directly distinguish truly normal embryos in PGD.

Summary answer: Our results suggest that microdissecting junction region (MicroSeq) can accurately evaluate the balanced or normal status of each embryo of chromosome balanced rearrangement(CBR) carriers.

What is known already: Preimplantation genetic diagnosis (PGD) is widely applied in chromosome balanced rearrangement(CBR) carriers to increase the chance for a successful live birth. However, carrier embryos were seldom discriminated from the normal ones mainly due to the technique restriction.

Study design, size, duration: This was a prospective study for 56 CBR carrier couples, who were treated by D5 or D6 biopsed PGD-CCS between July 2014 and December 2016.

Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya. Totally 56 carrier couples were recruited, including reciprocal translocation(n = 44), Robsonian

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translocation(n = 8), and inversion(n = 4). Before performed PGD, rearrangement breakpoint region and adjacent specific SNPs were characterized by next-generation sequencing following microdissecting junction region (MicroSeq) from 56 carriers. In the clinical phase of embryo analysis, specific SNPs of junction region were chosen for linkage analyses to identify the truly normal embryos.

Main results and the role of chance: Totally performed 63 OR cycles and biopsied 259 blastocysts(actually detected 233 blastocysts). Totally 101 blastocysts diagnosed to be chromosomal balanced, 57 blastocysts were identified to be carriers and 44 to be normal.The proportions of normal karyotype embryos obtained in reciprocal, Robertsonian and inversion carriers' cuoples were 13.33% (24/180), 33.33%(11/33) and 45%(9/20), respectively. The proportions of carriers karyotype embryos obtained in reciprocal, Robertsonian and inversion carriers' cuoples were 23.89% (43/180), 30.30%(10/33) and 20%(4/20), respectively. Cumulative 38 FET cycles were carried and resulting 24 pregnancy.Eight cycles late prenatal diagnoses(PND) for five carriers and three normal fetus confirmed the carrier diagnosis results. Other predicating eight carriers and five normal fetus is still in pregnancy without performe PND.

Limitations, reasons for caution: The final outcomes of 10 PGD cycles still have not been obtained.We cannot exclude differences between the final data and the data in the present submitted abstract.

Wider implications of the findings: MicroSeq technique is suitable for almost all CBR carrier couples PGD treatment.

Trial registration number: Not applicable.

P-589 Relationship between ´´high normal´´ CGG repeats of the FMR1 gene and response to gonadotropin stimulation in fertile women

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Study question: Is there an association between a specific range within the high normal CGG repeats (36-45) of the FMR1 gene and response to gonadotropin stimulation?

Summary answer: The range between 36-45 CGG repeats on the FMR1 gene was not correlated with suboptimal response to gonadotropin stimulation and impaired oocyte maturation.

What is known already: The Fragile X mental retardation 1 (FMR1) gene contains a variable number of trinucleotide (CGG) repeats. The majority of the population has 29-32 CGG repeats (normal range). Full mutation and premutation with >200 and 55-200 CGG repeats, respectively, are associated with diminished ovarian reserve. Within the high normal range (>32) smaller expansions of CGG repeats generate different clinical manifestations. Therefore, the relationship between 36-45 CGG repeats (included in the high normal CGG repeat zone) anti-Müllerian hormone (AMH), response to gonadotropin stimulation and oocyte maturation following retrieval, has yet to be assessed.

Study design, size, duration: A retrospective study involving 65 egg donors participating in a total of 82 donation cycles, who donated to a large private sperm and egg bank from March 2016 to January 2017. The egg donors were categorized into two groups i) donors with ≤35 CGG repeats in allele 1 and ii) donors with ≥36 CGG repeats in allele 1 of the FMR1 gene.

Participants/materials, setting, methods: All egg donors underwent FMR1 screening and AMH testing. Once approved, the egg donors began controlled ovarian stimulation utilizing a gonadotropin-releasing hormone (GnRH) antagonist protocol following a GnRH agonist (GnRHa) to induce oocyte maturation when follicles reached >17 mm in diameter in order to schedule oocyte retrieval, 36 hours later. Oocytes were denuded and metaphase II (MII) oocytes were assessed and vitrified. Serum estradiol (E_2) and progesterone were measured on cycle day 1, 4 and 7.

Main results and the role of chance: The majority of egg donors (59%) were Caucasian with mean age 24 ± 3.7 years (range 18-30 years), non-smokers with BMI ≤ 27 kg/m^2. The AMH (7.4 vs 6.6 ng/mL) and age (24.6 ± 3.7 vs 21.1 ± 3.1 years) were not significantly different between the two groups (p> 0.05). In contrast to allele 2 (26.6 ± 4.3 vs 30.7 ± 0.8; p> 0.05), the number of CGG repeats in allele 1 of the FMR1 gene for groups i) and ii) differ significantly (30.9 ± 1.4 and 41 ± 3.2; p < 0.05). There was no significant correlation between the amount of exogenous follicle-stimulating hormone (FSH) required for ovarian stimulation (184.2 ± 66.1 vs 220.80 ± 71.8 IU) and duration of stimulation between the two groups (9.4 ± 1.6 vs 9.30 ± 1.3 days; p> 0.05). The peak E_2 was not significantly associated with the number of CGG repeats (2147.8 ± 905 vs 2205.6 ± 1021.6 pm/mL; p> 0.05). No statistical difference was observed in the number of oocytes retrieved between the two groups, however a significant relationship was evident between increased number of CGG repeats and proportion of MII oocytes (74.2 vs 82.0 %; p < 0.001).

Limitations, reasons for caution: A limitation is the sample size of this still ongoing study, extended data will be available at the ESHRE meeting. In addition, egg donors is a homogeneous selected group so these results might not be extrapolated to other groups of women undergoing fertility treatment.

Wider implications of the findings: This is the first study to investigate in a population of healthy women, a specific range of the high normal CGG repeat numbers of FMR1 allele 1 that doesn't impair ovarian function, highlighting the importance of donor candidate assessment and provides valuable information on predicting risk towards premature ovarian insufficiency.

Trial registration number: N/A.

P-590 Comparative analyses of AZF microdeletions in leukocytes and testis tissue of TESE patients reveals cellular mosaicism impairing their sperm prognosis

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Study question: Why do patients with non-obstructive azoospermia and complete AZFa,b,c deletions found in leukocytes do have variable testicular pathologies ranging from Sertoli-cell-only syndrome (SCO) to hypospermatogenesis?

Summary answer: Genomic extensions of AZF deletions in patients Y DNA extracted from leukocytes and testicular tissue are variable due to cellular heterogeneities and probably mosaicisms.

What is known already: Azoospermia Factor (AZF) locus causes male infertility when disrupted. It includes 14 protein encoding Y genes located in three distinct Y regions designated AZFa, AZFb, AZFc, respectively. They are expressed during different phases of human spermatogenesis. Accordingly, complete deletion of an AZF region is associated with a distinct testicular pathology. This can be used for sperm prognosis of patients suffering from non-obstructive azoospermia who decided for testicular sperm extraction (TESE). Complete AZFa or AZFb deletions suggest, they have no sperms in their testicular tubules, whereas patients with complete AZFc deletion are believed to have a good prognosis for sperm detection.

Study design, size, duration: Comparative analysis of the extension of AZF microdeletion found in the genomic DNA samples of leukocytes and testicular tissue extracted from patients with non-obstructive azoospermia asking for testicular sperm extraction. Extension of PCR multiplex assays according to EAA/EMQN guidelines to recognize each AZF gene deletion separately and to distinguish the genomic breakpoints in the proximal and distal AZF deletion border regions.

Participants/materials, setting, methods: From 110 TESE patients DNA samples from leukocytes and testis tissue samples were collected between years 2009-2016. Comparative analyses for presence of partial or complete AZFa,b,c microdeletions were performed according to Vogt & Bender (Meth. Mol. Biol. Vol 927: 187-204, 2012). Any Y gene deletion result was confirmed by single STS PCR assays. Novel genomic AZF breakpoint regions were elaborated by PCR cloning and confirmed by sequence analyses.

Main results and the role of chance: Comparison of genomic extensions of AZF deletions in patients Y DNA of leukocytes and testicular tissue revealed

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some heterogeneities suggesting cellular mosaicism. We found one complete AZFa deletion, one partial AZFb + complete AZFc deletion and 7 complete AZFc deletions in leukocytes but not any of these AZF deletions in the same patients' genomic testis DNA. Quantitative analysis of the AZF gene markers used in testis tissue by appropriate TaqMan assays indicated cellular heterogeneities. In most but not all cells in the testis tissue the Y chromosome did not display the same AZF deletions found in leukocytes and maybe even absent. By FISH analysis with appropriate Y-DNA probes we confirmed this cellular heterogeneity. We assume that probably only germ cells do have the AZF deletions found in the patients leukocytes. The molecular mechanisms causing AZF microdeletions by non-homologous recombination events may thus only occur in germ cells due to complete decondensation of the human Y chromosome before meiosis.

Limitations, reasons for caution: Comparative analyses of extensions of AZF deletions in leukocytes and testis tissue of 110 TESE patients includes till now only 9 AZF mutation events in the total cohort. Large heterogeneities of their testicular tissue pathologies might also depend on the variable age of these patients probably interfering with our results.

Wider implications of the findings: Our results can probably explain the large heterogeneity of testicular pathologies found in patients with complete AZF deletions in their leukocytes and are important for their clinical counseling when asking for sperm prognosis before performing their testis biopsy.

Trial registration number: Not applicable.

P-591 Pregnancy rate per transfer in women over 38 years is improved by PGD-A after Polar Body (PB) biopsy. A study on 159 embryo transfers

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Study question: Is PGD-A on PB a valid alternative to trophectoderm biopsy in order to increase the pregnancy rate per transfer?

Summary answer: PGD-A on PB was shown to increase the pregnancy rate per transfer in older women, via selection of euploid oocytes.

What is known already: PGD-A is an established technique for increasing the chance of pregnancy, especially in women older than 36. The techniques for both genetic analysis and for biopsy have greatly improved. Blastocyst biopsy and comprehensive analysis of all chromosomes using aCGH or NGS has come to be considered the gold standard. Despite its safety and suitability for screening for maternally-derived aneuploidy, PB biopsy has been almost abandoned because of the higher costs and the higher biopsy skills requested. Consequently, there is a shortage of data concerning the effectiveness of PGD-A on PB.

Study design, size, duration: The result in terms of percentage of pregnancy rate (biochemical and ongoing pregnancy) in 80 embryo transfers after PGD-A on the first and second PB and resulting from 66 ICSI cycles was retrospectively compared to 79 embryo transfers without PGD-A, resulting from 79 matched randomized ICSI cycles performed in the same period in our clinic (2014-2016). All cycles were considered except for patients requesting PGD for monogenic diseases or chromosomal translocations.

Participants/materials, setting, methods: First and second PBs were taken simultaneously about 20 hours after ICSI. PB were individually amplified by WGA and chromosomes were analysed by array-CGH. One or two embryos were transferred freshly at day 3, or frozen 22 hours after ICSI and transferred after thawing. Pregnancy was considered if bHCG was higher than 10 IU/L at day 14. Pregnancy rates in control and PGD-A groups were compared by Fisher's two tailed exact test

Main results and the role of chance: Mean patient age was 37 years for both groups. 967 PB from 488 oocytes were analysed. 40% of PB were euploid, 47% were aneuploid and for 12% the result was not available (indeterminate). By considering all the transfers (n = 80), the pregnancy rate per transfer in the PGD-A group was 38% versus 31% in the control group (p = 0.05). Considering only women ≥38 years at pick-up (n = 43 in PGD-A group, n = 41 in control group), the pregnancy rates were 47% (PGD-A) versus 22% (controls; p = 0.0003). For women ≥39 at pick-up (n = 34 in both groups), pregnancy rates were 45% (PGD-A) versus 18% (controls); p = 0.0004).

Limitations, reasons for caution: This study is based on a limited number of subjects (80 cases, 79 controls). It will be necessary to confirm these data on a larger cohort of subjects.

Wider implications of the findings: PGD-A is a widely-used screening method in older women to prevent IVF failure caused by chromosomally-abnormal oocytes, and to avoid aneuploid pregnancies. Aneuploidy testing in PB is an effective alternative. Main advantages are 1) the possibility of transfer at day 2-3; 2) the absence of artifacts due to embryonic mosaicism

Trial registration number: none.

P-592 Alterations of methylation are reversible by developmental reprogramming in kidney tissue of Intracytoplasmic sperm injection (ICSI) mice

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Study question: Does the effect of Intracytoplasmic sperm injection (ICSI) on methylation continue during the development?

Summary answer: We found that ICSI manipulation and early embryo culture resulted in alterations of methylation, and the alterations were reprogrammed by developmental reprogramming.

What is known already: Although the prevalence of ICSI has increased year by year, there remains concern about the safety of these procedures because of reports of the increased risk for imprinting disorders. Previous research has demonstrated that gonadotropin stimulation contributes to an increased incidence of epimutations in ICSI-derived mice. However, the epimutations in ICSI offspring after removing the effect of gonadotropin stimulation and the possibility that epimutations are reversible by developmental reprogramming has not been investigated.

Study design, size, duration: Kidney tissues form twenty two-cell embryos transfer (TCET) mice (10 adults and 10 olds) and sixteen ICSI mice (8 adults and 8 olds) were analyzed. We quantitatively examined the DNA methylation patterns of a number of CpGs in the differentially methylated region (DMRs) of imprinted genes, since these are involved in kidney diseases.

Participants/materials, setting, methods: We used mice derived by TCET as control group to exclude the effect of gonadotropin stimulation. A key difference between ICSI and TCET mice is that manipulation and injection of oocytes and culture during the ICSI program are removed during the TCET process and replaced by in vivo fertilization. Kidney tissues obtained from adults or olds were used to analyze DNA methylation and expression of kidney-disease related imprinted genes including *H19*, *Snrpn*, *Mest* and *Peg3*.

Main results and the role of chance: We found reduced methylation and up-regulated expression of the imprinted genes, H19, Mest and Peg3, in adult ICSI mice (e.g. H19 methylation 58.71% ± 1.49% in ICSI mice vs. 62.77% ± 1.56% in TCET, p < .01, H19 mRNA expression 1.80 ± 0.35 in ICSI mice vs.1.00 in TCET, P < .01). But the above alterations observed in adult mice were not detected in old ICSI mice (e.g. H19 methylation 63.29% ± 1.97% in ICSI mice vs. 61.67% ± 0.98% in TCET, p>.05, H19 mRNA expression 1.03 ± 0.10 in ICSI mice vs.1.00 in TCET, p>.05). At the Snrpn DMR, methylation status was not altered in adult ICSI-derived mice (methylation 53.00% ± 2.30% in ICSI mice vs. 54.50% ± 0.70% in TCET, p>.05), but hypermethylation and correlated down-regulated expression of Snrpn were observed in old mice (methylation 57.70% ± 1.50% in ICSI mice vs. 54.10% ± 1.05% in TCET, p < .01, mRNA expression 0.85 ± 0.08 in ICSI mice vs.1.00 in TCET, P < .01).

Limitations, reasons for caution: Assessment of renal function in the blood and immunohistochemistry of the kidney tissue were not been carried out, which might provide more evidences for the alteration.

Wider implications of the findings: We are the first to find that alterations of methylation caused by ICSI itself exist after excluding the effect of

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gonadotropin stimulation, and the alterations might be corrected by developmental reprogramming in kidney tissues. But the mechanism under the reversible change and other tissues still needs further investigation.

Trial registration number: Not a clinical trial.

P-593 Comparison of livebirth delivery rates as a function of PGD use by IVF centers in the United States: Analysis of new national CDC data

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Study question: What is the impact of including PGD with IVF on reproductive outcome in the United States?

Summary answer: Livebirth delivery rates were not significantly different among IVF clinics with high uptake of PGD compared to clinics with low PGD use.

What is known already: Recognizing the limitations of embryo selection based on morphology alone, PGD has emerged as another assessment method aiming to improve IVF efficiency. Array comparative genome hybridization is a widely used PGD method, but this is gradually being replaced by massive parallel sequencing. In the meantime, there is still controversy regarding the clinical value of PGD even as it becomes used more often with IVF.

Study design, size, duration: This analysis was based on the 'Fertility Clinic Success Rates Report" from U.S. CDC. Data were reviewed from 208,604 treatment cycles performed at IVF units in the United States (n = 458) during 2014.

Participants/materials, setting, methods: Patient data from reporting clinics were stratified by level of PGD utilization: Group 0=PGD not performed, Group 1=PGD used in ≤5% of IVF, and Group 2=PGD used in >5% of IVF. Delivery rates for the three categories were compared by Chi-squared test.

Main results and the role of chance: For Group 0 (n = 7761), delivery rate after IVF cycle was reported as 37.9%. Patients in Groups 1 (n = 24897) and 2 (n = 6915) had IVF delivery rates of 36.7% and 37.6%, respectively (p = 0.27, for all three groups).

Limitations, reasons for caution: This dataset did not stratify PGD application by biopsy stage, infertility diagnosis, or patient age, and the PGD technique was unspecified. Data on fresh vs. frozen embryo transfers after PGD also could not be disaggregated. Other factors besides PGD uptake will influence reproductive outcome with IVF.

Wider implications of the findings: While preimplantation assessment of embryos can help select euploid embryos for transfer, more information is required to quantify exactly how this impacts outcomes with IVF. More precise ascertainment of PGD applications by national reporting platforms is essential to provide proper surveillance, particularly as PGD becomes more widely utilized.

Trial registration number: N/A.

P-594 Introduction of a novel, universal NGS-based research method for Preimplantation Genetic Diagnosis and Screening

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Study question: Is it possible to design a universal end-to-end NGS workflow that enables performing PGD (for Single Gene Disorders and Translocations) combined with PGS in the same embryo biopsy?

Summary answer: Our approach provides a universal end-to-end NGS workflow for PGD and demonstrates the power of combining PGD with PGS on the same embryo biopsy.

What is known already: Over the past few years, several new technologies have been developed for the high resolution molecular cytogenetic analysis of embryos prior to implantation. Although technologies such as STR-PCR, SNP arrays and aCGH have been utilized for the detection of mutations associated with Single Gene Disorder (SGD) and/or translocations for Preimplantation Genetic Diagnosis (PGD), significant challenges persist. To overcome these challenges, we have developed a novel NGS-based method coupled with specific algorithms that utilize parental haplotype information to detect SGD-associated mutations and translocations.

Study design, size, duration: In this retrospective research study, we compared the results obtained with the proposed approach to the results obtained with a validated reference method that is run in a reference lab. The analyses were performed throughout 2015-2017 on a diverse set of embryos from families with various PGD indications.

Participants/materials, setting, methods: We processed embryo biopsies harboring SGD-associated mutations, translocations and aneuploidies. Biopsies included blastomeres and trophectoderm cells. The data were analyzed using the novel algorithms we developed, and the results were compared to those obtained using a validated technology in a reference lab. Additionally, we also evaluated the performance of our assay using cell lines that harbor aneuploidies.

Main results and the role of chance: Our analysis on a diverse set of embryos that included single blastomeres and trophectoderm biopsies showed excellent concordance with results from the previously validated reference method. Additionally, we observed excellent concordance for a wide range of chromosome aneuploidies from cell lines. Our approach does not require gene-specific spike-ins, thereby making it a highly versatile assay. As our approach can concurrently detect aneuploidies, this effectively combines PGD and Preimplantation Genetic Screening (PGS) in one method.

Limitations, reasons for caution: The presented data represent the results obtained during a retrospective research study. Further verification and validation will be needed to assess the performance of the product.

Wider implications of the findings: This represents a novel Preimplantation Genetic Test approach that will enable combining PGD and PGS on the same embryo biopsy using a universal end-to-end NGS workflow that does not require gene-specific spike-ins.

Trial registration number: NA.

P-595 A cost-effectiveness analysis of preimplantation genetic screening with in vitro fertilization in Ontario, Canada

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Study question: What is the cost-effectiveness of preimplantation genetic screening (PGS) with IVF compared to IVF alone in terms of additional live births?

Summary answer: PGS becomes increasingly cost-effective as both the age of the woman and the number of available blastocysts increases.

What is known already: Many embryo transfers fail to result in an ongoing pregnancy because the embryos are aneuploid, and this occurs more as female reproductive age advances. PGS allows the transferable cohort of embryos to be limited to euploid embryos, which have been shown to have a higher implantation rate and lower early pregnancy loss rates when compared to the transfer of an unscreened embryos. Very little is known, however, about the cost-effectiveness of PGS compared to IVF alone for women of varying ages and blastocyst cohort numbers.

Study design, size, duration: A decision analytic model was created using TreeAge Pro to compare IVF with PGS to IVF alone for women of different ages with one to ten blastocysts from the societal perspective, encompassing all relevant costs including time off work. Time to conception was not modelled. Sequential single embryo transfers of all blastocysts from a single oocyte retrieval were modelled until all blastocysts were exhausted, treatment was discontinued or a live birth was achieved.

Participants/materials, setting, methods: Clinical parameters were obtained from provincial and national databases, peer-reviewed literature and expert opinion. Cost parameters were obtained from the Ontario Case Costing Initiative, the Ontario physician schedule of benefits and fertility clinics in Ontario, Canada. Productivity costs were estimated using data from Statistics Canada and expert opinion. One-way and probabilistic sensitivity analyses were

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performed to characterize the uncertainty around the estimates and to test the robustness of the model to various input parameters.

Main results and the role of chance: Compared to IVF alone, the cost-effectiveness of PGS depends both on female age and the number of available blastocysts. PGS became both incrementally less expensive and more effective as the number of blastocysts and the age of the woman increased. IVF alone was the superior strategy – more effective and less costly - for all women under age 35. This was also true when 3 or fewer blastocysts were available regardless of age. For women aged 35 to 40, PGS was more expensive than IVF alone regardless of blastocyst cohort size but PGS was slightly more effective in this age group once the cohort size exceeded 7-8 blastocysts. For women aged 41 to 42, IVF alone was the superior strategy when the cohort size was 5 or fewer. The two strategies were comparable when 6 – 8 blastocysts were available. PGS was the superior strategy only once the cohort size reached 9 blastocysts. For women above age 42 with a cohort size of at least 4, PGS was less costly with comparable effectiveness compared to IVF alone. One-way sensitivity analysis showed that the model was most sensitive to the implantation rates and early pregnancy loss rates.

Limitations, reasons for caution: There was a lack of high quality data to inform some of the model inputs, including implantation and pregnancy loss rates after PGS, to which the model was most sensitive. Data on dropout rates and time off work due to treatment and potential complications were also limited.

Wider implications of the findings: Using currently available data, PGS does not appear to be cost-effective in most scenarios when examined from a societal perspective in Ontario, Canada. PGS may be cost-effective for women over age 35 with a large number of blastocysts. Future research should focus on creating more robust population-based PGS outcome data.

Trial registration number: N/A.

P-596 Clinical application of low coverage massively parallel sequencing for chromosome aneuploidy and copy number variation screening in 6333 embryos

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Study question: Evaluation in terms of Preimplantation genetic screening (PGS) for aneuploidy and CNV detection, how the next-generation sequencing along with low-pass whole-genome sequencing, which provides the ability to assess many biopsied trophectoderm cells in order to confirm which normal embryos are ready-to-transfer in an IVF cycle.

Summary answer: Next-generation sequencing(NGS) is an emerging technology that provides a high-throughput parallel analysis of multiple embryos and high-resolution data for chromosomal analysis.

What is known already: Chromosome abnormality is a leading cause of repeat implantation failure and recurrent miscarriages. PGS enables the assessment of the numeral and structural chromosomal errors of embryos before transfer in patients undergoing IVF. NGS has been demonstrated to be an accurate PGS method and in present is thought to be effective.

Study design, size, duration: Retrospective cohort study of 6333 embryos obtained from 1699 couples performed between Feberary 2013 and December 2016 at Beijing Genomics Institute, Shenzhen, China.

Participants/materials, setting, methods: Trophectoderm biopsy were performed for the embryos. NGS was used for chromosome aneuploidy and copy number variation analysis, with an average of 0.07 depth and 5.5% coverage of the human genome.

Main results and the role of chance: Reliably identified with the NGS-based protocol were 3044 (48.1%) abnormal derived from 6333 embryos, with the smallest detectable chromosomal segment being 4 Mb in size. Within the 3044 abnormal embryos analysed, 1187(38.99%) were aneuploidies only, 1374 (45.12%) were just CNVs, 483(15.87%) were both CNV and aneuploidy, In terms of aneuploidy abnormality, chromosome anomalies, including chromosome 22(10.68%), 21(8.05%), 16(7.62%), 15(5.98%), 13(5.19%), 14(5.19%), X (4.04%) were of high frequencies.

Among all the couples, 513(513/1699) couples were balanced rearrangement carries, 3054 embryos obtained from them, 1381(45.22%, 1381/3054) were abnormal(translocation alone, related aneuploidies), 405(13.3%,405/3054) were spontaneous and inherited abnormalities. Also, the most common abnormalities were CNVs, followed by whole autosomal trisomies and monosomies, segmental imbalances of nontranslocation chromosomes.

But within non-balanced rearrangement couples, 3279 embryos obtained, 1258(38.4%,1258/3279) were abnormal. Compared with two groups, couples with balanced rearrangement showed higher frequency to produce chromosome anomaly embryos.

Limitations, reasons for caution: This method detects chromosomal aneuploidy and DNA deletion or duplication larger than 4 Mb. This method cannot detect point mutation, balanced translocation, inversion, ploidy change, low proportion mosaicism, uniparental disomy, methlytion alternation, and other variations that are not covered by this method.

Wider implications of the findings: NGS combined with WGA and bioinformatics analysis can effectively detect aneuploidies and CNVs in trophectoderm cells, meanwhile NGS will play an increasingly significant role in clinical laboratories for human-assisted reproduction applications and studies. This strategy is rapid, highly accurate, robust, sensitive, easily reproducible, and cost-effective in contrast to other methods.

Trial registration number: no.

P-597 Factors associated with chromosomal mosaicism in trophoectoderm cells

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Study question: Which paternal and maternal variables do increase the probability of chromosomal mosaicism in the trophectoderm cells?

Summary answer: Paternal age and embryo quality are associated with embryonic mosaicism.

What is known already: There is a high incidence of chromosome abnormalities in human embryos that leads to failure of IVF cycles. The chromosomal mosaicism consists of a mixture of more than one cell line with different karyotype in the same embryo. The main factors that may increase the incidence of embryo mosaicism have not yet been established.

Study design, size, duration: We retrospectively reanalyzed array-CGH results from trophoectoderm biopsies of day 5 and 6 blastocysts (from January 2013 to January 2017). We analysed 1923 blastocysts from 704 IVF cycles. We considered a mosaic embryo when the percentage of mosaicism, calculated by the log2 ratio, was higher than 25%. We evaluated the relationship between several paternal and maternal factors and embryo mosaicism.

Participants/materials, setting, methods: Chromosomal comprehensive screening was performed to couples who attended the Instituto Bernabeu with advanced maternal age, abnormal sperm FISH and/or a clinical history of recurrent miscarriage or implantation failure. Array-CGH analysis was performed using Agilent SurePrint G3 8×60 K CGH microarrays with previous whole genome amplification of genomic DNA. The association between variables and mosaicism was evaluated by logistic regression and chi-square (SPSSv20.0).

Main results and the role of chance: Trophoectoderm biopsies on day 5 and 6 blastocysts (n = 1923) were analysed by array-CGH. We have detected chromosomal mosaicism in 239 blastocysts (12.4%).

There are not statistically significant differences between the maternal ages of mosaic versus non-mosaic embryos (32.56 vs 32.77; p = 0.679). On the other hand, the age of the male is higher in the group of mosaic embryos (40.83 years) compare to non-mosaic embryos (39.93 years); (p = 0.037). In fact, the percentage of mosaic embryos for men aged 40 years or older is 14% and 10.7% when the male age is less than 40 years (p = 0.030).

Male factor is not related with blastocysts mosaicism. No significant difference was reported according to semen parameters (WHO 2010 criteria), sperm FISH (7 chromosomes analyzed) and sperm DNA fragmentation (p = 0.667, p = 0.176 and p = 0.292).

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Finally, the embryo quality is associated with the trophoectoderm mosaicism. A better embryo quality in D+5 is related with a lower percentage of mosaic embryos. Embryos of quality A (Istanbul Criteria) have a mosaic percentage of 8.2%, B of 13.8%, C of 24.1% and finally D of 22.4% ($p = 0.000001$).

Limitations, reasons for caution: The study is limited by its retrospective nature. It is unknown if the mosaicism is confined to trophoectoderm. A prospective randomized design should be used in future studies to corroborate the current findings. More data are needed to conclude which mechanisms are involved in the development of these mosaic embryos.

Wider implications of the findings: The knowledge of the parameters that determine the mosaicism are of great relevance so that patients who undergo an IVF cycles know the risk of appearance of mosaic embryos.

Trial registration number: Does not apply.

P-598 Comprehensive analysis of methylation of whole genome of blastocysts by culturing with four different constituents after In Vitro Fertilization in mice

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Study question: Would methylation of Imprinted gene be specifically disturbed by different constituent of amino acid in medium?

Summary answer: Different constituent of amino acid in medium may cause aberrant methylation at specific gene locus.

What is known already: Since Assisted Reproductive Technology(ART) established, various methods were examined to improve rate of implantation and further subsequence: terms of ovum collection, type and quantity of ovarian stimulation, technic of fertilization such as In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI), and medium. Pregnancy rate has been improved for long term investigation and examination, whereas imprinting disorders have been pointed out. This is because that ART would perturb methylation of specific imprinted gene. Relationship of ART and imprinting disorders have studied, whereas examination doesn't reach clear answer.

Study design, size, duration: First of all, we prepared four media which contained different constituent of amino acid. embryo was cultured with each media by blastocyst stage. Because rate of methylation reaches lowest level due to reprogramming. If different constituent of amino acid influence reprogramming, we would discover aberrant condition of methylation. Furthermore there is no study of comprehensive methylation analysis with culturing by blastocyst with different amino acid constituent.

Participants/materials, setting, methods: Oocytes and sperms were collected from 8 weeks ICR mice. Fertilization was operated by In Vitro Fertilization. Embryo was cultured by four different constituent media: KSOM medium, KSOM medium with essential amino acid, KSOM medium with non-essential amino acid and KSOM medium with essential and non-essential amino acid. Derived blastocysts were analyzed, and rate of methylation was calculated by next generation sequencer through Reduced Representation Bisulfite Sequence(RRBS).

Main results and the role of chance: At all media, there is less methylation change in promoter and exon than other region such as intergenic. This implies that genetically important region would have methylation protection mechanism from outer stimulation. Furthermore, there is more hyper-methylated region in NEAA than other medium. In other word, non-essential amino acid would protect methylation status from outer stimulation such as reprogramming.Next, we extracted all imprinted gene from in mapped 3 million CpG site. As a result, 3304 points in paternal gene and 2777 points in maternal gene were extracted. In addition, we extracted specific points which changed over 25% methylation change compared with NoAA in promoter and exon. Then imprinted gene, Mcts2, Nnat, Nespas, Peg10, Mest, Peg3, Snrpn, Snurf, H19, Igf2r and etc were extracted. These genes were previously reported as relationship between ART and aberration of methylation

Limitations, reasons for caution: RRBS focuses on point of CpG rich region and CpG island such as promoter and exon. This means that analysis of methylation at other region such as intergenic would be poor study compared with whole genome bisulfite sequencing.

Wider implications of the findings: Management of constituent of amino acid with media would improve rate of fertilization, quality of blastocyst, and reduce aberrant methylation condition and frequency of occurrence of imprinting disorder.

Trial registration number: This study is not clinical trials.

P-599 Polymorphism on chromosome 21 does not affect ovarian reserve but clinical outcomes after in vitro fertilization treatments

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Study question: To assess the effect of polymorphic chromosome variants in female fertility and assisted reproductive treatment (ART) outcome.

Summary answer: Polymorphisms on chromosome 21 affect oocyte and embryonic quality leading to worse gestational outcomes in women undergoing ART cycles.

What is known already: Polymorphic chromosome variants are more common in infertile women and seem to influence clinical outcomes after ART. Polymorphisms were considered as a variant when the chromosome region was greater or smaller than the same region on the homologous chromosome; as a minimum, twice the size on the other homologue. The apparently lower implantation and clinical pregnancy rates observed in female carriers has been related to a higher oocyte and embryo aneuploidy rate. However, not all authors find this negative effect, so it remains a controversial subject to date.

Study design, size, duration: This retrospective study was performed in 11 private clinics belonging to IVI group from January 2012 to December 2016. We included 985 women, of which 45.6% (n = 449) had normal karyotype and were considered as a control group; 37.2% (n = 366) were carriers of a polymorphism; and 17.3% (n = 170) showed the common polymorphic inversion for chromosome 9. All women underwent a fresh autologous ICSI cycle. Statistical analysis was performed by ANOVA and Chi-square where applicable.

Participants/materials, setting, methods: The cytogenetic study was performed by culture of peripheral blood lymphocytes stimulated with phytohemagglutinin and subsequent staining with trypsin-Giemsa (GTG bands). 15 metaphases were evaluated for each case and the banding resolution was 400–550 bands per haploid set. All cases were carried out according to the International System for Human Cytogenetic Nomenclature Guidelines.

Main results and the role of chance: Polymorphic variants for chromosomes 1 (46,XX,1qh+), 9 (46,XX,9qh+), 13 (46,XX,13 ps+), 14 (46,XX,14 ps+), 15 (46,XX,15 ps+), 16 (46, XX,16qh+), 21 (46,XX,21 ps+) and 22 (46, XX,22 ps+) were included, as well as the most frequent inversion of chromosome 9, 46, XX, inv(9)(p11q13). Basal data showed no differences among the three groups for the female ages, infertility years, days of stimulation, number of oocytes retrieved, number of metaphase II oocytes and number of transferred embryos. However, we observed statistical differences in antral follicle count between control group (3.2 ± 0.7), polymorphism carriers (4.6 ± 0.7) and chromosome 9 inversions (3.7 ± 1.0), $p = 0.008$. And for the implantation rate being the results as follows 34.7%, 31.6% and 26.0%, $p = 0.048$ for the control, polymorphism and inversion 9, respectively.

Focusing on chromosome 21 because its greater clinical relevance (n = 53), we found that female characteristics were similar compared to the control group, and despite demonstrating a similar response to stimulation, significant differences were noted in implantation rate (35.3% vs. 44.4%, $p = 0.036$); pregnancy rate (44.8% vs. 60.0%, $p = 0.008$) and miscarriage rate (22.5% vs. 53.3%, $p = 0.005$) between women with normal versus 21 ps+ polymorphism karyotype, suggesting that the higher miscarriage rate coud be related to higher embryo aneuploidy rate in these patients.

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Limitations, reasons for caution: Despite the advantages that our data set confer the analysis, limitations still remain. One consequence of a retrospective study is that not all pertinent risk factors are likely to have been identified and subsequently recorded. Therefore, only association, and not causation, can be inferred from the results.

Wider implications of the findings: Although the female carriers of the polymorphism on chromosome 21 have better implantation and pregnancy rates than women with normal karyotypes, the high miscarriage rates suggest a female aneuploid factor to be taken into account in women who are seeking pregnancy.

Trial registration number: It does not apply.

P-600 mitochondrial DNA Levels in Euploid Blastocysts and Pregnancy Outcome

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Study question: To evaluate the relation between mtDNA level and pregnancy outcome.

Summary answer: No correlation between mtDNA levels and pregnancy rate.

What is known already: Aneuploidy is one of the most important factors of implantation failure and early miscarriage, especially in old age females. Preimplantation genetic screening (PGS) is recommended to improve the pregnancy outcome by screening embryos for aneuploidy and selecting only euploid blastocysts for transfer. Mitochondria are the generator of the energy in the cell. Mitochondrial DNA (mtDNA) is located in the mitochondrial matrix. Some studies showed that, there is a correlation between high mtDNA level in the blastocysts and failure of implantation.

Study design, size, duration: This is a retrospective study conducted at the private New Life Fertility clinic. We have identified and reviewed health record of 78 patients who underwent FET in 2016. In which, preimplantation genetic screening was performed and all of embryos transferred were euploid blastocysts.

Participants/materials, setting, methods: We divided the cycles into two groups based on BHCG results, Group A: +ve BHCG, Group B: -ve BHCG. We analyzed clinical data, including demographic information, medical history, quality and number of embryos transferred, the ratio of mtDNA to nuclear DNA in each embryo, endometrial preparation protocol and pregnancy outcome. The primary outcome measures were the pregnancy rate and mtDNA level. Secondary outcome is viable ongoing pregnancy.

Main results and the role of chance: A total of 81 FET cycles for 94 euploid good quality blastocysts.

Group A: 56 FET cycles, Group B: 25 FET cycles. No significant difference between both groups regarding the age: 36.75 ± 3.70 vs 37.76 ± 2.84 (P = 0.227), endometrial thickness: 10.13 ± 1.89 vs 9.46 ± 1.50 (P = 0.254) or BMI: 25.71 ± 5.59 vs 24.50 ± 3.38 (P = 0.433).

Pregnancy rate: (56/81) [69.13%], clinical pregnancy rate: (43/81) [53%], Implantation rate:

(48/94) [51%] and ectopic pregnancy: 1.

Our results, showed that no difference in mtDNA level in both groups.

Limitations, reasons for caution: The sample size in the present study was limited. Retrospective nature of the study.

Wider implications of the findings: No impact of mt DNA level in blastocyst on pregnancy outcome.

Trial registration number: A retrospective study, no trial registration number.

P-601 Successful Polar Body-based Preimplantation genetic diagnosis for Fragile-X syndrome and Gaucher Disease

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Study question: Do oocyte polar bodies (PB) provide a valid and effective alternative to blastocyst biopsy for PGD for maternally-transmitted monogenic disease? A proof of concept on Fragile-X and Gaucher Disease.

Summary answer: PGD with PB biopsy can be considered to allow the selection and transfer of healthy embryos during IVF treatments of women carrying genetic disease.

What is known already: PGD is used worldwide to test embryos for specific genetic diseases; its main advantage is that it provides an alternative to selective pregnancy termination. PGD can be performed on different embryonic cells or on polar bodies.

Study design, size, duration: We present our experience of PB-PGD for two female patients carrying different genetic disorders; Gaucher disease (case 1) and fragile X syndrome (FRAX, case 2).

Participants/materials, setting, methods: A 35-year-old woman and a 37-year-old woman underwent ART treatment in our clinic. The first underwent ICSI with PB biopsy for PGD for Gaucher disease (GBA gene). The second woman underwent ICSI with PB biopsy for PGD for fragile X syndrome (FMR1-premutation). Analysis of the first and second PB was performed by multiplex PCR of 6 informative STRs flanking the gene (distal and proximal); for Gaucher Disease, the mutation was also directly tested using SNaPshot.

Main results and the role of chance: In these case reports, we describe two live births in Switzerland after PB biopsy and PGD. In case 1 (Gaucher), 14 MII oocytes underwent sequential first (day 0) and second (day 1) PB biopsy and 11 cleavage embryos were cryopreserved. The PGD analysis resulted in 7 embryos without the maternal mutation. A fresh-embryo transfer of two embryos was performed at day 2 and a healthy baby was delivered (39 weeks, male, 3460 g, 53 cm). In case 2 (FRAX), 8 MII oocytes underwent sequential first (day 0) and second (day 1) PB biopsy and 8 cleavage embryos were cryopreserved. Genetic analysis identified 3 embryos carrying the normal maternal FMR1 allele. After endometrial priming, a first frozen-thawed single embryo transfer (FET) was performed at day 3, followed by a negative bHCG test. A second FET was performed with the same protocol and a healthy baby was delivered after caesarean section (38 weeks, male, 3600 g, 50 cm).

Limitations, reasons for caution: By its nature, PB-PGD can only detect maternally-transmitted mutations. This is not a disadvantage in X-linked disorders but could lead to lower numbers of embryos for transfer in autosomal-recessive diseases. PB biopsy is also sometimes considered more technically demanding for the embryologist.

Wider implications of the findings: PB-PGD is a valuable tool for diagnosis of maternally-transmitted genetic diseases and transfer of healthy embryos. PB-PGD has two major advantages compared to trophectoderm testing: 1) shorter time of the embryos in culture, and 2) earlier testing leaving plenty of time for genetic analysis while allowing fresh embryo transfer.

Trial registration number: None.

P-602 young poor responders: should we screen for FMR1 mutations and cytogenetic aberrations?

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Study question: Do young women with a reduced ovarian response have an increased risk of cytogenetic aberrations and mutations in the *FMR1*?

Summary answer: We found a markedly increased prevalence of *FMR1* mutations and X chromosome mosaicism in young poor responders compared with known prevalence in the background population.

What is known already: Poor response to FSH stimulation (POR) is a reliable indicator for reduced ovarian reserve in young women indicating an increased risk of premature menopause. Women with premature menopause

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are known to have an increased incidence of mutations in *FMR1* and of cytogenetic changes. Thus, younger women with POR may also have an increased risk of cytogenetic changes and mutations in *FMR1*, both with possible risk for the offspring.

Study design, size, duration: Retrospective cohort study based on 43 young idiopathic poor responders identified in a retrospective database search comprising a total of 13.431 women attending ART in three Danish fertility clinics between 2006 and 2016.

Participants/materials, setting, methods: 239 women fulfilled criteria for inclusion (age ≤37 years at oocyte pick-up, ≤5 oocytes harvested in >2 cycles, FSH dose (>225 IU), no previous ovarian surgery, chemotherapy, or irradiation). Only 43 accepted to participate. *FMR1* mutations were analysed by PCR (FragilEase™ kit, Perkin-Elmer). Karyotyping was performed on peripheral lymphocytes (Q-band staining). Two metaphases were analysed in a digital karyotyping system (Icarus). Abnormal cases were analysed by fluorescence in situ hybridization (FISH) (50 cells).

Main results and the role of chance: 13 out of 43 (30.2%) had an X-chromosome mosaicism. One patient (2%) had a *FMR1* premutation (> 55 repeats), and 7 (16%) had a so-called grey zone repeat number (41-54 repeats). *FMR1*-premutations are found in 0.004 % (1/250) in the general female population and grey zone alleles in 1.7 %. No structural chromosome aberrations were identified. X-chromosome mosaicism was found in 16 women (37.2%), in most cases of low grade, but in two cases (5%) with 16 and 18% mosaicism. Similarly Lakhal et al (2010) found X-chromosome mosaicism in 6.2% of 1000 women with premature ovarian failure. The prevalence of X chromosome mosaicism in the general population is increasing by age reaching a maximum of 0,4% at the age of 70 years.

Limitations, reasons for caution: The number of participants is relative small given the study period of 10 years. Nevertheless, the finding of both *FMR1* abnormalities and X-chromosome mosaicism in our study was far higher than in the general population.

Wider implications of the findings: The present results indicate that young patients with idiopathic POR share similarities with women undergoing premature menopause, and indicate that POR patients should be screened for *FRM1* aberrations since abnormal repeat lengths in the mother can expand to the next generation with a risk of Fragile-X syndrome in the offspring.

Trial registration number: Not applicable.

P-603 Actual levels of mosaicism detection by construct reference curves

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Study question: The problem of whether the low levels of mosaic embryos can be transferred has always existed.

Summary answer: And different levels of mosaicism have different clinical phenotypes.

What is known already: Mosaic embryos which are characterized by the presence of a mixture of diploid and aneuploid cells, are not usually transferred because they are deemed to be abnormal. And the phenotypic manifestations of the mosaicism embryos are dependent on mosaicism level, the chromosome involved, and tissue distribution. PGDIS suggested that mosaic embryos for trisomies capable of liveborn viability (chromosomes 13/18/21) are of lowest priority when mosaic embryo is being considered for transfer. And suggest cut-off point for definition of mosaicism is 20-80% (aneuploid-euploid mosaics). Recently, array CGH and NGS that have accurately measured mosaicism level by quantifying the copy number change

Study design, size, duration: Here, 5 cell lines were purchased from https://catalog.coriell.org/ with known karyotype (47, XX, +18; 47, XX, +21; 47, XY, +13; 46, XY and 46, XX). DNA that extracted from these cell lines were constructed libraries and sequenced on BGISEQ-500, and the karyotypes of sequencing were matched with the expected.

Participants/materials, setting, methods: The study involved mixing experiments with different ratios of aneuploid and euploid samples lines under micromanipulation, including 0:8, 1:7, 2:6, 3:5, 4:4, 5:3, 6:2, 7:1, 8:0, respectively, each was prepared in triplicate. Experiments of a total of 81 samples were performed and these samples were amplified using the single cell SurePlex amplification kit (SurePlex, Rubicon) according to the manufacturer's protocol. After that these amplified DNA were constructed libraries and sequenced on BGISEQ-500.

Main results and the role of chance: As result, copy number ratios for each chromosome were calculated for the different mimic experiments and expressed as average ± SD. We obtain 0%, 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5%, and 100% mosaicism level, including chromosome 13, 18, 21, each level were triplicate. The copy number ratio of chromosome 13 were 0.96 ± 0.15 (0%), 1.02 ± 0.004 (12.5%), 1.06 ± 0.008 (25%), 1.10 ± 0.033 (37.5%), 1.15 ± 0.025 (50%), 1.25 ± 0.026 (62.5%), 1.27 ± 0.01 (62.5%), 1.34 ± 0.04 (75%), 1.36 ± 0.03 (100%) respectively. The linear relation of chromosome 13 between copy number ratio and mosaicism level is $y=0.4199x+0.959$, $R^2 = 0.9488$. $y=0.4676x+1.0151$, $R^2 = 0.9138$ and $y=0.3071x+1.0027$, $R^2 = 0.8581$ were linear relations of chromosome 18 and 21. Each linear relation is independent and applicable to mosaicism level detection of corresponding chromosome.

Limitations, reasons for caution: These three reference curve were just applicable to corresponding chromosome mosaicism level detection. Mimic mosaic samples have been validated by these curves. And we are collecting clinic samples to do validation by fluorescence in situ hybridization (FISH) methods.

Wider implications of the findings: Based on these mimic mosaic cell lines, the results showed that a new method to detect mosaic level of embryo.

Trial registration number: no.

P-604 clinical outcomes after transfer of embryos with low-level chromosomal mosaicism

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Study question: Can low-level mosaic embryos be transferred? Does the presence of chromosomal mosaicism affect clinical outcomes?

Summary answer: Transfer of mosaic embryo showed a significant lower pregnancy rate but it can improve the success rate for couples with no euploid embryos for transfer.

What is known already: Mosaic embryo is defined as the presence of more than one population of cells with different genotypes within the same embryo. The recent advances in comprehensive chromosome analysis using Next generation sequencing (NGS) provides the greatest dynamic range for detection of chromosomal mosaicism. Therefore, mosaic embryo has been classified and reported in many laboratories worldwide. However, the criteria for calling mosaic embryo may be different between laboratories and clinical outcomes of mosaic embryos are not fully understood.

Study design, size, duration: This retrospective observational study was performed in one private fertility clinic in Thailand between September 2015 and September 2016. Clinical outcomes obtained from 443 PGS cycles that transfer a single euploid or mosaic embryo were evaluated.

Participants/materials, setting, methods: The trophectoderm samples were subjected to whole genome amplification followed by comprehensive chromosome analysis using NGS method. Low-level chromosomal mosaicism was defined as the percentage of mosaicism between 20-50%. Genetic counseling regarding the risks and consequences of transferring a mosaic embryo was provided for all patients who elected to transfer mosaic embryos. The clinical outcomes, i.e. clinical pregnancy and biological pregnancy rate were followed. Statistical analysis was evaluated using the Fisher's exact statistical test.

Main results and the role of chance: There were a total of 406 cycles of single euploid embryo transfers (mean age 34.9 years, range 24-52) and 37 cycles of single mosaic embryo transfers (mean age 36.1 years, range 26-49). The clinical pregnancy rate, as confirmed by an intrauterine gestational sac with fetal heartbeat visualized by ultrasound examination, of transferring low level

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mosaic embryos was significant lower than those of euploid embryos (37.8% vs. 56.4%; *p = 0.0377*). Among cycles with transferring of a mosaic embryo, 14 (37.8%) were clinical pregnancy, 5 (13.5%) were biochemical pregnancy rate and 18 (48.6%) were not pregnant. Clinical pregnancy rate was not significant different between embryos with 30% and 40% mosaicism (44.4% vs. 38.8%). The number of chromosomal mosaicism was evaluated in pregnancy group and found that 11 (78.6%) were from embryos with one chromosomal mosaicism and 3 (21.4%) were from embryos with two or more chromosomal mosaicisms.

Limitations, reasons for caution: Due to the limited number of samples, more data and additional clinical data including prenatal diagnosis and live birth are required.

Wider implications of the findings: This study demonstrated that transfer of embryos with low-level mosaic slightly decreased the pregnancy outcome. However, it may potentially improve IVF success rate for couples with no euploid embryo available for transfer. The classification of mosaic embryo can improve embryo selection and clinical management strategies to optimize the reproductive outcome.

Trial registration number: Not applicable.

P-605 Pre-implantation genetic testing (PGT) is particularly more cost-effective in 41-42 yr-old women: A cost-effectiveness analysis of PGT in women with advanced maternal age

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Study question: Is pre-implantation genetic testing (PGT) more cost-effective to achieve live birth compared to standard in-vitro fertilization (IVF) in patients with advanced maternal age (AMA)?

Summary answer: Compared to standard IVF, PGT is more cost-effective to achieve live birth particularly in the 41-42-yr-old female age subgroup.

What is known already: Aneuploidy is the main contributor to implantation failure and increased risk of miscarriage in IVF, particularly in women with AMA. Instead of performing sequential and unscreened IVF cycles, PGT by trophectoderm biopsy and comprehensive chromosome screening might be undertaken to optimize results. Regarding PGT in AMA cases, other than the lack of randomized controlled trials (RCT) supporting efficacy, financial burden due to increased cost can be a limiting factor. There is paucity of data on the cost-effectiveness analysis of performing PGT in AMA cases, stratified by female age and number of blastocysts available to be biopsied.

Study design, size, duration: In AMA cases, a decision-analysis model was developed to compare cost per live birth of PGT (with freeze-all approach) versus standard IVF. All possible outcomes from ovarian stimulation up to embryo transfer from single started stimulation cycle were entered into the model. In PGT arm, up to 2 frozen single euploid embryo replacement (FSEER) cycles and in standard IVF, up to one fresh and one frozen embryo transfer were entered into the model.

Participants/materials, setting, methods: Data were derived from our database of 228 consecutive AMA patients undergoing PGT and 1107 consecutive AMA patients undergoing standard IVF. Blastocyst biopsy (Day5/6) and array-comprehensive genomic hybridization for 24 chromosomes were employed. Couples undergoing PGT due to genetic disorders or translocations were excluded. Patients were stratified according to female age as 38-40, 41-42 and 43-45. The main outcome was cost to achieve live birth exceeding 24 weeks of gestation. Sensitivity analysis was performed.

Main results and the role of chance: Overall, the live birth per one stimulation cycle along with up to two FSEER cycles was 14.4% in the PGT arm; the miscarriage, multiple and preterm birth rates were 12.7%, 0% and 8.6%. The respective figures were 11.6%, 28.2%, 28.2%, and 23.4% in the standard IVF arm. In all age subgroups, the costs per live birth were $13.119 and $13.960 in the PGT and standard IVF arms, respectively. When stratified by female age, in women 38-40-yr old, the cost per live birth was $11,045 and $10,789 in PGT and standard IVF arms. In women 41-42-yr old, cost to achieve live birth was $11,933 and $21,023 in PGT and standard IVF arms. Lastly, in women 43-45-yr old, these figures were $46,205 and $43,421 in PGT and standard IVF arms, respectively.

Limitations, reasons for caution: The analysis may vary with differences in pricing in different countries. Hidden additional costs of unscreened IVF arm, including i) increased time out-of-work, emotional burden and potential endometrial injury associated with miscarriage and curettage; ii) long-term morbidity associated with preterm birth due to multiple pregnancy have not been assessed.

Wider implications of the findings: In AMA cases, when compared with standard IVF, performing PGT is more cost-effective, only in the 41-42-yr old female age subgroup, but not in 38-40 and 43-45 yr-old subgroups. Decrease in miscarriage and multiple pregnancy rates remain to be the other main two advantages of PGT.

Trial registration number: None.

P-606 Preimplantation genetic screening 2.0: which indications can benefit? zygote number, female age and medical history are analyzed

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Study question: Which indications to follow for recruitment of patients in a Preimplantation Genetic Screening (PGS) program? Which is the limit not to overstep to have benefits?

Summary answer: There is a limit over that PGS has no benefit on patients. Indications, age and number of fertilized oocytes clarify which patients should be recruited.

What is known already: It is recognized that there is a new generation of PGS, the so-called PGS 2.0, identified by biopsy at blastocyst stage, comprehensive chromosome screening (CCS) and elective single embryo transfer (eSET) after vitrification and thawing. However, debate is still open regarding which patients can really benefit from this newest approach.

Study design, size, duration: Retrospective observational study carried out between May 2013 and December 2016 on 277 PGS cycles. The average female age was 40.4 years, ranging from 26 to 48. Cycles with complete PGS diagnosis were 166, 103 of which had embryo transfer. The performance of different groups of PGS patients was evaluated.

Participants/materials, setting, methods: Trophectoderm biopsy was performed on Day 5-6, followed by array-CGH and eSET.

The performance of PGS patients was evaluated in terms of eSET cycles per started cycles and main outcome measures.

Patients were assessed according to: 1) PGS indications: 218 AMA (advanced maternal age) patients; 45 RIF (repeated implantation failure) patients; 22 RM (recurrent miscarriage) patients; 18 SMF (severe male factor) patients; 40 patients without indication); 2) female age; 3) number of fertilized oocytes.

Main results and the role of chance:

- Regarding indications, the best outcome was obtained in SMF patients (ongoing pregnancy rate: 53.8%), while the worst, surprisingly/noteworthy, in patients with no specific indication (ongoing pregnancy rate: 17.9%), despite their significantly lower mean age ($P < 0.01$).
- According to female age, no significant difference in terms of ongoing pregnancy rate was found in the different age groups, although, as expected, younger patients had significantly higher cumulative pregnancy rate (68.2%) compared to the oldest ones (37.8%), ($P = 0.02$). Of interest is that all patients over 44 years old never reached embryo transfer: 76.2% of them because their embryos did not reach blastocyst stage and the remaining 23.8% because they did not produce euploid blastocysts.

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- If considering the number of fertilized oocytes, when it was ≤2, the eSET cycles per started cycles were only 17%, with a low cumulative pregnancy rate (12.5%).
- Finally, wondering how many fertilized oocytes would be necessary to obtain at least one euploid blastocyst, data showed 3.6 in women <38 years old, 5.4 in 38-40 years old women, 13 in 41-42 years old women and, remarkably, 55.9 in patients over 42 years old.

Limitations, reasons for caution: The study is referred to patients attending our Fertility Center, therefore it might not reflect the clinical scenario offered in other Centers worldwide. The low number of cases of different study groups represents another limitation. So it would be interesting to perform the analysis in a multicentric, large-scale study.

Wider implications of the findings: The study sheds light on specific biological parameters and clinical features which may help clinicians in the selection and management of patients for a PGS program.

Trial registration number: None.

P-607 Does the low-grade mosaicism detected by next-generation sequencing affect the live birth rate?

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Study question: Is the preimplantation genetic screening (PGS) by Next- generation sequencing (NGS) able to improve the birth rate compared with the array comparative genomic hybridization (aCGH)?

Summary answer: The NGS is able to detect low-grade mosaics undetected by aCGH, however the transfer of these embryos does not modify the live birth rate significantly.

What is known already: Chromosomal aneuploidy is highly prevalent in human embryos. It is a reason for the relatively low success rates of IVF cycles. PGS by aCGH has been used to identify euploid embryos in order to improve the clinical outcome of IVF treatments. However a percentage of morphologically normal euploid blastocysts fail to implant or result in a pregnancy loss. The chromosomal mosaicism, which consists of a mixture of diploid and aneuploid cells, has been considered as a feasible explanation for some failures after the transfer of euploid embryos.The incorporation of NGS to PGS has improved the mosaicism detection compared with aCGH.

Study design, size, duration: We reanalyzed by NGS trophoectoderm biopsies of day 5 and 6 blastocysts previously analyzed by aCGH (from February 2014 to March 2016). We included 85 euploid blastocysts analyzed by aCGH from patients who didn't achieve pregnancy (n = 46) and patients who resulted in live birth (n = 39). In all cases only one embryo was transferred. We considered a mosaic embryo by NGS when the estimated percentage of aneuploid cells was between 20% and 80%.

Participants/materials, setting, methods: The amplified genomic DNA from trophoectoderm biopsies (stored at -80°C) was reanalyzed by NGS. Next-generation sequencing was performed using the Veri-Seq protocol and MiSeq sequencer (Illumina). The analysis was performed using the BlueFuse Multi software (Illumina). The patients who didn't achieve pregnancy had β-hCG levels lower than 2 mUI/ml 14 days after the oocyte retrieval. The differences between the groups were evaluated using Pearson chi-square and t-student statistical tests (SPSSv20.0).

Main results and the role of chance: The DNA samples from trophoectoderm biopsies of day 5 and 6 euploid blastocysts analysed by aCGH (n = 85) were reanalysed by NGS. We detected chromosomal mosaicism in 24 blastocysts (28.2%). In all cases the percentage of aneuploid cells was between 20% and 40% (low-grade mosaicism). Within the group of euploid embryos that didn't achieve pregnancy (n = 46) we detected chromosomal mosaicism in 15 cases (32.6%), and we detected 9 mosaic embryos (23.1%) in the group of live births (n = 39). The percentage of low-grade mosaic embryos seems to be higher among the patients that didn't achieve pregnancy than in those who resulting in live births (32.6% vs 23.1%), however the difference was not statistically significant (p = 0.33).

There were no significant differences between the groups (no pregnancy vs live birth) with respect to maternal age (29.5 vs 29.31, p = 0.89), paternal age (41.8 vs 39.5, p = 0.07), MII oocytes retrieved (10.9 vs 10.7, p = 0.79) and percentage of top and good quality embryos transferred (p = 0.16).

Limitations, reasons for caution: This study was limited by the small sample size. Larger study is needed to stablish if the low-grade mosaicism detected by NGS could affect the live birth rate. In general, more data are needed to conclude the effect of the mosaicism in the embryo development.

Wider implications of the findings: The NGS is more efficient to detect mosaicism than aCGH. However, the transfer of low-grade mosaics detected by NGS does not affect the live birth rate, according to our data. Therefore, the transfer of these embryos should not be avoided until more data is obtained.

Trial registration number: not applicable.

P-608 Detection limit of partial insertions and deletions for PGS in terms of NGS by analyzing 242 embryos of couples with Robertsonian or reciprocal translocations

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Study question: What is the detection limit of next-generation sequencing (NGS) along with low-pass whole genome sequencing in the determination of partial insertion and deletions?

Summary answer: Up to 242 embryos from couples following IVF treatment due to altered karyotype were analyzed by using NGS Ion-Torrent™ PGM platform.

What is known already: Balanced translocations have a significantly impact in fertility and miscarriage risk. Preimplantation genetic screening (PGS) involves the genetic study of the embryo's genome in order to determine its chromosomal arrangement, prior to selection of euploid embryos and desired successful implantation rate in IVF. Initial attempts at preimplantation genetic diagnosis were limited by the inability to simultaneously evaluate aneuploidy and unbalanced translocations in all 24 chromosomes. Missdiagnosis reached up to 70% of aneuploidy in chromosomes unrelated to the rearrangement. Contemporary platforms are more accurate and less susceptible to technical errors.

Study design, size, duration: This retrospective trial involved genetic testing with NGS of 242 embryos from 47 patients with Robertsonian (17) or reciprocal (30) translocations recruited from January 2016 to December 2016. Aberrations with different sizes and most affected chromosomes (all but chromosomes 16, 19, 20 and X and Y) were analyzed.

Participants/materials, setting, methods: After human oocyte insemination, 242 embryo single-cell or trophoectodermal biopsy was performed on day 3 or 5. Whole genome DNA was amplified by Ion SingleSeq^{TN} Kit. Copy Number Variation (CNV) analysis was performed with Ion Reporter™ Software 5.2., which determined the ploidy status with less than 0.01X read coverage.

Main results and the role of chance: As a result of the analysis, we obtained that 179 of the total embryos analyzed (242) were aneuploid: 25 of 179 (14%) presented trisomies, 28 (15.6%) monosomies, 35 (19.6%) full gain and losses and 91 (50.8%) had partial imbalances; 58 (63.7%) of them presented the same imbalance than respective balanced translocation carriers and 33 of them (36.27%) presented others different. A total of 63/242 were euploid and suitable for embryo transfer. The implantation rate was 80 % and ongoing pregnancy rate at 20weeks gestation was 60 %. The smallest aberration detected by the software was 10 Mb in size. By manual inspection, we were able to identify aberrations as small as 5 Mb. We found differences depending on the chromosome analyzed.

Limitations, reasons for caution: Although reliability of NGS Ion-Torrent™ PGM platform along with low-pass whole genome sequencing for both aneuploidy and unbalanced translocations detection, the smallest fragment detected by NGS was 5 Mb.

Wider implications of the findings: PGS coupled to NGS is able to simultaneously identify aneuploidy events and unbalanced translocations, and increases

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the chances of obtaining a healthy newborn. It's also important to determine the detection limit of this technique, to identify not only the aberration arising from a balanced translocation, but also segmental chromosomal aberrations.

Trial registration number: None.

P-609 Expression of genes for PPAR-γ, COX-2 and pro-inflammatory cytokines in granulosa cells from women with polycystic ovary syndrome

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Study question: Is there the difference in expression of genes for PPAR-γ, COX-2 and proinflammatory cytokines (IL-6 and TNF-α) in granulosa cells from polycystic ovarian syndrome patients undergoing ICSI?

Summary answer: PPAR-γ and COX-2 mRNA were significantly down-regulated in the granulosa cells of PCOS women, but expression of IL-6 and TNF-α did not show significant differences.

What is known already: Although the etiology of PCOS is still obscure, granulosa cells dysregulation may affect ovarian follicular environment, which may be associated with poorer reproductive outcomes observed in PCOS patients. The existing literature has shown that there is a controversy about expression level of PPAR-γ and few studies on COX-2 expression in PCOS have been found in the literature. Infertile women with PCOS were found to have higher serum and folicular fluid concentrations of TNF-α and IL-6 than control women, suggesting that granulosa cells produce both cytokines.

Study design, size, duration: This was a prospective study in which 15 granulosal cell samples were obtained on the day of oocyte retrieval. Granulosa cells were collected from pooled follicular fluid and extracted mRNAs were frozen at -80°C till analysis.

Participants/materials, setting, methods: Nine patients with PCOS and 6 controls were enrolled for this study. Total mRNAs were extracted from granulosa cells. Reverse transcription was performed and quantification of gene expression levels was achieved by real-time quantitative PCR. Mann-Whitney test was used to compare the mean values of mRNA. Correlation between expressions of the different parameters was tested by nonparametric Spearman's correlation.

Main results and the role of chance: There were no significant differences in age, body mass index and total dose of gonadotropin except for LH/FSH ratio between PCOS and control group. PPAR-γ and COX-2 mRNA were significantly down-regulated in the GCs of PCOS women compared with controls ($p = 0.034$, 0.018, respectively), but expression of IL-6 and TNF-α mRNA did not show significant differences. No significant correlation was detected between expression of these mRNA and clinical characteristics, the number of retrieved oocytes, oocyte maturity, cleavage, or good-embryo rate. There was positive correlation between PPAR-γ, COX-2, IL-6 or TNF-α mRNA levels.

Limitations, reasons for caution: It should be noted that the results of this study were derived after analyzing a relatively small number of cases and PCOS study group did not contain various PCOS phenotypes.

Wider implications of the findings: Resulting data may provide novel clues for ovarian granulosa cells dysfunction in PCOS and indirectly provide evidence that the effect of PPAR-γ agonist in PCOS might come from altered ovarian follicular environment. Further studies with a larger sample size are required to confirm these.

Trial registration number: not applicable.

P-610 National reference materials of preimplantation genetic aneuploid screening based on next generation sequencing

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Study question: We want to establish one set of national reference materials which can effectively evaluate the effectiveness of PGS detection technology based on next generation sequencing(NGS)

Summary answer: Our reference materials have been verified by BGISEQ-500 platform and can be used for evaluating the accuracy of the PGS based on NGS.

What is known already: Developing with the maturity of NGS technology, due to its characteristics of high accuracy, high throughput, low cost and easy operation, PGS detection technology based on NGS has been more and more used in clinical practice. At present the main NGS platforms include Illumina, Life and BGI-SHENZHEN's BGISEQ series, and different companies based on these platforms developed a variety of PGS detection technologies, how to evaluate the accuracy of these PGS detection technologies becomes an urgent problem needed to solve.

Study design, size, duration: The set of national reference materials consists of 89 strains normal tarotype, CNVs(at least 1 M) and aneuploid cell lines, which includes 67 different CNVs cell lines, 1 data QC cell line, 9 normal karotype people's cell lines, 6 different sizes CNVs mixes mosaic cell lines and 6 trisomic embryo stem cell lines. All these cell lines were collected and validated from May 2015 to September 2016.

Participants/materials, setting, methods: All of the 89 strains cell lines with known karyotype bought from Coriell Institute or hospital and sorted into 0.2 ul PCR tube with 3-5 cells every tube by flow cytometry. We used EmbryoSeq kit, its software and BGISEQ-500 to verify this set of national reference materials. We used EmbryoSeq kit to construct libaries according to the manufacturer's protocol, send them to sequence on BGISEQ-500 and analyse sequencing data with automatic EmbryoSeq software.

Main results and the role of chance: 9 sets of this reference material were detected by BGISEQ-500, all of >4 M CNVs, aneuploid and normal cell lines can be detected correctly, the results were same with known karotype, and there are 15 strains 1-4 M CNVs and 80% of them can be also detected correctly. These results achieved reference materails'technical requirements. After BGISEQ-500 sequencer, other NGS platform also verify this reference materials, that have same serults with BGISEQ-500.

Limitations, reasons for caution: Due to the time and some cell lines were difficult to culture, this set of reference materials doesn't contain all of 24 chromosome aneuploid and CNVs, so those chromosome perhaps don't meet this set of reference materials' technical requirements if they don't be included in this set of reference.

Wider implications of the findings: Although this set of reference materials are validated only on several NGS platform now, but it can also be used for other new developed NGS sequencer, even maybe it can apply to the third generation sequencer or micro array platform.

Trial registration number: NO.

P-611 Does blastocyst biopsy technique effect aneuploidy rates?

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Study question: Do the inherent variables associated with the blastocyst biopsy procedure influence aneuploidy rates? Does the blastocyst biopsy technique produce equivalent results independent of mechanical variables?

Summary answer: Optimal trophectoderm biopsying involves minimal laser pulses and intact biopsy pieces free of appreciable cell lysis. Deviation from this ideal more often produces aneuploid results.

What is known already: Preimplantation Genetic Screening (PGS) offers patients the ability to know the ploidy status of embryos before implantation. The efficacy and invasive nature of this embryo procedure has made the use of PGS questionable in the past. Current technologies, such as trophectoderm biopsy, have been successfully applied to facilitate a complete chromosomal ploidy diagnosis. The blastocyst biopsy procedure has proven to not increase

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any known birth defects, appears to not damage embryo competence and is viewed as an improved method for obtaining embryo genetic material.

Study design, size, duration: Using a prospective, observational cohort design, 1204 blastocysts were biopsied between July 2015 through January 2016 from 220 cycles (average age: 37.2). Multivariate logistic regression was applied to derive models which predicted ploidy. Variables were selected for the model and analyzed using forward selection and backward elimination, with $p<0.05$ required to remain in the model. Pearson's chi-square test was then used to assess the association of nominal variables with observed embryo ploidy.

Participants/materials, setting, methods: Patient embryos were cultured in Life Global media with LGPS under tri-gas incubation conditions, and biopsied at or beyond the full blastocyst stage. Each embryologist self-reported biopsy parameter variables, including embryo quality, day of development, # of laser pulses applied (≤4, 5-9, ≥10 pulses), biopsy sample size (≤3 or ≥4 cells) and if the resulted biopsy sample appeared primarily lysed. For comparative analysis purposes, each blastocyst was classified as having an ideal or difficult biopsy.

Main results and the role of chance: 85% of all biopsies performed were classified as ideal, while only 175 blastocyst biopsies were considered difficult. Biopsy modelling variables revealed significance for age, embryo grade and the day of blastulation. The derived model also uniquely identified significant mechanical parameters including number of laser pulses and increased cell lysis as predictors of aneuploidy. A second model based only on biopsy mechanics identified cell lysis and the number of resulting biopsy pieces as significant predictors of ploidy. Comparing ideal to difficult biopsies, higher ($p<0.05$) aneuploidy rates occurred when biopsies were difficult. Embryo quality and day of development showed no significance for producing a difficult biopsy. Additionally, no difference was observed among the 5 technicians. An implantation rate of 72% was achieved for women ≤37 years old and 73% for women ≥38 years old following vitrified single euploid embryo transfers. The implantation potential of embryos derived from patients with difficult biopsies was not determined due to an insufficient sample size, however multiple pregnancies are ongoing. Spontaneous abortions were 5% across all age groups and no chromosomal errors were reported.

Limitations, reasons for caution: This study highlights an objective, observational analysis, but cannot delineate if aneuploid embryos are more susceptible to yielding a difficult biopsy. No differences in aneuploidy or prevalence of difficult biopsy parameters was observed between technicians, indicating that the embryo is the probable source of difficulty, not the technician micromanipulating.

Wider implications of the findings: The model derived in this study revealed independent mechanical variables that increased aneuploidy rates. We suggest that the variables identified by these models, describe a specific style of biopsy questioning if technique affects or is predictive of genetic outcomes. These variables appear to be linked and generally occur simultaneously.

Trial registration number: None.

P-612 Lessons learned from over 1,100 preimplantation genetic screening (PGS) cycles: blastocyst development and aneuploidy outcome analysis

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Study question: What are the relevant clinical outcome percentages for blastulation and aneuploidy in women using PGS in conjunction with their ART cycle?

Summary answer: Significant data was generated providing generalized aneuploidy percentages and cycle yield outcomes. With optimized embryology laboratory practices, most cycles achieve a euploid embryo.

What is known already: PGS offers patients the ability to know the ploidy status of embryos before implantation. Current technologies, such as trophectoderm biopsy, have been successfully applied to facilitate a complete chromosomal ploidy diagnosis. NextGen sequencing results are thought to correlate directly with the ICM status and subsequent pregnancy ploidy, but a conclusive link remains unattainable. The error rate of a PGS euploid embryo resulting in a chromosomally abnormal pregnancy remains extremely low, but the alternative of an aneuploid embryo resulting in a normal fetus is unknown. PGS testing has improved blastocyst utilization by facilitating single euploid embryo transfers.

Study design, size, duration: Retrospective analysis from a single center's 5 year (October 2011 to December 2016) PGS/Blastocyst biopsy program resulting in 1,087 autologous cycles, 88 donor cycles and 6234 embryos tested. Each cycle reported eggs retrieved, maturity, fertilization, usable blastocysts and aneuploidy results. All ICSI/PGS cycles achieving an oocyte retrieval cultured all embryos to blastocyst stage and were included in this analysis. No cycles were excluded for any reason.

Participants/materials, setting, methods: Patient embryos were cultured in Life Global media with LGPS under tri-gas incubation conditions, and biopsied at or beyond the full blastocyst stage. All embryos were biopsied on either day 5,6,7 and sent for array CGH (2011-2014) or NextGen sequencing (2015-2016). All patients were treated by a single physician and followed standard ART stimulation protocols. All cycles were cryo-all, with emphasis on subsequent single vitrified/thawed euploid embryo transfer.

Main results and the role of chance: The average patient (mean age=37.01) produced 15.1 eggs, with an 84.4% maturity rate and 76.4% normal 2PN fertilization. These cycles averaged 5.1 biopsied blastocysts per cycle and yielded 2.5 euploid embryos. Cycles generating blastocysts and resulting in at least one normal embryo occurred 89% of the time for patients ≤37 years old, and 59% for patients ≥38 years old. Blastocyst production rate was 56.1% for patients ≤37 years old and 50.1% for patients 38-42 years old. Blastocyst euploidy rates were 54.6% for patients ≤37 years old and 29.1% for patients ≥38 years old. Embryos made from donor oocytes achieved a 68.6% blastulation rate and euploidy rates of 66.1%. Blastocyst generation decreased and aneuploidy rates increased significantly with age. Top quality day 5 embryos yielded an average euploidy rate of 54% compared to 42% on day 6 and 27% on day 7. Top quality day 5 donor oocyte embryos achieved a 72% euploidy rate. No donor cycles failed to achieve a euploid blastocyst.

Limitations, reasons for caution: Aneuploidy rates are greatly influenced by patient selection and embryology biopsy criteria. Our center chose to perform no patient selection and enrolled all patients into a PGS cycle with modest biopsy requirements. The data in this analysis is necessary for further regression modeling to establish predictive parameters.

Wider implications of the findings: This study generated insightful data using a standardized approach to accurately report IVF derived embryo aneuploidy rates. Further validation is needed to determine whether each PGS tested embryo reflects an accurate diagnosis, however the donor data supports historic ART outcomes and provides significant predictive value.

Trial registration number: none.

P-613 Advanced maternal age (AMA) and preimplantation genetic screening (PGS) outcome after FET – controlled retrospective study

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Study question: Which is the benefit of PGS in patients with advanced maternal age (AMA) in compare to a similar group of patients that did not performed it?

Summary answer: The main benefit to perform PGS in AMA group is to increase the pregnancy and implantation rate/transfer, and reduce the miscarriage rate.

What is known already: Recent studies indicate that between 60% - 90% of miscarriages in the first trimester may be the result of chromosomal aneuploidy. Evidences suggest that aneuploidy rate increases with maternal age. Forthis reason, PGS may be a reasonable choice in order to improve efficiency in the selection of euploid embryos (chromosomally normal). At the present, the 22 autosomal pairs of chromosomes and the sexual pair can be evaluated by

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aCGH (Comparative Genomic Hybridization) or NGS (Next Generation Sequencing).

Study design, size, duration: It is a retrospective controlled study. Nighty three PGS cycles corresponding to AMA patients (A) were performed between 2013 and 2016. One hundred and thirty cycles were considered as a control group (B) between the same years.

Participants/materials, setting, methods: PGS was performed by aCGH and NGS at blastocyst stage (D5 or D6). All the patients performed IVF (ICSI) procedure at the same center. Delayed frozen embryo transfers were performed in both groups (at blastocyst stage). All patients had > 37 y/o. T-test and Chi square were performed for statistical analysis.

Main results and the role of chance: The overall average age was 40.5 ± 1.8 (A) and 40.0 ± 1.7 (B) (p>0.05). The average number of MII oocytes were similar 7.7 (A) vs. 6.9 (B). The fertilization rate and blastulation rate were also comparable 79.5% (A) vs. 79.6% (B), 42.4% (A) vs. 43.1% (B), respectively. In group A, the euploid blastocyst rate was 28.9% and embryo transfer cancellation rate was 57%. Comparing both groups, a significantly increased in the clinical pregnancy rate (51% vs. 34%) and implantation rate (53% vs. 23%) was seen (p < 0.05). Miscarriage rate was 7% in group A and 29% in group B (p<0-05).

Moreover, 3% of the embryos in AMA group had a single aneuploidy for chromosome 13, 18 and 21.

Limitations, reasons for caution: This is a retrospective study and all the clinical outcomes are per ET and not cumulative.

Wider implications of the findings: The overall rate of euploid blastocysts in PGS population is about 50%, this significantly decreased in AMA group, confirming that oocyte aging is the main cause of aneuploidies. ET cancellation is higher in AMA group. However, when a euploid blastocyst is selected the chance of pregancy and implantation is higher.

Trial registration number: None.

P-614 Preimplantation genetic diagnosis (PGD): the number of matures oocytes is determinant for Reciprocal but not for Robertsonian translocations

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Study question: The number of balanced embryos is the main limiting factor to obtain pregnancies after PGD. Is there a link with ovarian response?

Summary answer: An adequate ovarian response should be obtained for Reciprocal translocations but not for RT. For RCT below 12 mature oocytes, PGD outcomes are very poor.

What is known already: Previous studies showed that the unbalanced embryos rate after PGD is more elevated in RCT (almost 75%) compared to RT (almost 50%), consequently the chance to reach embryo transfer for a couple is lower in case of RCT. It is well established that the ovarian response to controlled ovarian stimulation (COS) was not impaired by the presence of a balanced translocation in women.

Study design, size, duration: This study was conducted retrospectively. We included an overall of 264 couples that benefited from a preimplantation genetic diagnosis (PGD) for a male or female balanced chromosomal translocation (172 couples for RCT and 92 for RT) between 2006 and 2015. We analyze the characteristics of 555 PGD attempts (201 for RT and 354 for RCT).

Participants/materials, setting, methods: The aim of the present study was to determine, for RT and RCT, if the ovarian response may have a predictive value on the number of unbalanced embryos. After a correlation study (Logiciel stat), we have compared, for RT and RCT, the PGD outcomes of three quartiles representing the number of mature oocytes (>25thpercentile, [25-75] percentile, >75th percentile).

Main results and the role of chance: The unbalanced embryo rates were significantly increased in case of RCT compared to RT (77.3 % vs 54.0%, $P < 0.0001$). Of note we found a significant increase of unbalanced embryo rates in female RT carriers compared to male (61.9% vs 49.8% $P = 0,002$). The number of mature oocytes was strongly correlated with the number of embryos biopsied and balanced embryos, regardless of the type of translocation. The quartile study for RCT couples showed that when more than 12 mature oocytes were available for PGD, the pregnancy rates obtained per pick-up and per embryo transfer were 26.3% and 37.3% respectively. Theses rates were significantly higher when compared to the 25th quartile (less than 6 matures oocytes, 6.7% per pick up and 14.8% per embryo transfer). For RT, the number of mature oocytes does not impact PGD outcomes and pregnancy rates remain not different even if less than 7 mature oocytes are available (25th quartile).

Limitations, reasons for caution: According to French low, genetic analysis was performed by fluorescent in situ hybridization with DNA probes targeting chromosomes involved in the translocation. Inter-chromosomal effect could not be assessed.

Wider implications of the findings: This study indicates that the management of COS for RT and RCT should not be the same in PGD. A real caution of ovarian reserve is necessary for RCT and oocytes pooling should be discussed when less than 6 matures oocytes are available for PGD procedure.

Trial registration number: NA.

P-615 A genetic predisposition for ovarian hyperstimulation syndrome? In-depth analysis of four non-PCOS cases

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Study question: Is there a genetic predisposition in non-PCOS women who developed the ovarian hyperstimulation syndrome after GnRH antagonist protocol with GnRH agonist trigger and freeze-all approach?

Summary answer: Whole exome sequencing (WES) of four non-PCOS patients with ovarian hyperstimulation syndrome (OHSS) showed the absence of a common genetic background.

What is known already: OHSS is an exaggerated response to ovarian stimulation, characterised by cyst enlargement of the ovaries, abdominal distention and pain, fluid shift from the intravascular space to the third space, which can result in ascites, pericardial and pleural infusions, and generalised oedema. This may lead to hypovolaemia, haemoconcentration, electrolyte imbalances and coagulation disorders and haemorrhage from ovarian rupture, ARDS, thromboembolism and renal failure. Reports on a total of only 7 patients who developed OHSS after GnRH agonist trigger and freeze-all approach have been published.

Study design, size, duration: Worldwide blood samples of 4 of the 7 previously published cases of OHSS after GnRH agonist trigger and a freeze-all strategy were gathered. These samples were analysed through WES, searching for (1) known causes of OHSS were investigated and (2) new causes present in at least two individuals.

Participants/materials, setting, methods: DNA samples of patients were collected after written consent. WES studies were performed according to standard procedures with an average sequencing depth of 75x. Further filtering, based on population frequencies (<1%) and type of alteration (only protein-changing mutation were selected) was performed by a scientist experienced in gene panel analysis.

Main results and the role of chance: In a first part of the study, we looked at the presence of mutations in genes already known to be involved in OHSS (i.e. FSHR, LHR/LHCGR, CYP11A1, CYP19A1, ESR1, ESR2, PGR, VEGFR1, VEGFR2, VEGF, AMH, AMHR, GDF9, BMP15, SOD2, SHBG, FOLR1, MTHFR, TP53, PAI and TNFalfa). In PGR and TP53 a heterozygous alteration was detected, each in one individual. PGR is predicted to be involved in progesterone resistance with a recessive inheritance pattern and was therefore not

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considered as being causal. TP53 is especially involved in cancer development. The detected alteration has a very low population frequency (~1/40000), but the functional consequences of the detected variant remain unknown.

In part 2 of the study, we looked at variants in genes previously not linked to OHSS. We especially focussed on genes with variants present in ≥ 2 patients. Although no evident link with OHSS was detected among the genes with variants in multiple patients, we have composed a list of genes which potentially contribute to OHSS. Among these are genes involved in oocytes maturation or genes expressed in ovaries.

Limitations, reasons for caution: Since OHSS after GnRH agonist trigger and freeze-all is an exceptionally rare disorder with only 7 patients reported so far; it is impossible to study large patient groups. Future functional studies are essential to define a more precise involvement of the detected variant in the development of OHSS.

Wider implications of the findings: Defining a genetic predisposition for OHSS is essential in view of prevention. If a common (risk) genetic factor is involved, preliminary screening could be offered.

Trial registration number: n/a.

P-616 Anti-Mullerian hormone (AMH) levels in the follicular fluid – a predictive marker of human blastocysts ploidy status

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Study question: To correlate AMH levels in follicular fluid (FF) with ploidy status of blastocysts in preimplantation genetic screening (PGS) cycles.

Summary answer: High level of AMH in the follicular fluid may be a good predictor of the development of euploid embryo.

What is known already: Follicular fluid is a product of secretory activity of granulosa and thecal cells and provides a very important microenvironment for the development of oocytes. Some biochemical substances found in the FF surrounding the oocyte may play a critical role in determining oocyte quality and consequently the capacity of fertilization and embryo development. Studies of the follicular fluid AMH levels have shown a more direct role of AMH as a regulator of human folliculogenesis. FF AMH levels are positively associated with embryo implantation, although direct and indirect effects FF AMH levels on in vitro fertilization (IVF) have not been clarified yet.

Study design, size, duration: Our study was planned as a prospective cohort research and consisted of 43 women (median age 36 years, range 33-40) with the diagnosis of infertility who were undergoing IVF treatment with PGS between August 2016 and December 2016 at INVICTA Fertility Centre, Poland.

Participants/materials, setting, methods: The follicular fluid sample was obtained from preovulatory follicle sized ≥ 18 mm of diameter from each ovary. AMH level in serum and FF was measured on day of pick-up by VIDAS® AMH assay (bioMérieux). A total of 87 blastocysts were evaluated with the NGS protocol. Ion Torrent Suite Software and Invicta Bioinformatics Team Script were used for chromosome copy number variation analysis.

Main results and the role of chance: All embryos were cultured separately to blastocysts stage. The trophectoderm biopsy was performed on day 5 or day 6 development. Among the 87 blastocysts, 39 (44.8%) were euploid, while 48 (55.2%) were aneuploid. The average level of follicular fluid AMH in normal embryos was 3,69 ± 3.1 ng/ml. The FF AMH levels were significantly higher ($p<0.03$) in euploid embryos compared to aneuploid, where the average level of FF AMH was 2.52 ± 2.4 ng/ml. The area under the curve (AUC) of FF AMH for prediction of euploid blastocysts was 0.63 ($p = 0.05$, 95% CI 0.506-0.749). A threshold of 2.75 ng/ml of FF AMH had a sensitivity of 58%, specificity of 72%. No significant differences were observed with respect to age of patients. There was also no significant differences in BMI, FSH, LH, baseline level of AMH in serum, measured on third day of menstrual cycle. No correlation were observed between serum AMH levels on the day of pick-up and ploidy status of embryos.

Limitations, reasons for caution: The study is limited by sample size. A higher sample size could be used in future studies to corroborate the current findings.

Wider implications of the findings: The present study revealed correlation between FF AMH and ploidy status of blastocysts. FF AMH might be a predictive marker for prognosis of genetic status of embryos created from oocytes from follicles with elevated FF AMH level.

Trial registration number: not applicable.

P-617 Clinical implementation of next-generation sequencing (NGS) for preimplantation genetic screening (PGS) improves pregnancy outcomes

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Study question: Does NGS for PGS improve pregnancy outcomes as compared to array comparative genomic hybridization (aCGH)?

Summary answer: NGS improves implantation and ongoing pregnancy rates/live birth rates compared to aCGH among patients undergoing in vitro fertilization (IVF) with PGS.

What is known already: Array CGH is widely used for IVF with PGS. NGS, a new platform for PGS, may be able to detect more cases of mosaicism and triploidy (69XXY) than aCGH. Genetic abnormalities detected by NGS are significantly higher among pregnancies resulting in miscarriage than live birth.

Study design, size, duration: This was a retrospective study of 1015 patients undergoing IVF with PGS followed by single thawed euploid embryo transfer (STEET) from 1/2014 to 12/2016 at a single university medical center.

Participants/materials, setting, methods: All IVF cycles with PGS and STEET were included. Oocyte thaws for biopsy, double embryo transfers (ET), mosaic ET, or cycles with incomplete outcome data were excluded. Primary outcomes: implantation rate (IR), spontaneous abortion rate (SABR), and ongoing pregnancy rate/live birth rate (OPR/LBR). Demographic data included age, gravidity, parity, number of prior IVF cycles, ovarian reserve testing, and infertility diagnosis. Student's t-test and Fisher's exact test were used for statistical analysis with $P < 0.05$ considered significant.

Main results and the role of chance: 424 patients underwent PGS with aCGH, and 591 patients underwent PGS with NGS. 18 patients were excluded from the NGS group for incomplete outcome data (11) or mosaic ET (7). There was no difference in baseline demographics between groups. The mean age of patients was 35.7 years. IR was significantly higher in the NGS group compared to the aCGH group (71.7% vs. 65.1%, $p = 0.027$). The OPR/LBR was also significantly higher in the NGS group (62.3% vs. 55.0%, $p = 0.023$). The SABR was decreased in the NGS group compared to the aCGH group, but this was not statistically significant (11.9% vs. 12.7%., $p = 0.813$).

Limitations, reasons for caution: This study was limited by its retrospective design. Prospective data on the outcomes of mosaic embryo transfers is needed to determine their true implantation potential.

Wider implications of the findings: PGS using NGS significantly improves pregnancy outcomes over PGS using aCGH. Mosaic embryos may have reduced implantation potential. Therefore, the exclusion of mosaic embryos with NGS may explain these results.

Trial registration number: N/A.

P-618 The price of a euploid embryo identified from preimplantation genetic testing for aneuploidy (PGT-A): A cost analysis

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Study question: To determine the expected age-related costs of undergoing IVF with PGT-A to obtain a 90% likelihood of having at least one euploid blastocyst.

Summary answer: The estimated cost to obtain a 90% chance of a euploid blastocyst increases with age, most substantially after 37y, and again after the age 40y.

What is known already: Many patients undergoing IVF for infertility treatment elect to undergo PGT-A hoping to optimize their chance of having a live birth and a reduced risk of miscarriage by selecting a euploid embryo for transfer. Oocyte aneuploidy increases with age, while the embryo yield per cycle tends to decrease with age. To minimize overall cost, some patients choose to undergo additional stimulation cycles and bank blastocysts prior to running PGT-A. The optimal number of blastocysts to bank, the number of cycles needed, and the cost of having a high likelihood of a euploid embryo are important issues for patient counseling.

Study design, size, duration: A retrospective analysis of 520 first, fresh, autologous IVF/ICSI cycles from women with normal ovarian reserve, treated from 1/2011-3/2015, was conducted. Age-specific probabilities of euploidy were estimated from 14,500 PGT-A results from an external testing laboratory. The expected number of blastocysts per cycle was calculated, and a cost analysis was performed to estimate the price of IVF/ICSI/PGT-A needed to achieve a 90% chance of having at least one euploid blastocyst, stratified by patient age.

Participants/materials, setting, methods: The expected number of blastocysts per cycle was calculated for patient age by yearly increments beginning at 34y using data from our University-based IVF center in the United States (US). Assuming an average cost of $12,500 USD for an IVF/ICSI cycle with biopsy, and $2,500 USD for PGT-A testing, the expected cost of treatment to obtain a 90% chance of having at least one euploid blastocyst was calculated.

Main results and the role of chance: To achieve a 90% chance of having one euploid blastocyst with PGT-A, a patient who is 34-36y would need 3 blastocysts, a 37-39-year-old would need 4 blastocysts, and a 40-year-old would need 5 blastocysts. Provided there is at least one blastocyst obtained following a cycle, a 34-year-old should expect to pay, on average, $19,500 US dollars (USD), while a 37-year-old should expect to pay $22,500 USD. The cost increases more significantly above the age of 37y. While a 37-year-old and 38-year-old both need 4 blastocysts to have a 90% chance that one is euploid, the 38-year-old would have to undergo more IVF/ICSI cycles, on average, to obtain 4 blastocysts, and should expect to pay approximately $30,000 USD; a 40-year-old who needs 5 blastocysts should expect to pay $44,000 USD.

Limitations, reasons for caution: The population used for this analysis includes only women with normal ovarian reserve, and the sample size is smaller for older women. This cost analysis is designed for patients who have at least one blastocyst following an IVF/ICSI cycle, though many do not. IVF/PGT-A costs vary greatly across clinics.

Wider implications of the findings: Our model should be useful when counseling patients presenting to infertility clinics for IVF/ICSI with PGT-A regarding the estimated number of blastocysts needed, the number of cycles to undergo, and the overall approximate cost of having a euploid embryo for transfer.

Trial registration number: Not applicable.

P-619 The association between thrombophilic gene mutations and recurrent pregnancy loss in Middle Eastern women

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Study question: Our objective is to evaluate the prevalence of thrombophilic gene mutations in Middle Eastern women with recurrent pregnancy loss (RPL) compared with women who had uneventful pregnancies.

Summary answer: Thrombophilia accompanied with Factor V Leiden (FVL) may plays a role in pathophysiology of RPL, suggesting that patients with RPL should be screened for FVL.

What is known already: RPL is a multifactorial condition, defined as two or more consecutive pregnancy losses and affecting 1-5% of reproductive-age woman. After chromosome abnormality, thrombophilia is one of the most important genetic factors that could cause RPL. However, the etiology of RPL remains unknown in ~50% of cases. Although numerous studies are available in literature, thrombophilia rate seems to vary from study to another due to different selection criteria and ethnicity of patients.

Study design, size, duration: A total of 300 Middle Eastern women were recruited in this case-control multicenter study between October 2012 and October 2015. Patients group included 200 women with RPL and the control group included 100 women from the same ethnic background and with at least one successful pregnancies and no history of pregnancy loss, which matched by age with patients.

Participants/materials, setting, methods: 200 women with RPL (who have two or more pregnancy losses) were recruited in this study, compared with 100 women without adverse pregnancy outcome, at Orient hospital, faculty of medicine of Damascus University and faculty of medicine of Baqubah University.

Genotyping of thrombophilic gene mutations were carried out by amplification Refractory Mutation System- PCR (ARMS-PCR) method after DNA extraction.

Main results and the role of chance: This study has shown that Factor V Leiden (FVL) is significantly associated with RPL (56/200)28% compared with controls (9/100) 9%, (p-values was 0.0036). And there were no statistically significant differences in the prevalence of methylenetetrahydrofolate reductase MTHFR (C677T and A1298C), prothrombin (G20210A) mutation and other mutations between RPL patients and controls. It is of interest to note that FVL constitutes a major risk factor if RPL is considered in comparison with controls [Odds Ratio were 4.13 (CI:1.43-5.85)].

Limitations, reasons for caution: We could not study some rare gene mutations as: factor XIII (V34L), β-fibrinogen (455 G → A), plasminogen activator inhibitor-1 (4 G/5 G) and human platelet antigen-1 (a/b9L33P).

Wider implications of the findings: Our results agree with Mitraui et al.; Finan et al.; and Mahjoub et al. and disagree with by Zahed et al.; Alfirevic et al.; and Howard et al., who found no correlation between FVL mutation and RPL. Moreover, FVL mutation prevalence was similar in patients and controls (Pauer et al).

Trial registration number: none.

P-620 PGS2.0 significantly decreases the miscarriage rate while maintaining similar live birth rate per oocyte retrieval cycle compared to conventional IVF/ICSI among women aged above 36

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Study question: Whether women with advanced maternal age (AMA) could benefit from preimplantation genetic screening (PGS) 2.0 treatment?

Summary answer: PGS 2.0 treatment could significantly decrease the miscarriage rate while not reduce the live birth rate per oocyte retrieval cycle (OCC) among women with AMA.

What is known already: It was well established that the miscarriage risks increased with maternal age, and embryo aneuploidy was the leading reasons of it. PGS 2.0 was widely used nowadays combined blastocyst biopsy and comprehensive chromosomal analysis to examine the complete chromosomes before embryo transfer to improve the pregnancy outcomes. Until now, numerous studies had reported that PGS 2.0 could decrease the miscarriage rate and increase the live birth rate among women with AMA. However, some

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researchers also argued that 'intent-to-treat" analysis was not adopted in these studies and patients not reaching transfer were neglected for evaluating the efficiency of new PGS strategy.

Study design, size, duration: This is a retrospective study comparing the pregnancy outcomes of women with AMA who underwent PGS treatment (n = 177) or conventional IVF/ICSI (n = 177) between November 2011 and April 2015 at the Reproductive and Genetic Hospital of CITIC-XIANGYA. Intent-to-treat analysis were applied in this study.

Participants/materials, setting, methods: Only patients whose age ≥ 36 and number of retrieved oocytes ≥1 were included in this study. The chromosome karyotype of the couples should both be normal and had no adverse pregnancy history. Patients using testicular or epididymal sperm were excluded, and those whose embryos were biopsied for twice or using donated oocytes or sperms were also excluded. Propensity score matching were applied to select proper controls with the ratio of 1:1

Main results and the role of chance: 105 (59.3%) OCCs were cancelled in PGS group, significantly higher than 26 (14.7%) in IVF/ICSI group (P < 0.001). No formed blastocyst and no euploid embryos after genetic testing were the main reasons of cycle cancellation in PGS group. After intent-to-treat analysis, the clinical pregnancy rate (CPR) and live birth rate (LBR) per OCC in PGS patients was comparable with IVF/ICSI patients (CPR: 20.3% vs. 27.7%, P = 0.087; LBR: 17.5% vs. 16.9%, P = 1.000). And the accumulative CPR was significantly lower in PGS patients (23.2% vs 36.2%, P = 0.006) but the accumulative LBR was not significantly different from IVF/ICSI patients (19.8% vs 23.7%, P = 0.450). However, the CPR and LBR per embryo transfer cycle (ETC) in PGS patients were higher (CPR: 47.6% vs. 34.7%, P = 0.043; LBR: 42.7% vs. 21.1%, P < 0.001), and miscarriage rate (MR) was lower (10.3% vs 39.1%, P = 0.001) when compared to IVF/ICSI patients, which was in accordance with previous studies.

Limitations, reasons for caution: This study was limited by the small sample size and retrospective design, a randomized double-blinded controlled trial was demanded to confirm this conclusion.

Wider implications of the findings: This study was limited by the small sample size and retrospective design, a randomized double-blinded controlled trial was demanded to confirm this conclusion.

Trial registration number: None.

P-621 Risk assessment for three way translocation (TWT) carriers

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Study question: Are the proportion of unbalanced embryos and the pregnancy risks of three way translocation (TWT) patients comparable to those of reciprocal translocation (RT) patients?

Summary answer: The proportion of unbalanced embryos and the pregnancy risk for TWT cases is similar to simple reciprocal translocation (RT) cases.

What is known already: Patients carrying structural chromosome abnormalities have a variety of reproductive problems, e.g. increase in spontaneous abortions and unbalanced offspring. The most common is the RT, carried by 0.2% of the population, with an estimated 70% imbalance rate in embryos. An RT is the interchange of genomic material between two chromosomes. Complex chromosome rearrangement (CCR) cases are more intricate. The most common CCR is the TWT: interchange of material between three chromosomes. Although the most common CCR, TWTs are poorly studied due to their rarity, though assumed to have poorer prognoses, as meiotic segregation possibilities are more complex and numerous.

Study design, size, duration: Three patients are included in this study. Case A is carrier of 46,XY,t(7;9;12)(p21;q22;p12.2), partner 33 years old (yo). Case B has a is 46,XX,t(1;2;3)(p3;p11.2;q27), 33 yo. Case C is carrier of 46,XY,t(5;16;10)(q33;q23;q24.1), partner 37 yo. All karyotypes were identified via evaluation for mut

The RT group had an average maternal age of 33 yo.

Participants/materials, setting, methods: Risk assessment obtained via pregnancy and family history. Embryos from cases A, B, and C underwent aCGH PGD. The proportion of 'normal/balanced" embryos was obtained, generating the probability of success for TWTs. Case A produced 17 embryos in two cycles; Case B produced 5 embryos and Case C produced 22 embryos, each in one cycle.

To compare successful PGD rates between TWT and RT, 162 embryos from 25 RT cases were reviewed.

Main results and the role of chance: All cases had histories of first trimester pregnancy loss (SAB); two had recurrent pregnancy loss. Two cases had pregnancies that were normal/balanced for the TWT; one with a healthy child, one with an aneuploid SAB. No additional fertility problems were reported

Case A produced 4 normal/balanced, 12 unbalanced and 1 aneuploid embryo. In Case B, all 5 embryos were unbalanced. Case C produced 3 normal /balanced, 14 unbalanced and4 embryos with aneuploidy unrelated to the translocation and 1 no-result embryo.

Table 1 shows the diagnoses of TWT cases, RT cases and statistical differences. The only significant difference is the percentage of unbalanced embryos.

Diagnosis	TWT (%)	RT (%)	p
Normal/Balanced	7(15.9%)	38(23.5%)	n.s.
Aneuploid	5(11.4%)	27(16.7%)	n.s.
Unbalanced	31(70.5%)	85(52.5%)	0.05
No Result	1(2.3%)	12(7.4%)	n.s.
Total	44(100.0%)	162(100.0%)	

In comparing TWT and RT, no significant differences were found in the proportion of normal/balanced embryos.

It is assumed that TWTs have poorer outcomes due to the increase in unbalanced gamete possibilities (64 vs. 16). This shows the proportion of transferable embryos is similar to RT cases. Scriven et al, 2014 found the proportion of alternate segregates was 27.6%, paralleled by this study, with 27.3% identified (5.9% normal/balanced, 11.4% aneuploid).

Limitations, reasons for caution: The low number of participants in the TWT group as well as the low number of their embryos limited to some extent the accuracy of the statistical comparison with the RT group. More cases should be included in order to asses a proper success rate for these PGD cases.

Wider implications of the findings: TWT meiosis likely generates a hexavalent ring, a stabilizing factor promoting alternate and adjacent 1 segregations. These aren't different segregations, but rather recombination events transforming one into another, together comprising 55.3% of PGD embryos. The success of TWT PGD may depend on recombinations in hexavalent critical regions, favoring alternate segregation.

Trial registration number: NA.

P-622 A validated approach to detect balanced chromosome abnormality based next generation sequencing at single cell level

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Study question: To establish an approach based next generation sequencing to detect balanced chromosome abnormality in preimplantation human embryos.

Summary answer: The results show that our approach can accurately detect BCA events at single cell level.

What is known already: Balanced chromosome abnormality(BCA) is a common chromosomal structural variation. The past most commonly used detection methods are based on cytogenetics. Current years, next generation

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sequencing has been reported to detect BCA-associated breakpoints. Preimplantation genetic diagnosis (PGD) is widely applied in BCA carriers to increase the chance for a successful live birth. However, Previous methods can only be used to detect BCA for couples had multiple abortions and need assisted reproductive, BCA carrier embryos were seldom discriminated from the normal ones mainly due to the technique restriction. we provide a next generation sequencing approach to detect BCA in Preimplantation human embryos.

Study design, size, duration: Six cell lines samples containing BCAs validated by karyotyping were cultured to get enough cells. Single cell from cell lines isolated by mouth control pipette, and amplified with QIAGEN REPLI-g Single Cell Kit. Genomic DNA from cell lines was extracted with the QIAamp DNA blood mini kit.

Participants/materials, setting, methods: Six single cell whole genome amplification DNA and genomic DNA were sheared into ~3000 bp fragments with a HydroShear DNA Shearing Device, and mate-pair libraries were constructed by an established protocol. Each DNA library was then sequenced on BGISEQ-500 as paired-end 50-bp reads. By clustering anomalous read pairs and filtering out the false-positive results with a control cohort and the concomitant mapping information.

Main results and the role of chance: By clustering anomalous read pairs and filtering out the false-positive results. BCA events in all six single cell samples could be detected with ~100 million reads pairs per sample, and corresponding genomic DNA samples from same cell lines also detect concordant BCA events. The BCA detection results of these samples were concordant with karyotyping, showing 100% accuracy. Our results suggest that our approach can accurately detect BCA events, even in single cell. Our approach advances the application of next generation sequencing for BCA detection, especially for evaluating the genetic risk of BCA carriers and carrier screen in later PGD treatment.

Limitations, reasons for caution: This approach need further validated using amount of clinical PGD samples that contained BCAs.

Wider implications of the findings: This approach can become a clinical applicable approach to detect precise breakpoint of balance chromosome abnormality and to further distinguish normal embryos in PGD.

Trial registration number: NO.

P-623 Genetical analysis of extra X in Klinefelter Syndrome:47XXY

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Study question: Does the extra X in Klinefelter Syndrome (KS) patients originate only paternally?

Summary answer: There might be a possible mechanism of production of extra X from disomic XX oocyte which would associate with low risk of KS after ICSI.

What is known already: It has been reported that the incidence of sperms with abnormal sex chromosome is higher in the testis tissue smear of KS patients and these sperms are the cause of KS.

Study design, size, duration: Blood samples from 12 KS patients were used for X-chromosome short tandem repeats (STR) analysis. The STR analysis also included data of the parents of the KS patients (8: both parents, 4: mother only, 0: father only) from January 2015 to December 2016. This study was conducted with the informed consent of all participating patients and approved by The Institutional Review Boards of the Saint Mother Obstetrics and Gynecology Clinic.

Participants/materials, setting, methods: Blood samples of 12 KS patients and one or both of their parents were used to determine the origin of the extra X chromosome using X-chromosome haplotype markers (short tandem repeats of 12 loci), according to the method by Shrivastava et al. With DNA extracted from the samples, multiplexed PCR amplifications of the 12 X-STR loci and AMELOGENIN were conducted using an Investigator Argus X-12 QS Kit (Quigen, Germany).

Main results and the role of chance: X-chromosomal STR DNA profiles were compared among KS patient and their parents. In 7 of the 12 KS patients, both two X chromosomes were maternal origin, showing that an extra X chromosome was left in an oocyte as a result of chromosomal non-disjunction at the 1st (4/7) or 2nd (3/7) meiotic division. In 5 patients, X-chromosomes were inherited from parents, suggesting that fertilization of XY-sperm is the cause of KS.

Limitations, reasons for caution: Further investigation with more X-STR is needed. It might be premature to draw conclusions about the origin of the extra X.

Wider implications of the findings: Although the sample number applied for X-chromosomal STR DNA profiling is not enough, the present data may indicate that contribution of XX oocyte to the production of XXY embryos is greater than XY sperm. Namely, a XX oocyte penetration by a Y sperm is the main cause of KS.

Trial registration number: UMIN Clinical Trials Registry: UMIN000024542.

P-624 Why do euploid embryos miscarry? A study analysing the factors of euploid embryo transfer(ET) diagnosed with comprehensive chromosome screening(CCS) that resulted miscarriage or ongoing pregnancy

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Study question: It is estimated that more than 50% of miscarriages are due to aneuploidy. This study investigates possible factors affecting the miscarriage after euploid embryo transfer.

Summary answer: Our study indicates that endometrial thickness and serum progesterone (P4) levels before progesterone replacement can play a significant role in miscarriages after CCS.

What is known already: CCS-based trophectoderm (TE) biopsy has been demonstrated to be a safe, accurate and reproducible approach. Also CCS for aneuploidy assessment has been shown to exert a beneficial effect on clinical outcomes; it increases the chance or implantation, decreases high order multiple gestation and decreases the likelihood of miscarriage associated with aneuploidy. Although CCS decreases the miscarriage rate, it can never eliminates it. Due to its highly unknown and multidimensional nature, miscarriages after euploid embryo transfers can create a more complex clinical environment for patients and clinicians that is need to be elegantly managed.

Study design, size, duration: This retrospective study compiled 363 controlled ovarian stimulation(COH) cycles of 247 patients undergoing preimplantation genetic screening(PGS) between October 2015 to October 2016. Next generation sequencing(NGS) was performed for CCS and clinical indications for PGS included recurrent implantation failure and advanced maternal age. A total of 2024 embryos were analysed and 886(43.8%) of these were euploid. Of these 363 cycles, 132(36.4%) were cancelled due to all-aneuploid results. In 193 patients, 200 ET cycles were further evaluated.

Participants/materials, setting, methods: All patients underwent COH with GnRH antagonist and rFSH or hMG. TE biopsy was performed on day 5 or 6. Blastocysts were cryopreserved using vitrification.Endometrial preparation was performed as artificial hormone replacement therapy. Single euploid embryos were transferred in frozen-thaw cycles. A clinical pregnancy was defined as the presence of a gestational sac within the uterus. Ongoing pregnancy was defined as confirmed fetal heart beat by ultrasonography and continued at least 12 weeks of pregnancy.

Main results and the role of chance: Following transfer of 200 single euploid embryos in 193 women, 155 had positive hCG levels (77.5%/ET) leading to 140 had clinically confirmed and 119 ongoing pregnancies (70%/ET and 59.5%/ET respectively). In 21 women, pregnancies ended up miscarriage (15%). Outcomes were compared between cycles with ongoing pregnancies and miscarriages. There were no statistical differences between ongoing pregnancies and miscarriages regarding female age(35,1 ± 4,5 and 34,5 ± 4,9 years, respectively), female BMI (23,6 ± 3,8 kg/m^2 and 23,5 ± 4,1 kg/m^2 respectively), antral follicle count (11,6 ± 6,7 and 13,6 ± 5,9 respectively), previous IVF attempt (2,9 ± 1,8 and 3,2 ± 3,1 respectively), day of stimulation(9.5 ± 0.73 and 9.7 ± 0.65 days,

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respectively), peak E2(1819 ± 1279.4 and 2032 ± 1100 pg/ml, respectively), and peak P4(0.87 ± 0.52 and 0.91 ± 0.48 ng/ml, respectively). The two groups were also similar in terms of the number of oocyte retrieved, and injected MII oocytes. A significantly higher peak endometrial thickness and lower P4 levels before progesterone replacement was observed in ongoing pregnancy group (9,0 ± 1,3 mm, 0,25 ± 0,26 ng/ml respectively) compared to miscarriage group (8,3 ± 1,1 mm, 0,54 ± 0,58 ng/ml; p: 0.026, p: 0.034 respectively). The two groups mean mitoscore levels, distribution morphological grading of embryos and day of blastocysts (day 5 or day 6) were also similar. Regression logistic analysis showed that endometrial thickness and P4 levels were the significant parameters that can predict ongoing pregnancy rates.

Limitations, reasons for caution: The study is limited by its retrospective nature. A higher sample size or a prospective design can be used in future studies to corroborate the current findings.

Wider implications of the findings: Our results suggest that endometrial preparation for frozen ET may have a significant role for prevention of miscarriages. Review of cut-off values for endometrial thickness and P4 levels in large groups may be useful.

Trial registration number: None.

P-625 single cell rapid testing technique for preimplantation genetic screening on the BGISEQ-50

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Study question: A rapid PGS (preimplantation genetic screening) technique, targeting at single cell, needs be developed on the BGISEQ platform.

Summary answer: A single cell rapid testing technique for PGS (rapid PGS) within 18 hours on BGISEQ-50 was successfully built.

What is known already: The controversy between fresh embryo transfer and frozen embryo transfer always existed. In some areas, doctors prefer fresh embryo transfer in IVF cycles. According to this situation, PGS would be better completed within 24 hours. BGISEQ-50 was published in the 11th ICG conference, on November 5, 2016. BGISEQ-50, which is powered by combinatorial Probe-Anchor Synthesis (cPAS) and improved DNA Nanoballs (DNB) technology, has fewer replication mistakes, and the whole process including sample preparation can be completed in 18 hours.

Study design, size, duration: Single cells of 67 different cell lines with known karyotypes, including 43 cell lines with different CNVs sizes (>4 M), 15 aneuploid cell lines, and 9 negative cell lines, were performed WGA (whole genome amplification). The library construction of above WGA products would use our new single cell rapid PGS method, and sequenced on BGISEQ-50. The results of the BGISEQ-50 sequencing would be compared with the known karyotypes.

Participants/materials, setting, methods: All the above cell lines purchased from the website https://catalog.coriell.org/. Single cell of different cell lines was picked up using micromanipulation. The picked 67 samples were amplified with the PicoPLEX® WGA Kit (Rubicon Genomics, Ann Arbor, MI, USA), according to the manufacturer's protocol. Then, we constructed the libraries using our new rapid method, the input of the WGA products could change from 1 ng to 100 ng. Finally, we sequenced these libraries on BGISEQ-50.

Main results and the role of chance: The yield of the WGA products was about 3 microgram, 50 ng DNA was inputted to perform subsequent experiments. The sequence data produced on BGISEQ-50 conformed to its quality control standard. In our test, we referred to perform windows selection, GC bias correction and copy number analysis on the sequencing reads. The whole process, including WGA, library construction, sequencing, and analysis, could be finished in 18 hours. The 43 cell lines with different CNVs sizes (>4 M), along with 15 aneuploid cell lines, and 9 negative cell lines could be all detected successfully on the BGISEQ-50. Our new <18 hours single cell rapid PGS technique could realize fresh embryo transfer.

Limitations, reasons for caution: For now, the throughput of BGISEQ-50 could sequence 16 samples in one run, which needs further optimization.

Wider implications of the findings: In the future, we will perform this single cell rapid PGS method in clinical utilization for fresh embryo transfer, after testing more samples.

Trial registration number: not applicable.

P-626 Inconclusive PGS results: go for a second biopsy!

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Study question: Is blastocyst re-biopsy and re-vitrification a reasonable and safe procedure to be applied in cases of inconclusive or no results after a PGS analysis?

Summary answer: Our preliminary results show that blastocyst re-biopsy and re-vitrification is a good approach that ultimately allows the transfer of euploid embryos and good clinical outcomes.

What is known already: One of the advantages of trophectoderm biopsy is the significant decrease in the percentage of undiagnosed embryos (from around 10% with blastomere biopsy to less than 5% with trophectoderm biopsy). However, patients with few or no euploid embryos can be specially affected by this residual percentage of diagnostic failure. Moreover, blastocyst vitrification has been reported to have a limited impact on embryo viability even in cases when blastocysts are re-vitrified.

Study design, size, duration: Embryos from PGS cycles were vitrified shortly after the biopsy procedure before the genetic diagnosis was achieved. From January 2015 to December 2016, PGS patients having embryos with inconclusive or no results were offered the possibility of warming such embryos and perform a second biopsy and vitrification.

Participants/materials, setting, methods: Twenty seven patients agreed to the warming of 29 blastocysts to perform a second biopsy. After warming, surviving blastocysts were cultured until re-expansion. Embryos not re-expanded on day 7 were discarded. Trophectoderm cells from re-expanding blastocysts were biopsied using laser thermolysis. Biopsied blastocysts were vitrified for the second time while waiting for the results. The genetic analysis was performed by aCGH using the kits and protocols provided by the manufacturer (Illumina, USA).

Main results and the role of chance: The survival rate after warming was 86.2% (25/29) although only 72.4% of the embryos re-expanded (21/29) and could be re-biopsied. After the second biopsy and analysis, 95.2% (20/21) of the embryos could be successfully diagnosed. The euploidy rate was 65% (13/20). Embryos with failed amplification after the first biopsy showed a 50% of euploidy rate (7/14). On the other hand, all embryos with inconclusive results that could be diagnosed (6/7) turned out to be euploid.

To date, 6 re-biopsied euploid embryos have been warmed for transfer in 6 patients with a 100% survival and re-expansion rate. Four clinical pregnancies have been achieved with 2 ongoing pregnancies and 2 miscarriages.

Limitations, reasons for caution: To date, few transfers of re-biopsied blastocyst have been performed. As data grows, the results obtained should be monitored.

There are more sensitive techniques than a-CGH that might have allowed to obtain diagnosis in some of the undiagnosed embryos without the need of performing a second biopsy.

Wider implications of the findings: Blastocysts with inconclusive diagnosis show suboptimal results regarding survival and re-expansion rates. This fact should be informed to patients. When the second biopsy can be performed, euploid re-biopsied re-vitrified blastocysts have a high implantation potential. Re-biopsy allows the rescue of euploid embryos thus enhancing the cumulative pregnancy rate per cycle.

Trial registration number: not applicable.

P-627 A systematic comparison on the bacterial colonization of paired endometrial tissue and fluid samples: Implication on endometrial microbiome studies in the future

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Study question: Are the profiles of bacteria (microbiomes) in endometrial fluid reflecting those in the endometrial biopsied tissue?

Summary answer: No, the kinds and abundance of the bacterial taxa (plural of taxon, i.e. species/genus) in endometrial biopsies are different from their corresponding endometrial fluid samples.

What is known already: Contrary to widely-held belief, the endometrium and endometrial fluid are not sterile but harbor bacteria. IVF patients with endometrial fluid not dominated by Lactobacillus are associated with significantly lower implantation rate (61% vs 23%), pregnancy rate (71% vs 33%), ongoing pregnancy rate (59% vs 13%) and live birth rate (59% vs 7%).

Study design, size, duration: This is a non-interventional observational study on 102 bacterial taxa in endometrial tissue sample and its paired endometrial fluid sample collected from three reproductive-age women.

Participants/materials, setting, methods: Endometrial biopsied tissue and its corresponding endometrial fluid were collected from the same participant attending our Assisted Reproductive Technology Unit. Genomic DNA was extracted, PCR-amplified of the 16 S ribosomal RNA gene, which is universally possessed by all bacteria, but not by human, and sequenced on the MiSeq-200 (Illumina) sequencing platform. Similar sequencing reads (>97% nucleotide identity) were clustered as one bacterial taxon (genus/species, plural: taxa.). Read counts were normalized across different samples before comparison.

Main results and the role of chance: We have sequenced endometrial biopsied tissue and their corresponding endometrial fluid samples from three women at a median depth of 29,597 reads (interquartile range, 7,106 reads to 84,166 reads) per sample.

We detected 40, 63 and 43 bacterial taxa in participants A, B and C, respectively. Of these, only 35 taxa (24% of 146 taxa) were detected in both the endometrial tissue and fluid samples. Sixty-nine taxa (47%) were detected only in the tissue samples. Interestingly, 42 taxa (29%) were detected only in the fluid samples. The most abundant taxon in the tissue samples was a Bifidobacterium, while that in the fluid samples was a Lactobacillus.

Further, the Pearson product-moment correlation coefficients between the abundance values of taxa in the tissue samples and those in the fluid samples were 0.64, 0.13 and -0.24, and the p-values were $<1\times10^{-4}$, 0.31 and 0.12, respectively, for participants A, B and C.

Hence, among >100 bacterial taxa detected in this study, there were no correlation between their abundance values in the endometrial fluid and the endometrial tissue in two of the three participants. There was only a moderate correlation in one participant.

Limitations, reasons for caution: The limitation on this pilot study is that it involved only three patients and two samples from each of them, albeit each sample was sequenced at moderately high read depth.

Wider implications of the findings: The endometrial tissue harbors bacterial taxa not detectable in endometrial fluid and vice versa. The abundance values of taxa detected in the two endometrial samples are weakly correlated. Additional qualitative and quantitative information can be acquired in microbiome analysis of both the endometrial and fluid samples from the same woman.

Trial registration number: Not applicable for this basic science study.

P-628 Mosaic embryos: is it worth re-biopsying them?

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Study question: Is the performance of a second biopsy and analysis useful and efficient in mosaic embryos?

Summary answer: Performing a second analysis in mosaic embryos provides additional information for decision making and counseling about the possible transfer of such embryos.

What is known already: Trophectoderm biopsy allows the detection of certain levels of mosaicism. However, its clinical significance is yet to be determined although healthy live births have been generated by the transfer of mosaic euploid-aneuploid embryos. The decision to transfer euploid-aneuploid mosaic blastocysts should be taken considering the chromosomal anomaly and chromosomes involved.

Study design, size, duration: In our Preimplantation Genetic Screening (PGS) program, biopsied blastocysts are vitrified until diagnosis is performed. From January 2015 to December 2016, PGS patients with mosaic embryos were offered the possibility of warming such embryos and perform a second biopsy to obtain more information about their chromosomal constitution and better assess their possible use for transfer.

Participants/materials, setting, methods: Seventeen patients agreed to the warming of 20 mosaic blastocysts to perform a second biopsy. After warming, surviving blastocysts were cultured until re-expansion. Embryos not re-expanded on day 7 were discarded. Trophectoderm cells from re-expanding blastocysts were biopsied using laser thermolysis. The analysis was performed by aCGH using the kits and protocols provided by the manufacturer (Illumina, USA).

Main results and the role of chance: The survival rate after warming was 100% (20/20) although only 85.7% of the embryos re-expanded (18/20) and could be re-biopsied. After the second biopsy and analysis all the embryos could be diagnosed and mosaicism was confirmed in 8/18 blastocysts.

The first analysis in those 18 blastocysts showed that 11 had whole chromosome mosaicism and 7 had been diagnosed as segmental mosaics. Seven out of 11 embryos presenting whole chromosome mosaicism were confirmed to be mosaic after the second analysis. Three embryos showed a euploid result. One was mosaic for a different chromosome.

Regarding embryos with segmental mosaicism, its presence was only confirmed in 1/7 embryos. The rest showed a euploid profile after the second analysis.

Even based in a limited number of cases, our results show that segmental mosaicism is less confirmed than whole chromosome anomalies after a second biopsy.

Embryos with segmental mosaicism should be those first selected in cases of considering a transfer of a mosaic embryo.

Limitations, reasons for caution: Small sample size.

Array CGH is not an optimal tool for low degree mosaicism detection.

Embryos firstly diagnosed as mosaic that resulted euploid after a second biopsy should still be considered as mosaic. Patients' decision to transfer them must be taken after proper genetic counseling.

Wider implications of the findings: More sensitive analysis techniques are leading to the detection of more mosaic embryos in PGS programs, thus increasing the difficulty to decide embryo eligibility for transfer. It is of importance to collect as much information as possible about the actual chromosomal constitution of these embryos and the significance of mosaicism.

Trial registration number: Not applicable.

P-629 Higher incidence of chromosome 11 abnormalities associated with male embryos in cycles undergoing oocyte donation

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Study question: Are there any gender-specific differences in distribution of chromosomal abnormalities between female and male embryos obtained in cycles undergoing oocyte donation?

Summary answer: Chromosomal distribution between female and male embryos shows similar pattern except chromosome 11, which showed a significantly higher abnormality rate towards male gender.

What is known already: It is known that oocyte donation programs yield high pregnancy and take home baby rates. Although it is generally believed that such high rates are mainly due to superior gamete quality, recent studies have reported a high rate of chromosomal abnormality in donor cycles. However, studies in which the incidence and distribution of genetic abnormalities in

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embryos have been assessed and documented according to the gender of the embryo is scarce. To the best our knowledge this study is the first study to investigate distribution of genetic abnormalities in male and female embryos generated from donor oocytes

Study design, size, duration: This retrospective study was performed in British Cyprus IVF Centre between January and October 2016

Participants/materials, setting, methods: In this study, a total of 62 cycles of 62 patients that had undergone oocyte donation and comprehensive chromosomal screening (CCS) cycles for gender selection were included. All male partners showed normal semen parameters according to World Health Organisation. Mean age of oocyte donors was (24.5 ± 2.4). From 365 biopsied blastocyst-stage human embryos, 319 were analysed and 312 were efficiently diagnosed.

Main results and the role of chance: Of these 312 embryos diagnosed, 133 were found to be chromosomally abnormal (42.6%). Due to complex aneuploidy, gender of 11 embryos has not been reported (21%) hence excluded from the analysis. In total 3504 chromosomes in female embryos and 3744 in male embryos were assessed. Of female embryos, 0.66% were found to carry monosomy; 0.60% were trisomic and 0.31% had segmental imbalances. On the other hand, 0.69% of male embryos had monosomy; 0.77% displayed trisomy and 0.43% had segmental imbalances. There was not any difference between male and female embryos with respect to the incidence of monosomy, trisomy and segmental imbalances ($p = 0.739$, $p = 0.103$, and $p = 0.151$, respectively). A chromosome-specific analysis revealed more abnormalities in chromosome 11 in male (5.9%) than female (0.7%) embryos ($P = 0.020$).

Limitations, reasons for caution: This investigation is a retrospective study and due to the limited number of samples, further data with larger sample sizes should be evaluated in order to confirm our findings.

Wider implications of the findings: Chromosome 11 carries very important genes associated with epigenetic/imprinting disorders and its full or partial loses/gains can be very important in reproductive genetics. If confirmed in prospective studies with larger sample sizes, our results may indicate the novel yet unknown pathway for gender-specific early demise of male embryos before implantation.

Trial registration number: non applicable.

P-630 Validation of a high throughput, low cost NGS PGS assay: impact of library preparation and read length on resolution

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Study question: With the aim of developing a high throughput, low cost PGS NGS assay, we evaluated the impact of library preparation kit and read length on single cell and 5-cell inputs.

Summary answer: The quantity and quality of sequencing data is highly dependent on multiplexing, library preparation method and read length. Their optimization makes PGS highly scalable.

What is known already: Limited WGA technologies suitable for copy number detection are commercially available. DOPlify™ (Reproductive Health Science Ltd) has been specifically developed for Next Generation Sequencing (NGS). The time and financial efficiencies offered by NGS of clinical samples is mediated by multiplexing, with the cost per sample decreasing as more samples are multiplexed. However, this can also negatively impact the test resolution and quality of results. When limited source material for genome wide evaluation is available, effective fragmentation, highly efficient adapter ligation and optimal read length is imperative to maximize the reads per sample available for interrogation.

Study design, size, duration: The aim of this study was to compare NGS run and sequencing read metrics from a range of library preparation kits as models for a PGS workflow. Using a common input DOPlify™ WGA product, kits from Kapa Biosystems, Illumina and Bioo Scientific were used in parallel according to manufacturers' instructions. The prepared libraries were sequenced together to eliminate any inter-sequencing variability.

Participants/materials, setting, methods: Individually sorted single cell and 5-cell aliquots from four aneuploid cell lines (Coriell Institute for Medical Research) were used as test material. DNA libraries were prepared from four individual DOPlify™ WGA DNA samples that were created from a pool of eight individual WGA reactions to create a uniform input template. The libraries were subsequently sequenced (paired-end) using a NextSeq (n = 16; 150 bp) platform according to standard protocol (Illumina).

Main results and the role of chance: More than 12,250,000 reads per sample were generated using this workflow. Random downsampling to approximately 300,000 reads per sample was performed to mimic low pass NGS and sequencing data was bioinformatically aligned to hg19, before mapping rates and mapping quality were determined for single-end reads truncated to 36, 50, 75 and 150 bp. In addition, the generation of artificial aneuploidy samples with whole chromosome gains or losses, and segmental duplications or deletions were used to bioinformatically determine the resolution of the pipeline. The number of mappable reads increased as the read length was extended from approximately 64% for 36 bp to 70%, 78% and >80% for 50, 75 and 150 bp length reads respectively for Kapa HyperPlus kit samples. At a read length of 36 bp, Covaris shearing resulted in an approximate 40% increase in the number of unmappable reads compared to enzymatic fragmentation. Bioinformatic modelling using artificial copy number variations (CNVs) demonstrated a 5Mbp detection limit with binning 300,000 reads into 1Mbp bins. It was also demonstrated that increasing the limit of detection to 2Mbp was possible when 600,000 reads were binned into 500kbp bins. Kapa HyperPlus NGS genomic coverage was x0.01; 96 sample run (1×75 bp) and increased to x0.05; 48 samples (1×150 bp).

Limitations, reasons for caution: This data has been generated from limited NGS runs, and therefore increasing the number of sequencing runs is necessary to increase the number of samples per sequencing parameter/condition and to confirm intra-sequencing results.

Wider implications of the findings: Differences in resolution were evident with choice of library preparation kit and read length. By selecting RHS DOPlify™ and Kapa HyperPlus kits, we have validated a protocol that multiplexes 48-96 samples per PGS MiSeq NGS run and provides sensitivity down to 2 Mb using longer read lengths.

Trial registration number: not applicable.

P-631 Identification of novel mutations in PLCZ1 in two patients with fertilization failure after ICSI

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Study question: Are abnormalities in the phospholipase C zeta (PLCZ1) gene responsible for failed fertilization after ICSI?

Summary answer: PLCZ1 mutational screening in patients with a history of failed or low fertilization after ICSI resulted in the identification of three mutations in two patients.

What is known already: In mammals, sperm-oocyte fusion leads to the oocyte activation process, which is initiated by calcium oscillations in the oocyte cytoplasm. A sperm specific phospholipase C zeta (PLCZ1) is known to induce this series of specific calcium oscillations, which hallmarks the start of oocyte activation. Loss of PLCZ1 function is known to cause oocyte activation failure. To date, there are only three missense mutations reported in two patients with fertilization failure.To obtain more insight in the role of PLCzeta in fertilization, a larger screening of the gene in patients experiencing failed or low fertilization after ICSI is warranted.

Study design, size, duration: Patients (n = 25) with previously low or total failed fertilization after routine ICSI were enrolled in the study after written consent. The genomic DNA was sequenced in these patients to check for abnormalities in the PLCzeta gene. In addition, RT-PCR was performed on the sperm

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of a patient with splice mutation to see the effect of splice mutation at transcriptional level. The patients also underwent Assisted Oocyte Activation (AOA) after ICSI to obtain fertilization and pregnancy.

Participants/materials, setting, methods: Genomic DNA was extracted from the patient saliva samples. PCR was performed using primers designed to amplify all the 15 coding exons and the exon-intronic boundaries. The amplified PCR products were sequenced using illumine MiSeq. RNA from one patient was isolated using Picopure RNA isolation kit, cDNA synthesis was performed using iscript and RT-PCR with primers spanning exon 3 and 5. For AOA, sperm was injected together with calcium followed by a double ionomycin exposure.

Main results and the role of chance: PCR amplification and Illumina sequencing of the PLCZ1 gene revealed a known missense mutation c.698 A>T; H233L and a novel truncating mutation c.964 A>T; K322* in a compound heterozygous state in one patient.

In a second patient, a rare heterozygous variant c.422 G>A; R141H was found.

Finally, we detected a novel heterozygous invariant splice mutation c.136-1 G>C in a third patient.

The 136-1 G>C substitution is predicted to lead to loss of splice acceptor site by three different splice prediction programmes. The loss of splice acceptor site leads to loss of exon4. To analyze the effect of this mutation at transcriptional level, we extracted RNA from the sperm sample of the patient. RT-PCR on the patient cDNA confirmed the loss of exon4.

AOA was performed during ICSI on all the three patients oocytes to restore fertilization. This resulted in 80% fertilization rate and a successful live birth in the patient with the splice mutation

The second patient with compound heterozygous mutations had 5 cycles of AOA with a mean fertilization rate of 24.63% and a live birth was obtained.

The third patient with a rare variant had two cycles of AOA, which resulted in 33.33% fertilization but no pregnancy could be established yet

Limitations, reasons for caution: We still need to verify further the functional effect of these mutations by making recombinant protein of these mutations and injecting them into oocytes to verify its effect on activation rate, calcium pattern and embryonic developmental potential.

Wider implications of the findings: Identification of mutations in two patients with failed fertilization after ICSI confirms the crucial role of the PLCZ1 protein in the fertilization process. Screening of more patients with failed fertilization after ICSI will give us more insight of the frequency of PLCZ1 mutations in these patients.

Trial registration number: not applicable.

P-632 From prenatal diagnosis of fetal abnormality to preimplantation genetic diagnosis (PGD) by using next-generation sequencing (NGS) technologies: A case study for skeletal dysplasia PGD

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Study question: Couple with two abortions due skeletal dysplasia, from initial diagnostic to complete preimplantational study. A reliable method to avoid malformation in subsequent pregnancies is needed.

Summary answer: We applied Trio whole exome sequencing (Trio-WES), involving both parents and fetus, combined with PGS/PGD and informativity testing in order to obtain healthy embryos.

What is known already: Skeletal malformations and dysplasias involve more than 300 syndromic and non-syndromic disorders, characterized by considerable phenotypic and genetic heterogeneity (recessive, X-linked disorders, or spontaneous mutations). If chromosomal abnormalities are not detected, a powerful technique for genetic testing of gene mutations associated with those observed abnormalities is needed, where Trio-WES is indicated. After that, if causative point mutations are detected, PGD coupled with informativity testing is mandatory before in vitro fertilization (IVF) in order to transfer disease-free embryos successfully.

Study design, size, duration: A couple with two consecutive abortions due to skeletal dysplasia came to our laboratory for genetic counseling. Since there was no previous medical history in the family for this pathology, we applied Trio-WES to the couple as to the fetus. We found that both members were genetic carriers of Jeune syndrome. After that, two IVF cycles were performed combining PGS and PGD (August-October 2016). A total of 2 euploid disease-free embryos were selected to transfer.

Participants/materials, setting, methods: Trio-WES study was performed on DNA isolated from both the second abortion material and parental blood samples. Targeted-WES was performed using Ion-AmpliSeq™ Exome Kit on Ion Proton™ platform. Two mutations were detected in compound heterozygosity state in *DYNC2H1* gene (pathogenic p.(Thr2106fs*7), and likely pathogenic p.(Asp3015Gly)). After oocyte insemination and day-5 biopsy, DNA whole genome amplification was followed by PGS/PGD/informativity testing in all embryos. SNPs detected through exome sequencing were used for the informativity test.

Main results and the role of chance: We present the case study of a healthy couple, with a previous history of two abortions due to undiagnosed skeletal dysplasia. A protocol for preconception assessment based on genetic diagnosis of an affected abortion was developed, in order to prevent inheritance diseases in subsequent pregnancies. In the set-up phase, we performed genetic diagnosis on malformed abortion by Trio-WES using Ion-AmpliSeq™ Exome Kit on Ion-Proton™ platform. After 5-day embryo biopsy, a combined PGD/PGS cycle was carried out. Along informativity testing, SNP-genotyping linked to gene regions involved by mutations was performed according to original Trio-WES data, giving us an accurate genetic profiling of embryos before implantation. A total of 65 informative SNP markers were selected in 2000 Kb flanking region on both sides of DYNC2H1 gene and Ion-AmpliSeq™ primer pools were designed. Targeted next-generation sequencing on Ion-PGM™ System along with Ion-AmpliSeq™ and Ion-ReproSeq™ workflow was performed. After IVF, among the seven embryos analyzed, 2 were euploid and 5 aneuploid. Both euploid ones were also healthy carriers for only one mutation, therefore they were suitable for transfer. This has not been performed yet due to gynecological issues in the patient. Presented framework will improve whole PGD/PGS in terms of turnaround-time, reliability and cost-effectiveness.

Limitations, reasons for caution: Limited to couples with an affected child or previous abortions due to genetic disease. Even a more accurate genetic diagnosis is required; a truthful one can only be achieved if the gene was previously implicated in a similar condition. The diagnostic yield from Trio-WES is reported to be approx. 15-30%.

Wider implications of the findings: Couples with undiagnosed pathologies could be benefited from this combined Trio-WES-PGD assay. We decided to couple the most powerful genetics tools available, with the aim of offering a very reliable and cost-effective method for those patients without previous clinical history reported.

Trial registration number: This is not a clinical trial so it is not linked to a trial registration number.

P-633 Accurate detection of small segmental aneuploidy using next generation screening in preimplantation genetic diagnose and screening (PGD/PGS)

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Study question: To identify whether genome amplification (WGA) method combined with next generation sequencing (NGS) could efficiently detect segmental aneuploidy less than 16 Mb in blastocysts from PGD/PGS?

Summary answer: The PCR-based WGA methods are superior than the multiple displacement amplification (MDA) to detect the small segmental aneuploidy in sequencing depth of 0.1× data.

What is known already: Previous studies have indicated the NGS-based method for PGS/PGD could accurately detect segmental aneuploidy above 16 Mb. The small segmental aneuploidy, may inherited from a carrier of structural abnormality or can be generated de-novo in preimplantation development, could lead to miscarriage or born chromosomally abnormal fetus with micro-deletion and micro-duplication syndrome. Reliable and accurate PGD/PGS by NGS is dependent on efficient whole genome amplification of a representative biopsied sample. But currently, there is no available data to show which WGA method and to what extent the sequencing depth is required to efficiently detect segmental aneuploidy less than 16 Mb.

Study design, size, duration: This study was designed to evaluate three commercially available methods of WGA (Sureplex, DOP-PCR and MDA) in TE biopsies. Totally twenty blastocysts and ten genomic DNA samples with known small segmental abnormalities were used. The study was approved by the ethics committee (LL-SC-SG-2014-011), and all blastocysts with small segmental aneuploidy were donated for research with informed consent from the couples.

Participants/materials, setting, methods: Twenty blastocysts and with the small segmental aneuploidy which related to translocation were thawed and re-biopsied. Three TE biopsies were collected from each single blastocyst and subject to DOP-PCR, MDA and Sureplex amplification and then detecting the small segmental aneuploidy by NGS, respectively. Then, ten genomic DNA samples harbouring known chromosome disease CNVs (range of 1.49 - 5.79 Mb) identified by SNP array were subjected to Sureplex amplification and for NGS analysis for further validation.

Main results and the role of chance: Twenty blastocysts with variable CNVs sized between 1.29 Mb to 10.5 Mb were thawed and subjected to three amplification methods (DOP-PCR, SurePlex and MDA) respectively. The chromosome mapping of the unique reads were 50%, 60% and 50% respectively. Both PCR-based WGA methods (Sureplex and DOP-PCR) exhibited more uniformity and potentially less regional amplification bias than the MDA-based WGA system. Also, the PCR-based WGA products detected all the CNVs with sizes and map intervals similar to those detected in FISH. In contrast, from the MDA products, NGS only identified the three larger CNVs (10.5, 8.63 and 6.28 Mb) and failed to completely detect the remain smaller CNVs. Furthermore, a number of false positive results were present in the MDA products. Since the two PCR-based WGA methods applied to TE biopsies provided a more sensitive and specific detection of CNV, we further evaluate the reproducibility of Sureplex with variably sized (1.8, 2.2, 1.74, 1.67, 1.5, 1.49, 2.8, 4.8, 3.75 and 5.79 Mb) as the test CNV. All this CNVs was detected from all three replicate Sureplex WGA products with the correct copy number.

Limitations, reasons for caution: Nonetheless, further validation studies using blinded samples and different sequencing depths are warranted to determine the sensitivity and specificity of NGS for detection of a wider range of pathogenic CNV that can arise in embryos in different locations of the genome.

Wider implications of the findings: When NGS was applied to TE biopies WGA products derived by either Sureplex or DOP-PCR amplification, we showed that known disease CNVs in the range of 1-16 Mb could be reliably and accurately detected at the correct genomic positions. But the MDA methods may lack the capacity.

Trial registration number: not applicable.

P-634 The likelihood of transferring a euploid embryo after PGD-Aneuploidy cycles depends not only on female age but also on the number of biopsied blastocysts

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Study question: Could the number of biopsied blastocysts compensate for the age effect in cycles with aneuploidy screening?

Summary answer: Until 40 years of age a good ovarian reserve providing a higher number of biopsied blastocysts can compensate for the age effect leading to aneuploidy.

What is known already: Aneuploidy is the most common type of chromosome abnormality and the leading cause of implantation failure, miscarriage and congenital abnormalities. Three RCTs have already demonstrated higher clinical pregnancy rates in young good prognosis patients with single embryo transfer, but investigations in other groups are limited to observational studies. Although scientific principles of comprehensive aneuploidy screening are widely accepted, controversy remains about the clinical and, particularly, economic effectiveness of this approach (Lee et al., 2014). Thus, patients and especially advanced maternal age cases need to be informed properly regarding their realistic chances of producing euploid embryos in different clinical scenarios.

Study design, size, duration: This retrospective study comprised a period between August 2011 and December 2016. A total of 1154 patients were subjected to aneuploidy screening, due to advanced maternal age (≥38), recurrent pregnancy loss (≥2) and repeated implantation failure (≥3).

Participants/materials, setting, methods: Patients were grouped according to female age (<35: n = 208; 35-37: n = 138; 38-40: n = 320; 41-42: n = 299 and ≥43: n = 189). They were also analyzed regarding the number of biopsied blastocysts (1: n = 273; 2-3: n = 456; 4-5: n = 276; ≥6: n = 149). Trophectoderm biopsy samples were analyzed with array CGH.

Main results and the role of chance: Regression and multivariate analyses performed designated female age and the number of biopsied blastocysts (BB) as being the most significantly correlated with the likelihood of finding at least one euploid blastocyst (LFLOEB) ($p<0.0001$). Mainly, five groups exist and the highest probability is found for females <35 and between 35-37 years of age for 4-5 and ≥6BB (90.5%, 94.4%, 90.0%, 93.3% respectively), but also for the 38-40 age category with ≥6BB (92.3%). LFLOEB is elevated for patients <35 with 2-3BB and females between 38-40 with 4-5BB, but also for patients between 41-42 years of age with ≥6BB (78.7%, 74%, 73.9%, respectively). Likewise, the LFLOEB is similar for females <35 with only 1BB, for patients between 38-40 years of age with 2-3BB, or 41-42 with 4-5BB and ≥43 with ≥6BB (57.9%, 56.6%, 57.6%, 61.1%). A diminished LFLOEB is obtained for females between 35-37 years of age with 1BB, for patients between 41-42 with 2-3BB, for the 38-40 age category with 1BB and for ≥43 with 4-5BB (33.3%, 34.6%, 41.7% and 46.7%, respectively). The worst scenario is for patients of 41-42 years of age with 1BB and for those ≥43 with 1 or 2-3BB (14.4%, 12.9% and 16.5%, respectively).

Limitations, reasons for caution: Results were obtained in an IVF program aiming prolonged culture and blastocyst transfer to select viable embryos. Cancellation rates of patients aiming at PGD-Aneuploidy but not reaching the blastocyst stage were not addressed in this study.

Wider implications of the findings: When counselling patients for PGD-Aneuploidy, maternal age and ovarian reserve should be considered together to inform them about their realistic chances of achieving a viable pregnancy. The couple should be well aware of the medical and psychological aspects of miscarriages and of terminations due to chromosomal aneuploidies.

Trial registration number: Not applicable.

P-635 Patient characteristics influence the outcome of embryo aneuploidy testing cycles

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Study question: Do patient characteristics influence outcomes of aneuploidy testing cycles? Is there a particular patient group who might benefit more from preimplantation testing for aneuploidy (PGT-A)?

Summary answer: Patient characteristics influence embryo aneuploidy. Given consistently elevated aneuploidy rates, women of advanced reproductive age (ARA), irrespective of additional indications, may benefit most from PGT-A.

What is known already: PGT-A aims to assist couples that have experienced repeated implantation failure, or recurrent miscarriages, or women of

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advanced reproductive age to achieve pregnancies and healthy births. Various methodologies are used, but the most widely applied examine the entire chromosome complement of trophectoderm (TE) samples biopsied from blastocysts. Randomised controlled trials (RCTs) have suggested that PGT-A via comprehensive chromosome screening is associated with positive outcomes. However, these studies have been criticised due to the patient groups included (generally good prognosis). We aimed to assess whether aneuploidy rates are influenced by PGT-A indications, and determine which patient group might benefit the most.

Study design, size, duration: This was a retrospective analysis of the chromosome constitution of TE samples removed from 1966 of embryos. These embryos were generated by 323 couples undergoing 366 PGT-A cycles. 149 of these couples were referred for advanced female reproductive age (ARA, average age 40.8 years), 61 for recurrent miscarriage (RM, average age 38 years), and 113 for repetitive IVF failure (RIF, 36.3 years). The corresponding data was collected from 2015 to 2016.

Participants/materials, setting, methods: All TE samples initially underwent whole genome amplification. Cytogenetic assessment took place by next generation sequencing (NGS). NGS has greater sensitivity and dynamic range, compared to other comprehensive cytogenetic methods. It can therefore reliably detect euploid embryos, as well as those with aneuploidies present in all of the TE cells (non-mosaic), and those with chromosome errors present in some TE cells (mosaic). Statistical analysis took place with ANOVA and Fisher's exact test.

Main results and the role of chance: 433 (22%) blastocysts were euploid, with the remaining 1,533 (78%) carrying different abnormalities. Of the aneuploid TE samples, 516 (26%) had errors present in all of the TE (non-mosaic), 486 (25%) had errors present in part of the TE (mosaic), and 531 (27%) had a combination of mosaic and non-mosaic abnormalities. There were significant differences ($P = 0.015$) in the euploidy rate among the referral groups with ARA generating the fewest (19%, vs. 24% in RM and RIF). ARA also generated significantly more ($P < 0.0001$) blastocysts with non-mosaic aneuploidy, compared to RIF (62% vs. 45%), but not when compared to RM ($P = 0.7$, 62% vs. 50%). The RIF and RM groups generated significantly more embryos with chromosome mosaicism, compared to the ARA group ($P = 0.0106$, 48% vs. 54%). Irrespective of PGT-A indication, the abnormality rate significantly increased with advancing female age ($P < 0.0001$, 67% for women of 36 years or younger vs. 83% for women of 37 years or older). Moreover, the no transfer rate for the younger patient group (≤36) was 11.6%. For those patients ≥37 the no transfer rate was 41%.

Limitations, reasons for caution: Cytogenetic characterisation of blastocysts was based upon NGS results of a single TE sample. As not all cells in the embryo are tested, some mosaic embryos will inevitably be classified incorrectly. However, any error is likely to be evenly spread across all groups and have little impact on the conclusions.

Wider implications of the findings: Differences in euploidy and aneuploidy rates were observed among the patient groups investigated. This suggests that patient characteristics influence embryonic chromosome constitution. PGT-A would likely be of most benefit for reproductively older women, irrespective of indication, due to the large number of abnormal blastocysts they tend to produce.

Trial registration number: Not applicable.

P-636 Embryo asymmetry on day 2 is associated with blastocyst mosaicism after trophectoderm biopsy

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Study question: What is the relationship between embryo quality parameters at cleavage stage and the appearance of mosaicism after trophectoderm biopsy?

Summary answer: The presence of blastomeric asymmetry on day 2 increased the rate of embryo mosaicism at the blastocyst stage.

What is known already: Anaphase lagging is the main process by which mosaicism arises in the preimplantation embryo. We know that mosaicism is increased in the cleavage divisions compared to the blastocyst stage, where it is still prevalent despite the variability in results. It seems that mosaicism and aneuploidies are routine during the first cleavage divisions in human embryos (Taylor *et al.*, 2014). Moreover, the greater sensitivity of the techniques of genetic diagnosis makes the detection of mosaic embryos possible. Therefore, it would be important to know at what point of the embryo development begins to generate cellular lines with different chromosome copy number.

Study design, size, duration: Retrospective analysis of 1039 day 5/6 blastocysts biopsied in our institution from January 2014 since December 2016.

Participants/materials, setting, methods: Before trophoectoderm biopsy, we registered some morphological parameters at the cleavage stage (day 2/3): number of cells, asymmetry and fragmentation degree. The quality of the embryos was graded as A, B, C or D (ASEBIR criteria). An embryo was considered as asymmetric when a difference of 20% in blastomeric size was found between the biggest and the smallest one. Data were analyzed and related to chromosomal diagnosis, obtained by array-CGH in our PGS program.

Main results and the role of chance: The embryo quality on day 2 and day 3 was not associated with the presence of mosaic blastocysts ($p = 0.504$ and $p = 0.544$, respectively). The degree of embryo fragmentation at day 2 and 3, also did not reveal a relationship with mosaicism ($p = 0.769$ and $p = 0.804$, respectively).

Regarding assymetry we stablished 3 groups: group A: symmetric, group B; slightly asymmetric (a difference less than 20% in blastomeric sizes), and group C: asymmetric. On day 2, the percentages of mosaic embryos in each group were 12.2%, 5%, and 25%, respectively, with statistical significance ($p = 0.004$). However, on day 3, the percentages of mosaic embryos in each group were not statistically significant (12.8%, 11.5%, and 8.6%, respectively, $p = 0.748$). Finally, non-statistical differences were observed in the number of cells on day 2 or 3 and the presence of mosaic embryos.

Limitations, reasons for caution: The main limitation is that this is a retrospective study.

Wider implications of the findings: Morphokinetics could be related to chromosomal haplotype in preimplantation embryos. There are many parameters that could influence in different cell lines during embryo development, but probably asymmetry could seriously affect the final result of chromosomal status. The way in which the first divisions occur could determine a future mosaic embryo.

Trial registration number: No trial registration.

P-637 Combined preimplantation genetic diagnosis, informativity testing and aneuploidy screening with human leukocyte antigen (HLA)-Matching by a novel single nucleotide polymorphism-genotyping in terms of Next-Generation Sequencing

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Study question: Is our NGS strategy coupled to HLA-allele prediction from SNP-genotyping reliable to achieve successful birth of genetically suitable donor-baby(ies) and hematopoietic stem cell transplantation (HSCT)?

Summary answer: We've implemented a novel methodology for predicting HLA-alleles from SNP-genotyping combined with Ion-AmpliSeq™ sequencing for the PGD of β-thalassemia in order to ensure sufficient informativity.

What is known already: The major histocompatibility complex (MHC) containing the classical HLA system is among the most polymorphic and diverse regions in the human genome. Combination of in vitro fertilization (IVF), PGD for haemoglobinopathies, and HLA-typing, allows the birth of healthy children

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who are potential donors of stem cells for their affected siblings through HSCT. Nowadays, multiplex short tandem repeat polymerase chain reaction (STR-PCR) within both the HLA and β-globin loci is routinely performed in PGD-HLA. Analysis of HLA-SNPs and SNPs linked to β-globin gene mutations with NGS technology enables an all-in-one informativity testing, PGS and PGD shortly after embryo biopsy.

Study design, size, duration: A thalassemia trait couple with an affected child was referred for thalassemia-HLA PGD. A preclinical set-up study was first conducted after written informed consent from parents about whole procedure and its outcomes. Complete study comprised two IVF-cycles with PGS/PGD (May-December 2016). Mother carried a common β-globin gene (HBB) mutation, known as hemoglobin (Hb) Lepore-Washington-Boston (g.63632_71046del). Father carried other frequent HBB mutation (g.70842 C>T, Cd39C> T). Affected child was therefore double heterozygous for both parental gene mutations.

Participants/materials, setting, methods: Imputation from HLA-SNP genotyping was predicted from family blood samples within 9 HLA-antigen loci (Class I {-AI-BI-C }; Class-II {-DPA1I-DPB1I-DQA1I-DQB1I-DRB1I-DRA}) using SNP2HLA software. In parallel, informativity testing for β-globin locus was performed. Haploview provided SNPs-linkage disequilibrium. Potentially informative SNPs up to 200Kb flanking regions were used for Ion-AmpliSeq™ primer-design and subsequent targeted-NGS on Ion PGM™ platform. After oocyte insemination and day-5 biopsy, DNA-whole genome amplification was followed by PGS/PGD/informativity testing in embryos.

Main results and the role of chance: A single-custom set of 160 SNPs typed across HLA and HBB loci has been studied in order to ensure a sufficient informativity. Genotype data for all SNPs with European ancestry was found in The International HapMap Project. SNPs were only selected for four-digit prediction accuracy ≥90% at each locus in HLA-typing. Mismatch in SNP-haplotype and recombination rate between informative flanking markers was estimated in less than 5% and 0.05%, respectively. Around 17% of SNPs studied were fully informative in order to select mutation-free and HLA-matched embryos. Along two β-thalassemia/HLA-PGD cycles, a total of 16 embryos resulted. NGS-Ion PGM™ System along with Ion-ReproSeq™ workflow determined that 8 were euploid, 5 aneuploid and 3 presented a chaotic arrangement. No amplification failures, neither false positives nor negatives were detected. The overall ADO rate was ≤3%. Finally, PGD/SNP informativity testing in euploid embryos showed 3 mutation-free ones and 1 was found to be both healthy and HLA-compatible with the affected sibling. To date, embryo transfer is pending. Following the ESHRE-PGD recommendations about covering 5 HLA regions together with at least 2 markers linked to HBB locus, 20 different STRs on both HLA and HBB loci ensuring enough informativity were used to validate SNP-based approach.

Limitations, reasons for caution: Number of HLA-PGD studies is limited to couples with affected children by genetic or acquired immune system diseases. Also, some SNPs within HLA-region can show strong LD to particular alleles. Improving the HLA-allele prediction algorithm would optimize the selection of informative SNPs based upon maximizing the predictability of SNP haplotypes.

Wider implications of the findings: This SNP approach is extremely useful regarding to time-consuming and cost-effectiveness along PGD-HLA cycles, against laborious, expensive and case-specific STR-PCR protocols. Also, HLA-SNPs genotyping combined with Ion-AmpliSeq™ NGS-technology could be applicable for preimplantation-HLA selection alone or in combination with genetic diseases or acquired immune system disorders, only curable by HSCT.

Trial registration number: This is not a clinical trial so it is not linked to a trial registration number.

P-638 The quality of the inner cell mass and trophectoderm is correlated with mosaicism

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Study question: Is there any association between the quality of the embryo and the position of the inner cell mass (ICM) with the appearance of mosaic blastocysts?

Summary answer: We found a relationship between the quality of the blastocyst and the diagnosis of mosaicism.

What is known already: Anaphase lagging is the main process by which mosaicism arises in the preimplantation embryo. In the blastocyst stage, this phenomenon is still prevalent despite the variability in results (Taylor *et al.*, 2014). Moreover, the greater sensitivity of genetic diagnosis techniques makes possible the detection of mosaic embryos. These embryos may have a lower implantation rate and a greater risk of genetic abnormalities, so the detection of mosaic embryos prior to transfer is important. Although a correlation between different morphokinetic parameters and embryo ploidy has been observed, we do not find any study evaluating mosaicism.

Study design, size, duration: This is a retrospective study evaluating 663 blastocyst biopsied on day 5 or 6 from January 2014 since March 2016.

Participants/materials, setting, methods: The quality of the trophectoderm (1-3) and the ICM (1-3) was assessed independently, according to Gardner's criteria prior to embryo biopsy. The position of the ICM at the moment of biopsy (outside, inside or in the herniation) was also evaluated. Data were analyzed and related to chromosomal diagnosis, obtained by array-CGH in our PGS program.

Main results and the role of chance: The quality of the ICM and the trophectoderm was associated statistically with the appearance of mosaic embryos. The rate of mosaicism for the ICM quality was: 8.3% (grade 1), 17.2% (grade 2) and 23.1% (grade 3) ($p = 0.001$). The appearance of mosaicism for the trophectoderm quality was: 6.1% (grade 1), 17.8% (grade 2) and 13.9% (grade 3) ($p = 0.0001$). So, blastocysts with high quality (grade 1 ICM and grade 1 trophectoderm) had the best options not to be mosaics. We do not found statistical differences in relation to the position of the ICM and the diagnosis of mosaic embryos (11.9%, outside; 12.8%, inside; 8.2% herniation; $p = 0.705$).

Limitations, reasons for caution: The main limitation is the retrospective nature of the study. We also have to consider that the assisted hatching procedure performed on day 3 makes that the blastocyst morphology, mainly the trophectoderm, could be slightly different, so the blastocyst assessment could differ from the standard blastocyst classification.

Wider implications of the findings: The quality of the ICM and trophectoderm may be a marker of mosaicism. To our knowledge this is the first work evaluating blastocyst quality and mosaicism.

Trial registration number: Not applicable.

P-639 The effect of complete ban of comprehensive chromosome screening on the outcome of in vitro fertilization of patients with advanced maternal age in Hungary

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Study question: How does prohibition of comprehensive chromosome screening affect clinical outcomes of patients with advanced maternal age (AMA)?

Summary answer: Based on our results using CCS increases sustained implantation rates while reduces miscarriage and multiple pregnancy rates and the number of unnecessary embryo transfers.

What is known already: In 2015, due to a legislative change in Hungary, classification of CCS from recommended for patients with AMA changed to experimental, that led to its complete ban. Since then several meta-analyses showed that comprehensive chromosome screening is a useful tool to select embryos that results in a higher implantation and a reduced miscarriage rates. The aim of this longitudinal retrospective analysis is to investigate the effect of embryos selection on clinical outcomes using CCS or conventional morphology-based selection.

Study design, size, duration: Data were collected from all stimulated, autologous cycles where female patients had an advanced maternal age (37-42) between January of 2013 and May of 2016 at our clinic. Patients with known

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chromosome rearrangements were excluded from the analysis. Retrospectively two groups were formed based on the type of embryo selection.

Participants/materials, setting, methods: In the CCS group the transferred embryo(s) were all euploid (array-CGH, day-3 biopsy), while in MBS group only conventional morphology-based selection was applied. In the CCS group 52 fresh embryo transfers were carried out with 64 embryos and in MBS group 102 embryos were transferred in 70 fresh cycles. We analyzed only one cycle from all patients to ensure the independence of observations.

Main results and the role of chance: The mean maternal age did not differ in the two groups (39,00 ± 1,833 in CCS group, 39,16 ± 1,603 in MBS group) while the mean number of embryos transferred was significantly higher in MBS group (1,46 ± 0,440 vs. 1,25 ± 0,582; p = 0,0393). Despite the difference in the number of transferred embryos, clinical pregnancy rates did not differ (40,00% (28/70) in MBS vs. 49,02% (25/51) in CCS group). We found a significantly higher miscarriage rate in MBS group (40,63% (13/32) vs. 15,39% (4/26), p = 0,0455). These embryos had a 4,54-fold risk (95%CI=1,1193-18,4123, p = 0,0342) for miscarriage after a detected heart-beat compared to the euploid embryos. The sustained implantation rate were significantly higher in the CCS group (40,63% (26/64) vs. 20,59%(21/102), p = 0,0077). We did not find a significant difference in case of ongoing pregnancy rates (43,14%(22/51) in MBS vs. 27,14%(19/70), p = 0,0812). Furthermore, in the MBS group one pregnancy were terminated due to a trisomy on chromosome 21.

Limitations, reasons for caution: This analysis is partially longitudinal meaning after the ban of CCS only conventional methods were used. Also, despite of analyzing more than 3 consecutive years of data, the number of observation lower than expected.

Wider implications of the findings: Our results demonstrate that CCS is a more effective technique to select embryos for transfers than conventional morphology-based selection for patients with AMA. Using CCS, less patients experience miscarriage that is considered a major benefit compared to conventional IVF/ICSI treatments.

Trial registration number: None.

P-640 Concordance rates between single blastomere day-3 (D3) and trophectoderm biopsies (D5) using Next Generation Sequencing (NGS)

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Study question: To evaluate the concordance rates of NGS findings by analyzing the same, non-transferred embryos, after day 3 and day 5 biopsies.

Summary answer: NGS results on D3 and D5 embryo biopsies, showed concordance rates of 92.4% for whole-chromosome aneuploidies and 94.9% for segmental aneuploidies. In total 87.3% concordances.

What is known already: Previous publications, using array-CGH and comparing D3 versus trophectoderm biopsies to full analysis of aneuploid embryos, showed similar high concordance rates per embryo diagnosis for D3 biopsies (98 %) and trophectoderm biopsies (96.6 %). This blinded study was conducted to re-analyse 109 embryos previously diagnosed as aneuploidy. PGS was performed using array-CGH on D3 (n = 50) or D5 (n = 59) biopsies. After the two biopsy strategies, reanalysis of whole blastocysts was carried out with same array-CGH protocol (Mir ,2016).

Capalbo et al using aCGH found that the sensitivity of D3 biopsy predicting the blastocyst chromosomal constitution was 86.4% and specificity was 95%

Study design, size, duration: Observational study, including 35 patients (118 embryos) undergoing PGS at IVI Abu Dhabi from August 2016 to January 2017, to validate the chromosomal status of D5 embryos previously diagnosed on D3 with NGS technology. Double biopsies (D3 and D5) were performed to all embryos, reaching blastocyst stage on D5 and not being selected for transfer (including surplus euploid embryos, that could not be vitrified according to the country law).

Participants/materials, setting, methods: Infertile patients with normal karyotype undergoing PGS with fresh oocytes, with more than four embryos to biopsy on D3, women aged between 18 and 45 years old, with a body mass index between 19 and 30. For the cases of D3 embryo biopsy, only embryos with five or more nucleated blastomeres and less than 25 % fragmentation were biopsied and for blastocyst biopsy, only hatching blastocyst were biopsied. NGS was performed after whole genome amplification.

Main results and the role of chance: Concordance rates for segmental and whole chromosome aneuploidies, determined on D3 and D5 biopsies were blindly evaluate. We calculated two types of concordance rates: the concordance rate per analyzed chromosome, where we considered the total number of chromosomes independently if they were called as euploid or aneuploidy (24 chromosomes per embryo); and the concordance rate per embryo diagnosis, where we considered discrepancies in the embryo diagnosis as euploid or aneuploid.

The overall concordance rate per analyzed chromosome (24 chromosomes x 118 embryos) was 98.4% (2787/2832 analyzed chromosomes). For whole-chromosome aneuploidy, the concordance rate was 99.0% (2803/2832) and for segmental aneuploidies was 99.3% (2813/2832).

The concordance rates per embryo diagnosis were 92.4% (108/118) for whole-chromosome aneuploidies and 94.9% (112/118) for segmental aneuploidies. Therefore, false positive rate per D3 embryo diagnosis (euploid/aneuploid) was 11.9% (14 out of 118 embryos). From which 6.8% (8/118) were whole-chromosome discrepancies between D3 and D5, and 5.1% (6/118) were segmental aneuploidies. False negative rate per embryo diagnosis (aneuploid/euploid) was 0.8% (1 out of 118).

The concordance rates per embryo diagnosis for aneuploid/aneuploid embryos were 83.1% (98/118), representing embryos aneuploid at both biopsy stages, but with and additional aneuploidy either on D3 or D5.

Limitations, reasons for caution: The difficulty to discriminate the origin of the discrepancies, that could be attributed to D3 or D5 mosaicism, or to technical artefacts. Single cell genetic technologies are still biased by amplification artefacts. Further inner cell mass analysis could also identify discrepancies with trophectoderm and bring additional information.

Wider implications of the findings: Is important to know the limitation of D3 and D5 biopsies from biological and technical point of view to consider the proper stage of embryo biopsy. The reported mosaicism incidence in preimplantation embryos is wide (between 4 and 90%). Further studies are needed to elucidate the real rate of mosaicism.

Trial registration number: not applicable.

P-641 New algorithm for a precise determination of the level of mosaicism in Preimplantation Genetic Screening (PGS) with next-generation sequencing (NGS)

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Study question: Are we capable to improve the accuracy identifying genome-wide aneuploidies based on NGS, using a new filter-duplicates and a copy number variation (CNV) calling algorithms?

Summary answer: Our algorithm can accurately identify percentage of aneuploidy for embryo-biopsies, improving confidence-value and reducing risk of misdiagnosis. Less reads are required enabling high-level multiplexing.

What is known already: With the advent of NGS for PGS purpose, bioinformatics has become a key area of development. Filtering PCR-artifacts, normalization and aberrations calling are common tasks implemented in any software designed for analyzing NGS data. However, most of the algorithms implemented to date have been developed for post-natal diagnosis, where NGS has recently become a useful but not fully compatible approach for PGS. Previous methods based on whole-

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genome sequencing (WGS) require high-depth coverage on the whole genome scale and are not cost-efficient. Moreover, they do not determine precisely the level of mosaicism, because this is very uncommon in post-natal diagnosis.

Study design, size, duration: *In-silico* and *in-vitro* approaches were achieved in order to evaluate the percentage of mosaicism. Firstly, *in silico* mosaic-embryos were designed controlling the percentage of mosaicism from sequencing alignment data of 25 euploid/aneuploid embryos. *In vitro* mosaic-embryos were mimetized from DNA of cell-culture.

To determinate minimum reads number per embryo required for a correct PGS forecast, a random sequence selection within these embryos was carried out to perform *in silico* embryos from 100,000 to 5000 reads.

Participants/materials, setting, methods: Sequencing data from previous biopsied day-3 and day-5 euploid/aneuploid embryos was used to perform *in silico* sets. DNA was isolated from aneuploid lymphocyte cell-culture lines showing trisomy18, Turner syndrome and euploid and mixed to create *in vitro* set.

Biopsied and *in vitro* embryos were sequenced on Ion-PGM™ platform and analyzed using Ion-ReproSeq™ workflow and our own-designed algorithm.

MAPD quality values and ploidy stage per embryo were thoughtfully evaluated.

Main results and the role of chance: While Ion-ReproSeq™ workflow needs at least 100.000 reads per embryo for a successful analysis, our algorithm accurately predicted the ploidy stage of submitted embryos with only 10.000 reads per embryo. Also, Ion-ReproSeq™ workflow was able to detect the mosaicism stage when it was greater than 40% of aneuploidy, and to diagnose embryos as aneuploid; otherwise aneuploidy was hidden and undetected. Our algorithm has been able not only to detect the whole mosaicism spectrum (from 90 to 10%), but also to indicate the exact percentage of aneuploidy in an analyzed mosaic cell. This has allowed us to be more consistent with the final diagnosis of the embryos submitted. Moreover, our approach would provide the possibility to discover the biological significance of mosaic embryos along IVF cycles and to discuss the convenience of transfer according to its mosaic state. Finally, we can assert that our algorithm is reliable regarding to accuracy improvement, time-consuming and cost-efficiency, which enhances PGS service and allows us to offer it specifically for every couple and cycle. Even, it is limited not only to discover variations within exons, but also on whole genome.

Limitations, reasons for caution: MAPD-quality values greater than 0.4 can difficult diagnosis, by modifying the percentage of mosaicism showed by our algorithm. However, mosaic embryos never could be wrongly identified as euploids. This limitation is semi-controlled by applying the filter-duplicates algorithm that decreases reads-variability while quality of copy number calls increases.

Wider implications of the findings: Our algorithm is more accurate than Ion-ReproSeq™ workflow one. It shows the exact ploidy-rate in mosaic-embryos, enabling detection even at very-low mosaicism. This approach could improve implantation chances, giving us a deep-knowledge about the developmental potential of mosaic embryos generated by *in vitro* fertilization-cycles and biological-implications derived from transferring themselves.

Trial registration number: None.

P-642 Quantitative polymerase chain reaction (qPCR)-based patterns consistent with mosaicism/partial aneuploidies(PA) indicate blastocysts with slightly lower reproductive competence: preliminary results from a non-selection study

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Study question: Can we set qPCR parameters and thresholds to infer mosaicism and/or large PA in blastocysts on trophectoderm-based analysis' patterns and predict embryos' reproductive competence?

Summary answer: The developed criteria for qPCR data analysis may indicate mosaicism and/or large PA in trophectoderm biopsies and identify blastocysts with a slightly lower reproductive competence.

What is known already: Mosaic blastocysts are made of cells with different karyotypes. Recently, targeted-qPCR has been reported to inherently identify whole chromosome mosaicism in cell-mixture models of trophectoderm biopsies with a considerable sensitivity and specificity. PA are imbalances involving a portion of a chromosome, which targeted-qPCR may also identify when the imbalance involves a large chromosomal region. In this study, we tested whether specific parameters and thresholds compatible with mosaicism and PA applied to clinical blastocyst stage preimplantation genetic diagnosis for aneuploidies (PGD-A) cycles can be valuable to identify embryos with lower reproductive potential.

Study design, size, duration: Prospective non-selection study involving 369 vitrified euploid single blastocyst transfers between March 2015 and September 2016. Aneuploidy testing performed through qPCR on trophectoderm biopsies according to a previously published method. Euploid blastocysts to be transferred were chosen independently from putative mosaicism/PA.

Participants/materials, setting, methods: Only euploid blastocysts whose qPCR profile plot showed an overall concurrence ≤0.3 and an inter-chromosome standard deviation ≤0.32 and ≤0.18 for male and female embryos, respectively, were considered as a first inclusion criteria. Putative mosaicism was defined as copy number (CN) ≤1.65 or ≥2.35 and an intra-chromosome assays' concurrence ≤0.25. Putative PA were defined as two consecutive assays on a chromosome arm with a CN ≤1.65 or ≥2.35 and an intra-chromosome assays' concurrence≥0.4.

Main results and the role of chance: 3.5%(n = 13/369) and 28.2% (n = 104/369) of the transferred blastocysts were not included due to an overall concurrence and inter-chromosome standard deviation above the defined thresholds, respectively. 78.6% of the blastocysts included in the study showed a qPCR profile not compatible with mosaic and/or PA(n = 198/252;95% CI=73.0%-83.5%), and 21.4% were classified as "putative mosaic" and/or "putative PA" for at least one chromosome(n = 54/252,95%CI=16.5%-27.0%). Specifically, 15.1% "putative mosaic"(n = 38/252;95%CI=10.9%-20.1%), 6.3% "putative PA"(n = 16/252; 95%CI=0.4%-10.1%) and 0.4% both (n = 1/252;95%CI=0%-2.2%.). No correlation was found between blastocyst morphology and qPCR profiles compatible with mosaicism/PA (p = 0.4).

The positive pregnancy rates per SET were 52.6%(n = 20/38;95%CI=35.8%-69.0%) and 43.8%(n = 7/16;95%CI=19.8%-70.1%) for "putative mosaic" and "putative PA", respectively. The biochemical pregnancy loss rates were 10.0%(n = 2/20;95%CI=1.2%-31.7%) and 14.3%(n = 1/7;95%CI=0.4%-57.9%). The miscarriage rates were 22.2%(n = 4/18;95%CI=6.4-47.6%) and 16.6%(n = 1/6;95%CI=0.4%-64.1%). The ongoing pregnancy rates (>12 gestational weeks) per SET were 36.8% (n = 14/38;95%CI=21.8%-54.0%) and 31.3%(n = 5/16;95%CI=11.0%-58.7%).

When combining "putative mosaic" with "putative PA" and comparing the clinical outcomes with non-mosaic/PA, the positive pregnancy test rate per SET were 50.0%(n = 27/54;95%CI=36.1%-63.9%) and 60.6%(n = 120/198;95%CI=53.4%-67.5%), respectively. The biochemical pregnancy loss rates were 11.1%(n = 3/27,95%CI=0.2%-29.2%) and 8.3%(n = 10/120;95% CI=0.4%-14.8%). The miscarriage rates were 20.8%(n = 5/24;95%CI=7.1%-42.2%) and 9.1%(n = 10/110; 95%CI=4.5%-16.1%). At last, the ongoing implantation rate per SET was significantly lower for "putative mosaic/PA" blastocysts versus non-mosaic/PA ones (p = 0.05): 35.2%(n = 19/54; 95% CI=22.7%-49.4%) and 50.5%(n = 100/198;95%CI=43.3%-57.7%).

Limitations, reasons for caution: Mosaicism on trophectoderm biopsies does not predict mosaicism in the whole blastocyst due to an unavoidable sampling bias. Putative mosaicism/PA were not confirmed on products of conceptions or ongoing pregnancies. Additional biological and technical issues can produce CN profiles resembling mosaicism and have not been tested in this study.

Wider implications of the findings: These preliminary outcomes highlighted a slightly lower reproductive potential for embryos showing an intermediate CN consistent with mosaicism/PA. These data need to be corroborated in a higher sample size to define whether the newly developed bioinformatic algorithm can be incorporated in the clinical management of qPCR-based PGD-A cycles.

Trial registration number: none.

P-643 Detection of segmental aneuploidy and mosaicism in the human preimplantation embryo by next generation sequencing (NGS) methodologies

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Study question: Can MiSeq-based (Veriseq) and personal genome machine (Reproseq)–based next-generation sequencing (NGS) accurately detect segmental aneuploidy and embryonic mosaicism in trophectoderm biopsies?

Summary answer: Segmental imbalances as little as 4.5 Mb and 10 Mb, and chromosomal mosaicism ≥20% and ≥30% were reliably detected with the Veriseq and Reproseq-based NGS, respectively.

What is known already: Preimplantation genetic screening (PGS), specifically with NGS, for aneuploidy testing on embryos has become a common practice to improve outcomes in patients undergoing treatment for infertility through in vitro fertilization. As technology has evolved, detection of segmental aneuploidy and embryonic mosaicism has become possible. Although possible to diagnose, the limits of detection for segmental aneuploidies and mosaicism between different NGS platforms has not been examined.

Study design, size, duration: This study evaluates the ability to detect segmental aneuploidy and mosaicism between two NGS platforms. Samples composed of specific euploid/aneuploidy cells ratio (10–90% mosaicism) of 10 (n = 54) and 100 (n = 54) total cells were obtained and analysed with both platforms. In addition euploid (n = 6) and aneuploid (n = 6) cell lines were used as control. Duplicates of eight cell lines with different structural abnormalities (from 4.5 Mb to 17 Mb) were used for segmental aneuploidy assessment.

Participants/materials, setting, methods: Female euploid/male trisomy 21 cell line were used for one set, and a male euploid with either female trisomy 18 or a female trisomy 21 cell line were used for another. Sensitivity and specificity of aneuploidy detection at each level of mosaicism was determined and compared between platforms. CNV analysis was accomplished by current version of BlueFuse software (Veriseq) or IonReporter Software(ReproSeq). Benefit in terms of time, and user-friendliness were determined and compared between platforms

Main results and the role of chance: In total, 120 samples were assessed for mosaicism detection, 108 chromosomal mosaics (true positive) and 12 aneuploid or euploid (true negative) samples. Sensitivity was 90% (95% confidence interval [95% CI]: 83.18% to 94.73%) and 81.82% (95% confidence interval [95% CI]: 74.17% to 87.99%) for Veriseq and Reproseq, respectively. The 12 false negative results obtained with VeriSeq were from samples with 10% mosaicism. The 24 false negative results obtained with ReproSeq were from the samples with <30% mosaicism. Specificity was 100% (95% confidence interval [95% CI]: 73.54% to 100.00%) for both platforms. These studies indicated a different limit of detection for mosaicism detection with a limit of detection (LOD) of 20% and 30% for VeriSeq and ReproSeq, respectively. Analysis of segmental aneuploidy in the cell lines demonstrated that ReproSeq could identify a segmental imbalance as small as 10 Mb in size, while VeriSeq, using a manual call, identify microdeletion as little as 4.5 Mb. No additional segmental imbalances were identified. ReproSeq protocol was more rapid (<15 h) compared to VeriSeq. VeriSeq was judged by our operators to be more user-friendly than ReproSeq.

Limitations, reasons for caution: Further data and broad-based clinical application are required to confirm the limit of detection for mosaicism level and segmental aneuploidies of NGS platforms.

Wider implications of the findings: These findings demonstrate that VeriSeq-based NGS platform has a much higher resolution for segmental aneuploidies and a higher level of accuracy at a lower level of mosaicism compared to ReproSeq.

Trial registration number: none.

P-644 Embryo selection of euploid embryos by an automated time-lapse prediction is superior to conventional morphological analysis: a retrospective study

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Study question: Is the time-lapse prediction useful in a Preimplantation Genetic Screening (PGS) program to select among euploid embryos the one with the highest implantation potential?

Summary answer: The automated time-lapse prediction provides valuable information for embryo selection, significantly increasing the chances of pregnancy and accurately predicting the implantation potential of euploid embryos.

What is known already: Previous studies confirmed that genetic screening of human blastocysts significantly improves pregnancy rates per transfer diminishing the likelihood of miscarriage. Unfortunately, there is still a considerable number of euploid embryos that fail to implant. New embryo selection markers to improve ART-outcomes have been developed. Time-lapse imaging (TLI) has emerged as an interesting non-invasive tool to identify competent embryos. No conclusive data about this technology utility for chromosome abnormalities determination is available. In the present study we investigate if combined TLI and PGS embryo selection may be a valuable strategy to identify those embryos with the best chances of success.

Study design, size, duration: This unicentric and retrospective study included 81 patients seeking PGS-cycles with autologous oocytes and 163 with donated eggs between September 2013 and July 2016. The control group (*PGS-only*) comprised cycles in which embryos had been selected for transfer following euploidy criteria only. The study group (*PGS+Eeva*) comprised cycles in which embryo selection for transfer was based on combined PGS and Eeva predictions.

Participants/materials, setting, methods: All embryos were cultured, biopsied, and vitrified at the blastocyst stage. Genetic analyses of trophectoderm biopsies were performed by NGS. Single euploid blastocyst transfers were performed in all cases. Transfers were differed and under HRT. Within the control *PGS-only* group, the best morphological euploid embryo available was transferred. Within the *PGS+Eeva* group, the euploid blastocyst with the highest Eeva-prediction available was prioritized for transfer. Ongoing pregnancy rates of study groups were statistically compared.

Main results and the role of chance: Clinical characteristics were comparable between groups. There were no significant differences in terms of MII, fertilized eggs, blastocyst rate, high quality blastocyst rate and euploidy rate between groups. However, significant differences ($p<0.05$) were found when comparing ongoing pregnancy rates of the *PGS-only* and *PGS+Eeva* groups independently in both autologous oocytes and egg donation cycles. Significantly higher pregnancy rate was achieved in the PGS+Eeva group in transfers were *high* Eeva prediction in addition to PGS were used as criteria for embryo selection compared to the PGS-only group after the transfer of the best morphological quality euploid embryo available in both autologous oocytes cycles (75% vs 35,1%, $p<0.05$) and egg-donation cycles (67.2% vs 50% $p<0.05$)]. These results show that Eeva Test provides valuable information for euploid embryo selection, significantly increasing the chances of pregnancy in cycles where chromosomally normal embryos predicted by Eeva as *high* are prioritized for transfer. An improvement by selecting *high*-Eeva-classified euploid embryos was also observed when only high morphological quality embryo transfers (AA, BB or BA or BB according to Gardner's criteria) were analysed. The TLI+PGS combined selection of embryos strategy rendered 2x pregnancy rates compared to the ones obtained by the selection of high morphological quality euploids.

Limitations, reasons for caution: The unicentric and retrospective design of this study may be a reason for caution. Further data regarding follow up outcomes must be included to confirm the predictive value of the analysed parameters.

Wider implications of the findings: This analysis reveals the ability of early cleavage time-lapse parameters to predict embryo implantation potential and suggests that normal chromosome status determination is not enough to guarantee a viable pregnancy. Other biomarkers such as automated time-lapse-based parameters can add valuable information to embryo selection strategies.

Trial registration number: A trial registration number was not required due to the retrospective study design.

P-645 'Batching' of multiple IVF cycles combined with preimplantation genetic testing for aneuploidy: how does the number of cycles undertaken affect outcomes?

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Study question: When undertaking several sequential IVF-PGS cycles, what outcomes are anticipated? Is there a threshold number of embryos beyond which additional cycles are of no benefit?

Summary answer: Having multiple IVF-PGS cycles significantly increases the likelihood that a euploid embryo will be identified. Undergoing two cycles may represent the most cost-effective strategy.

What is known already: Patients and clinics sometimes choose to batch embryos from several IVF cycles. This can potentially reduce the costs of genetic testing and increase the likelihood of finding a euploid embryo for transfer. The strategy also allows production of multiple embryos in a relatively short space of time, an important consideration if the patient is of declining fertility due to age. Batching of embryos has become a routine practice in many clinics. Nonetheless, it is not clear how many cycles are optimal for maximising the desired outcome of identifying a euploid embryo, leading to transfer, and ultimately a healthy live birth.

Study design, size, duration: We investigated the effect of female age, referring clinic, and IVF indications on embryo outcomes in patients who have undergone at least two IVF cycles in combination with preimplantation genetic testing for aneuploidy (PGT-A) via next generation sequencing (NGS). A comparative analysis of cycles was carried out in order to determine the proportion of euploid embryos, aneuploidy, and mosaicism rate present in trophectoderm biopsies. Patients were referred by 8 European IVF clinics.

Participants/materials, setting, methods: In total, 635 embryos generated by 61 patients in 141 cycles were analysed. These patients underwent 2 (n = 49), 3 (n = 6), 4 (n = 5), or 5 (n = 1) IVF/PGT-A treatments. The trophectoderm samples were subjected to comprehensive chromosome screening using NGS. Maternal age ranged from 29-47 years (mean 39.8) and patients' reasons for referral included advanced maternal age, recurrent implantation failure, and recurrent miscarriage, with the average of 2.7 months between the first and second IVF treatments.

Main results and the role of chance: As expected, aneuploidy significantly increased with advancing maternal age (P = 0.001). No euploid embryos were found in women aged 44 or over regardless of IVF cycle number (0/35 blastocysts in total). Female age had a strong influence on aneuploidy rate, but the incidence of mosaic abnormalities was not associated with age. Statistical assessment of the first two cycles (n = 61) suggested a significant increase in the proportion of euploid embryos in the second cycle (12.4% and 20.1%, in cycles 1 and 2, respectively, P = 0.03). This difference may be due, in part, to the production of greater numbers of embryos, perhaps a consequence of tweaks to the stimulation protocol during the second cycle. In the 40 patients with no euploid embryos in cycle 1, 50% had at least one euploid embryo in cycle 2. Of the 21 patients who had at least one euploid embryo in the first cycle, 13 produced at least one additional euploid embryo in cycle 2 (4 patients produced two euploid embryos, 2 patients produced three and 1 patient produced four euploid embryos). No association was observed between the proportion of euploid embryos and reason for referral or the clinic treating the patient.

Limitations, reasons for caution: This study looked at the incidence of aneuploidy and the likelihood of identifying euploid embryos after sequential IVF cycles. However, clinical outcomes of PGS, including pregnancy rates per cycle, implantation and miscarriage rates, were not considered. Those outcomes should ideally be evaluated as part of a randomised control trial.

Wider implications of the findings: While all cycles contribute to the probability of finding a chromosomally normal embryo, most patients who will ultimately produce a euploid blastocyst have done so after the two cycles. The findings have potential implications for optimisation and management of batching procedures and consideration of costs versus benefit of such strategies.

Trial registration number: not applicable.

P-646 Comparing rates of blastomere and trophectoderm cells biopsies for Preimplantation Genetic Diagnosis using the array-CGH technique

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Study question: Purpose of this study was to compare rates after blastomere and trophectoderm biopsy using the same (array-CGH) technology, which has reliability, precision and permits determination of all chromosomes numerical abnormalities.

Summary answer: Both types of biopsies showed similar high pregnancy rates, as well as high ongoing pregnancy rates between the two groups.

What is known already: Preimplantation genetic screening (PGS) is a method to detect aneuploidies in preimplantation embryos. Those embryos diagnosed as chromosomally normal embryos will be candidates for transfer to the maternal uterus. The technique most commonly used for PGS is array-comparative genomic hybridization (CGH) Currently, blastomere and trophectoderm biopsies are the two main approaches used for PGS, but there is a trend towards trophectoderm biopsies, because the biopsy of trophectoderm cells would not adversely affect embryo development.

Study design, size, duration: A retrospective study was performed at a private clinic (Iakentro, Thessaloniki, Greece) during a period of 3 years (January 2014 to December 2016), conducted to analyze 479 embryos of 95 infertile couples. Preimplantation genetic screening (PGS) was performed using array-CGH. Embryo biopsy was performed either on day 3 or day 5. Only whole chromosome aneuploidies were considered.

Participants/materials, setting, methods: A mean number of 10-12 oocytes were fertilized by partner's sperm. Embryos were cultured in single step culture medium (Sage, 1-step) and the blastocysts developed on day5. Embryo biopsy was performed either on day 3 (44 patients, mean female age of 34.6) or day 5 (51 patients, mean female age of 32.9).All patients included in the study signed a consent form that includes all the possible pitfalls that may happen in PGS analysis

Main results and the role of chance: Pregnancy rate (40.9% vs 41.2%) and ongoing pregnancy rate (77.7% vs 80.9%) were similar in two groups. The PGS result was confirmed in the whole blastocyst in (a) 53/233 (22.7 %) normal embryos after day-3 biopsy and (b) 84/246 (34.1 %) normal embryos after trophectoderm biopsy. Concordance rates for both biopsy strategies and for individual chromosomes were evaluated by Fisher's exact test and showed no significant differences.

Limitations, reasons for caution: This study includes couples having repetitive implantation failures (RIF). The results of this study should not be extrapolated to any other group.

Wider implications of the findings: According to the results shown, both types of embryo biopsy are suitable approaches that can be used if required in a PGS program. Therefore, both types of embryo biopsies can coexist in the same PGS program and the patient's treatment can be customized according to the individual patient's requirements.

Trial registration number: The study was approved by the Institutional Review Board (Ref. no. 3/2015, granted 7 January 2015).

P-647 Unraveling the role of seminal fluid exosomes within the male reproductive tract

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Study question: To identify exosomes in the male reproductive tract and suggest putative signaling mechanisms in human ejaculates, epididymal and testicular tissue.

Summary answer: Exosomal vesicles are produced at different levels in the male genital tract and appear to control the seminiferous tubules' spermatogenic function.

What is known already: Exosomes are small membrane vesicles containing functional biomolecules such as proteins, lipids, RNA and DNA. They are shed from most cells and are present in various body fluids. Although they have been previously identified in seminal fluid, their precise source or role in the male reproductive tract remains puzzling. It is known that exosomes can mediate

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immunomodulatory influences on recipient cells and may indeed exert an effect on the female reproductive organs during the conception and implantation period. Exosomes may also play a role in the communication between Sertoli cells among seminiferous tubules and be responsible for modulating spermatogenic waves.

Study design, size, duration: From January to December 2016, 85 consenting men screened for infertility donated their 99 ejaculated, 2 testicular and 1 epididymal tissue specimens. Epididymal and testicular tissue specimens were surgically retrieved from men with obstructive azoospermia (OA) or non-obstructive azoospermia (NOA). Sperm samples were centrifuged 500x*g* for 10 min followed by 3,000x*g* for 20 min on the supernatant to remove cell debris. The supernatant was preserved at 4° C until exosome isolation.

Participants/materials, setting, methods: Seminal fluid was centrifuged at 12,000x*g* for 20 min to ensure removal of residual debris. The resulting supernatant was centrifuged at 100,000x*g* for 70 min for exosomal isolation. Exosomes were washed in 3 ml PBS and pelleted again by ultracentrifugation. The final exosome pellet was re-suspended in PBS and protein concentration was measured by BCA. The NanoSight LM10 nanoparticle analysis system was used for characterization of exosomes.

Main results and the role of chance: A total of 99 semen samples in 74 men had the following semen parameters: 69.6 ± 22 x 10^6/mL (concentration) and 46.1 ± 5% (motility). The number of seminal fluid exosomes isolated was 4.1 ± 3 x 10^8/ μL, with a mean size of 171 ± 16 nm in the samples that went through NanoSight analysis. The exosome protein content of the 100 μL seminal fluid sample was 96.8 ± 119 μg. The exosome protein content in the ejaculate of men showed a negative correlation of exosome protein per spermatozoon (R^2 0.14; $P < 0.001$) and exosome protein per motile spermatozoon (R^2 0.13; $P < 0.001$) in relation to the sperm concentration in the initial ejaculate. However, the exosome protein content in the ejaculate of oligoasthenozoospermic men (101.9 μg per 100 μL) was comparable to normozoospermic men (111.9 μg per 100 μL). Interestingly, men with OA due to vasectomy retained some exosome protein content, while men with congenital absence of the vas deferens (CBAVD) did not. Exosomal protein content in epididymal tissue of men with OA was 1.29 μg/100 μL and in testicular tissue of men with NOA was 25.0 μg/100 μL, possibly representing a proportional sourcing of exosomes from the different levels of the male genital tract.

Limitations, reasons for caution: The proportional contribution of exosomes in the testis, epididymis and seminal fluid in the same individual was not assessed. The current study only included men undergoing infertility screening. Thus, whether the profile in exosomal number, size, and protein content is comparable to normozoospermic men with proven fertility remains unanswered.

Wider implications of the findings: Analysis of seminal fluid exosomes may elucidate their role in immunomodulation within the genital tract during the peri-conception period. Exosomes may be involved in ordaining waves of spermatogenesis within the seminiferous tubule network. Exosomes are differentially produced at various levels of the male genital tract.

Trial registration number: Not applicable.

P-648 Identification of circular RNAs in human spermatozoa

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Study question: Are circRNAs (circRNAs) expressed in human spermatozoa?

Summary answer: We have identified a novel class of non-coding RNA, circRNAs that were expressed in human spermatozoa

What is known already: The RNA population carried by sperm is large, heterogeneous, and rich in non-coding RNAs (ncRNAs). However, for most of the thousands of transcripts carried by sperm, functions are completely unknown. Recently, a novel class of ncRNAs called circRNAs have been described, as produced in eukaryotic cells during post-transcriptional processes. They are ~100 nucleotide-long single-stranded RNA molecules forming a circle through covalent binding. A large number of circRNAs has been identified but their function awaits further investigations

Study design, size, duration: Spermatozoa were isolated from the semen specimen of a healthy fertile man by centrifugation and swim-up method. RNA extraction was carried out from 80 × 10^6 cells

Participants/materials, setting, methods: Total RNA was isolated using a spin column-based commercial kit. cDNA libraries were generated according to the Illumina TruSeq RNA-seq protocol and sequenced on Illumina HiSeq 2000 with 100 bp paired end reads. Only circRNAs with two or more supporting reads within single samples were kept. Furthermore, Real-Time PCR was performed to confirm results obtained, using primers designed as convergent primers to detect circular junctions, and to cross the backsplice junction

Main results and the role of chance: We performed a RNA-seq experiment on a normal semen sample (WHO 2010) from healthy, fertile patient. We mapped all the RNA-seq reads to the genome using Bowtie in single-end mode, allowing ≤2 mismatches. We extracted back-spliced ordering reads from the reads unmapped to the HG19 reference genome and ciRcus was used for circRNAs annotation and analysis. Three thousand possible circRNAs were predicted by at least two supporting reads. This represents a surprising number compared with those obtained for other types of cells and tissues. Based on the potential function in the reproductive process, we considered four of the most conserved circRNAs circ-nr2f1, circ-gigyf2, circ-insig1, circ-rmst, for the subsequent validation. We validated the authenticity of the identified circRNAs by two independent methods. Firstly, compared with linear RNAs, circRNAs are endowed with a strong resistance to exonuclease RNAse R. Thus, we quantified the RNAse R resistance of these candidate circRNAs and all of them showed > 5 times higher stability than the linear transcripts following RNase R treatment. Secondly, Real-Time PCR was performed with divergent primers detecting the circRNA junction and linear isoforms. We found that expression patterns of the four circRNAs considered were consistent with the RNA-seq data

Limitations, reasons for caution: This study must be confirmed in a larger cohort of patients

Wider implications of the findings: The nucleus of mature spermatozoa contains a complex population of RNAs despite its transcriptionally inert state that are thought to contribute extra-genomically to early embryonic development. The circRNAs seem to be the best candidates for a potential role of spermatozoal RNA content in embryogenesis activation

Trial registration number: N/A.

P-649 Effect of a severe male factor condition on chromosome segregation in spermatozoa from reciprocal translocation carriers

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Study question: Does the frequency of unbalanced segregation modes in spermatozoa from reciprocal translocation carriers change with the severity of the male factor condition?

Summary answer: There is no correlation between the occurrence of specific segregation modes and sperm indices.

What is known already: Carriers of reciprocal translocations have an increased risk of pregnancy loss or transmitting chromosomal abnormalities to their offspring because of the production of a higher number of unbalanced spermatozoa. They mainly result from two events: the unbalanced segregation during meiosis of the chromosomes involved in the translocation and the occurrence of additional aneuploidy events.

Study design, size, duration: From 2005, 45 sperm samples from carriers of reciprocal translocation were analyzed by Fluorescence In Situ Hybridization (FISH) to investigate the mode of segregation for the chromosomes involved in the translocation (2:2 alternate, 2:2 adjacent-I, 2:2 adjacent-II, 3:1 and 4:0). The distribution of frequencies of the segregation patterns was evaluated using the Kolmogorov-Smirnov test. In 29 samples, aneuploidy for 9 chromosomes was also tested.

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Participants/materials, setting, methods: When classified according top WHO criteria, 14 samples were normozoospermic, and 31 were oligoasthenoteratozoospermic (19 moderate-OAT, ≥5 million spermatozoa/ml and 12 were severe-OAT, < 5 million spermatozoa/ml). A combination of specific probes was used to determine the segregation mode in each rearrangement. In 29 carriers, multicolour FISH with probes specific for X, Y, 13, 15, 16, 17, 18, 21 and 22 was used to estimate the incidence of aneuploidy for chromosomes not involved in the translocation.

Main results and the role of chance: The frequency of unbalanced gametes was comparable in the two groups (63.6% ± 19.4 in normozoospermic and 58.9% ± 21.9 in OAT). Frequencies and dispersion of the five segregation modes were respectively 36.7% ± 19.4, 29.4% ± 15.9, 9.3% ± 7.9, 24% ± 12.8, 0.6% ± 1.0 in normozoospermic and 41.1% ± 21.9, 21.9% ± 14.3, 12.9% ± 13.1, 23.1% ± 33.2, 1.0% ± 1.5 in OAT.

In both patients' groups, the frequency of 2:2 alternate was significant higher than 2:2 adjacent-II ($P < 0.001$), and the frequency of 2:2 adjacent-I was significant higher than 2:2 adjacent-II ($P < 0.02$). The 2:2 adjacent-I pattern occurred at the same frequency than the 3:1, whereas the 2:2 adjacent-II was less frequent than the 3:1 ($P < 0.03$). The 4:0 segregation mode was by far the most rare segregation mode ($P < 0.001$).

In the OAT group, additional differences were found with the 2:2 alternate segregation occurring more frequently than the 2:2 adjacent-I ($P = 0.0022$) and 3:1 ($P = 0.014$).

Regarding aneuploidy events for the chromosomes not involved in the translocation, the frequency of aneuploidy in OAT patients (5.7% ± 4.0) was significantly higher when compared to normozoospermic patients (2.7% ± 1.6; $P = 0.028$).

Limitations, reasons for caution: This study included a restricted number of samples. Additional data would be necessary to corroborate our findings.

Wider implications of the findings: In translocation carriers, the frequency of unbalanced gametes is not affected by sperm indices, although segregation patterns may have different frequencies. As a male factor condition is associated with a higher incidence of aneuploidy, semen parameters in translocation carriers help estimating the global risk of forming chromosomally abnormal spermatozoa.

Trial registration number: none.

P-650 Analysis of PGD Uptake after Expanded Carrier Screening: Attitudes and Reproductive Decisions of High-Risk Couples

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Study question: For couples who were identified as "high-risk" through expanded carrier screening (ECS), what was the uptake of PGD and what factors influenced this decision?

Summary answer: 40% of "high-risk" couples pursued preimplantation genetic diagnosis (PGD); of those who did not pursue PGD, cost was the most influential factor.

What is known already: ECS assesses carrier status and reproductive risk for monogenic autosomal recessive and X-linked diseases. Couples in which both partners carry the same autosomal recessive disease, or couples in which the female partner carries an X-linked disease, are considered to be at high reproductive risk. 2.20% of couples who undergo ECS are identified as high-risk. ECS can afford high-risk individuals additional reproductive options, including the option to pursue preimplantation genetic diagnosis (PGD). While significant research has been conducted on PGD technologies, the patient-facing side of PGD, including the decision-making process and the emotional impact, remains largely unexplored.

Study design, size, duration: This retrospective study was conducted via an online survey. The survey contained questions regarding participants' family planning journey prior to ECS, their emotional reactions after ECS, their reproductive decisions after ECS, and the factors which drove those decisions. Patients identified as high-risk through the carrier screening laboratory within the last four years (2012-2016) were eligible for participation. A total of 127 participant responses were collected and included in analysis.

Participants/materials, setting, methods: The study population consisted of patients identified as high-risk through ECS. Eligible couples were contacted via phone and email to alert them of the study and invite their participation. Those wishing to participate completed an online survey. Responses were analyzed and statistical analyses were performed. Informed consent was obtained.

Main results and the role of chance: 40.4% of couples identified as high-risk through ECS pursued PGD, which demonstrates the significant clinical impact of ECS on high-risk couples' reproductive decisions. Of those who did not pursue PGD, 48.5% reported pursuing prenatal testing such as amniocentesis or CVS— procedures which carry a risk of miscarriage and may not be indicated had carrier status not been known, further speaking to the impact of ECS on high-risk couples' approach to clinical care. Cost was cited as the most influential factor in the decision not to pursue PGD; as cost declines, PGD may become an increasingly popular option. Additionally, analysis showed that 78.9% of respondents reported sometimes or often feeling relieved they had ECS. 81.4% of respondents reported never or rarely having difficulty making reproductive decisions after ECS.

Limitations, reasons for caution: The majority of patients included in this analysis reported having a high level of education (94.4% with a university degree or higher), and 80.6% of respondents reported an income of greater than $100,000. Uptake and awareness of PGD may be lower among patients from a different demographic.

Wider implications of the findings: Patients expressed satisfaction and relief after undergoing ECS. As ECS becomes an increasingly integral component of reproductive medicine, we should continue to investigate its utility and consider recommendations for ECS as routine care within and beyond the fertility clinic. Genetic counselors can play an important role in providing these services.

Trial registration number: NA.

P-651 Investigating the role of sperm-specific RNA to screen men with unexplained infertility

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Study question: To identify the compartmentalization of candidate gene clusters within the human spermatozoon that contribute to spermatozoal fertilization capacity and embryo developmental competence.

Summary answer: Functional assessment of gene clusters localized in various components of the spermatozoon may identify concealed reasons of unexplained infertility in men with normal semen parameters.

What is known already: Semen analyses are routinely used to investigate male infertility. However, they rarely predict spermatozoal function or reproductive outcomes. Men with seemingly normal semen parameters can therefore be infertile, suggesting the presence of an otherwise unknown or concealed genetic male factor. Querying spermatozoal RNAs has emerged as a novel technique, which enables identification and classification of sperm-specific RNAs. By quantifying vital RNA transcripts within the components of the spermatozoon, we can assess their contribution to male gamete competence, as well as the delivery of such RNA transcripts to the oocyte and subsequent embryonic development.

Study design, size, duration: In this prospective study, 31 consenting men screened for infertility donated their ejaculated semen samples during a 15-month period. Three men with proven fertility (natural conception) served as controls. Following RNA extraction from semen samples, differentially expressed RNA transcripts were identified and classified into gene clusters based on sperm-specific components i.e., the acrosome, nucleus, mid-piece, and flagellum. The expression of these transcripts were compared with standard semen parameters, and between the study and control groups.

Participants/materials, setting, methods: RNA was isolated from $25x10^6$ human spermatozoa using a commercially available spin column kit. Spermatozoal

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RNA concentration was subsequently assessed. Illumina stranded RNA sequencing (RNA-Seq) library preparation was used to construct paired-end libraries. Pilot sequencing was expanded to 50-60 M reads at 2x76 bp. RNA expression was calculated in fragments per kilobase of transcript per million mapped reads (FPKM). Functional assessment and classification of genes was performed via the Database for Annotation, Visualization and Integrated Discovery (DAVID).

Main results and the role of chance: Thirty-one men with a mean age of 39.6 ± 5 years had the following semen parameters: 46.3 ± 19 x 10^6/mL concentration and 44.8 ± 14% motility. The age of the female partner in the study and control groups was comparable. None of the couples in the study population conceived naturally or with intrauterine insemination. The expression of *APLF*, *CYUB5R4*, *ERCC4,* and *TNFRSF21* was found to be higher in men <40 years (47.2 ± 15 FPKM) compared to those >40 years (13 ± 9 FPKM; $P = 0.02$). On plotting the RNA transcript expression against semen parameters, a positive correlation between *AKAP4* and motility was noted (R^2 0.40; $P = 0.01$). Differentially expressed RNA transcripts between the study and control groups were identified and grouped into gene clusters based on the following sperm-specific components: *DPY19L2* and *ATP6V1E2* (acrosomal); *APLF*, *CYUB5R4*, *ERCC4*, *MORC1*, *PIWIL1*, *TNFRSF21*, *TSSK6* and *H1FNT* (nucleus); *ADCY10*, *SMCP*, *PLK4* and *AGPAT2* (mid-piece); and, *AKAP4* and *CATSPER1* (flagellum). There was a strong correlation between the expression of *ATP6V1E2* and fertilization rates (R^2 0.63; $P < 0.001$) in men who underwent ICSI. The gene cluster localized to the nucleus, which is known to regulate DNA repair mechanisms throughout spermatogenesis, was significantly under-expressed in the study group when compared to the control group ($P < 0.001$).

Limitations, reasons for caution: While these preliminary results are encouraging, prospective data in a larger cohort are required to elucidate the epigenetic mechanisms involved in male infertility and male gamete competence. Differential expression of RNA transcripts should be confirmed in more than a single ejaculated semen sample from each participant in the study group.

Wider implications of the findings: Functional assessment of the gene cluster specific to certain compartments of the human spermatozoon may supplement standard semen analysis and serve as a vital tool in the diagnosis of unexplained infertility. This may further facilitate tailoring of assisted fertility treatments, thereby reducing the time to pregnancy.

Trial registration number: Not Applicable.

P-652 The Impact of Genetic Ethnicity on Clinical Practice: A Comparison of American and European Fertility Patients

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Study question: Do we see differences in the genetically-predicted ethnicities of patients from European fertility clinics as compared to patients from United States (US) clinics?

Summary answer: We found that the genetically predicted ancestries of European and American fertility patients did not significantly differ, which may impact carrier screening recommendations.

What is known already: Genetic carrier screening is routinely offered to patients in US fertility clinics. While offerings were initially based upon patients' reported ethnicities, the recognition of increased genetic admixture and technological advances caused a shift to broader acceptance of expanded carrier screening (ECS) panels. Such panels are pan-ethnic and screen for over 200 diseases. ECS has not been widely accepted across Europe. The resistance is partially due to a belief that the US is genetically diverse from years of admixture while the European population is genetically homogeneous, making pan-ethnic screening unnecessary. We sought to determine if a difference in admixture was present.

Study design, size, duration: Our retrospective analysis examined genomic data from an ECS panel run on more than 10,000 patients over the past three years. Documented informed consent was obtained to use genotype information in a de-identified manner. Data was analyzed from a total of 7544 participants, 6297 from US clinics and 1247 from European clinics. A previously-validated genetic ancestry admixture analysis was run on all samples.

Participants/materials, setting, methods: Carrier screening for all samples was performed on the Illumina Infinium HD Custom Genotyping platform. Genetic ancestral origin was predicted by a statistical model based on 672 SNPs validated using samples from the 1000 Genomes Project, and admixture proportions were calculated for 6 ancestral populations (European, Oceania Native, Native American, East Asian, Sub-Saharan African, and South Asian). A comparison of predicted admixture proportions was made between patients in Europe against patients in the US.

Main results and the role of chance: For comparison, the European and American patients were further subdivided into four groups, based upon which ethnicity they self-reported on test requisition forms: European, Mediterranean, Latin American, or African. Across all four comparison groups, our results showed similar average admixture proportions. For example, European patients who self-identified as Mediterranean were genetically predicted to be an average of 86% European, and 5% Sub-Saharan African. American patients who self-identified as Mediterranean were genetically predicted to be an average of 81% European and also 5% Sub-Saharan African. European patients who self-identified as African were predicted to be an average of 78% African and 16% European, which was strikingly similar to the predictions for American patients who self-identified as African (78% African and 12% European). This similarity in admixture proportions was also seen between European and American patients who self-identified as European and Latin American. These results demonstrate that on a genetic level, both European and American patients demonstrate equal degrees of admixture. The European patient population may not be as genetically homogenous as previously believed. Pan-ethnic carrier screening panels could prove to be effective at identifying carriers in a European population, who would otherwise be missed.

Limitations, reasons for caution: Future studies with larger patient populations may strengthen the above findings. Additionally, more in depth details regarding ancestral origin could be obtained from comparison between a higher number of SNPs.

Wider implications of the findings: Our findings indicate that both European and American patients who identify as one specific ethnicity experience equal amounts of genetic admixture. Thus, pan-ethnic expanded carrier screening among reproductive patients may be more beneficial to European patients than previously believed.

Trial registration number: NA.

P-653 Mitochondrial DNA (mDNA) detection from blastocoelic fluid: a potential indication of blastocyst viability

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Study question: Could the blastocoelic fluid represent a source of embryonic mtDNA?

Summary answer: mtDNA can be isolated from blastocoelic fluid and the D-loop region can be sequenced

What is known already: The mtDNA copy number in blastocysts, as assessed by trophectoderm (TE) biopsy, has been associated with the potentiality of the embryo to implant. Several studies have shown that embryos with a content of mtDNA above a certain cut-off level rarely give rise to a term pregnancy. Other scientific works have demonstrated the presence of DNA in the blastocoelic fluid (BF). This DNA can be analyzed to provide information on the genetic status of the corresponding blastocyst

Study design, size, duration: To assess the presence of mtDNA in BF samples, qualitative analysis of mtDNA was performed on 10 BFs from expanded blastocysts on which aCGH analysis had already been completed on TE cells in our PGS program

Participants/materials, setting, methods: BFs was extracted by 10 aneuploid blastocysts from 10 couples with an age between 37 and 41 years. Patients had previously signed an informed consent to this additional procedure. The samples were amplified by WGA to test aneuploidy on BFs. An aliquot

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of the amplified product was processed to amplify the D-loop region by an operator blind to source of DNA

Main results and the role of chance: mtDNA was found in all tested BF with amplification bands specific for the tested region of the D-loop. Following sequencing, the prediction of the haplogroup was possible in all samples (mean probability of 84.35 ± 0.18%, range 50-100%)

Limitations, reasons for caution: This is a preliminary approach with a small number of samples to verify the presence of mtDNA in the BF. No quantitative analysis was performed, and only non-transferred embryos were tested

Wider implications of the findings: Blastocentesis can be a non-invasive method to evaluate not only the chromosome condition of the blastocyst, but also its competence. After proving the presence of mtDNA in the BF, we are now performing a quantitative analysis that would allow us to establish a possible correlation with implantation

Trial registration number: Not applicable.

P-654 Identification of High-Risk Carrier Couples With Limited versus Expanded Carrier Screening Panels

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Study question: Our aim was to determine the effectiveness of expanded carrier screening panels in a clinical setting.

Summary answer: Testing with only a limited panel of conditions missed 22% of carriers of high-impact conditions.

What is known already: Recent statements by United States professional societies have provided guidance on appropriate approaches to the clinical implementation of expanded carrier screening panels. However, formal recommendations from the same professional societies remain limited to a select few conditions based upon the patient's reported ethnic background. With such a large difference between guidelines and formal recommendations, and an increased awareness of genetic admixture across the globe, we sought to examine the effectiveness of expanded carrier screening to capture high-risk carrier couples by comparing the percentage of carriers identified on 3 panels of increasing scope.

Study design, size, duration: The retrospective analysis included data from more than 10000 patients who underwent carrier screening within the past five years. Carrier counts were tallied for each disease screened, and overall number of carriers was analyzed for 3 disease panels of increasing scope: (1) diseases currently recommended for screening by professional society guidelines; (2) diseases considered to be high-impact for reproductive decision making; and (3) a broad panel of 200+ diseases that range in severity.

Participants/materials, setting, methods: Documented informed consent was obtained to use de-identified genetic data from expanded carrier screening tests. Individuals were screened for over 200 recessive genetic diseases using Illumina's Infinium HD Genotyping Platform.

Main results and the role of chance: On the most limiting panel, which only screened for diseases currently included in professional society guidelines, 12% of patients were identified as carriers. When compared to a larger panel inclusive of 188 high impact diseases, our results indicated that limited screening failed to identify 22% of carriers in our patient population. An additional 10% of patients were identified as carriers when the panel was expanded further to include a total of over 200 diseases of varying impact. Expanded carrier panels are more effective at identifying patients at risk of having children with genetic conditions who thus may benefit from increased reproductive options.

Limitations, reasons for caution: Analysis among a larger population of patients, or with a greater number of SNPs for ethnic admixture analysis, may reveal different degrees of admixture than reported here; however, such differences are not expected to be significant.

Wider implications of the findings: Data from our ethnically diverse patient population demonstrate that screening for only traditionally recommended diseases failed to identify over a quarter of carriers. Such oversight might contribute to decreased detection of carrier couples, and thus the inability for patients to make fully informed reproductive decisions.

Trial registration number: NA.

P-655 DNA methylation at imprinted genes varies with infertility diagnosis in infertile men

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Study question: Are there DNA methylation abnormalities in different diagnoses of infertile men and are these abnormalities similar?

Summary answer: There are DNA methylation errors at imprinted genes in men diagnosed with oligozoospermia. Errors were not found in more severe forms of infertility (azoospermia).

What is known already: Previous studies have shown that men with oligozoospermia may show more DNA methylation errors at imprinted genes.

Study design, size, duration: Our case control study includes a total of 121 patients, which includes 20 fertile control men and 21 vasectomy reversal men to be used as controls for azoospermic men. The remaining 80 patients were divided into four categories based on infertility diagnosis: 24 with oligozoospermia (5-15 million sperm/ml), 33 with severe oligozoospermia (< 5 million sperm/ml), 15 with obstructive azoospermia, and 8 with non-obstructive azoospermia.

Participants/materials, setting, methods: We examined the methylation status of four imprinted genes (*H19*, *IG-GTL2*, *MEST,* and *LIT1*) and globally in the sperm of reproductive age men using bisulfite pyrosequencing. DNA fragmentation and *MTHFR* genotype were also examined.

Main results and the role of chance: For oligozoospermic men, DNA methylation was significantly different in the *MEST* gene compared to fertile control men ($P = 0.00046$). Similarly, for severe oligozoospermic men, DNA methylation was found to be significantly different in the *H19*, *IG-GTL2*, and *MEST* genes compared to fertile control men ($P = 0.0036$, $P = 0.037$, $P = 0.000086$; respectively). DNA fragmentation was also found to be significantly different in severe oligozoospermic men compared to fertile control men ($P = 0.0041$). Differences in *MTHFR* genotype were not found to be significantly different.

Limitations, reasons for caution: The number of patients in some subcategories may not be sufficient. There may be potentially significant findings in the other comparisons if the sample sizes were similar.

Wider implications of the findings: Our results provide further evidence of methylation defects at imprinted genes in the sperm of infertile men.

Trial registration number: Not applicable.

P-656 Importance of the Aging Male Gamete on ART Outcome

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Study question: To assess the effects of paternal age on the occurrence of sperm chromosomal aneuploidy and its relevance in pregnancy outcome.

Summary answer: Advancing paternal age affects spermatogenic meiosis of most autosomes, affecting the ability of the male gamete to fertilize and generate competent embryos.

What is known already: As we recognize the relevance of maternal age, the effect of aging on the male gamete has only recently been recognized. Aging men have a higher concentration of spermatozoa with fragmented chromatin and may be prone to meiotic error capable of affecting the conceptus' health.

Study design, size, duration: Cytogenetic analysis for chromosome X, Y, 13, 15, 16, 17, 18, 21, and 22 was performed on human spermatozoa from consenting men whose partners underwent at least one ICSI cycle. These men were categorized into one of 7 age ranges, and the rates of disomy and diploidy were recorded. ICSI outcomes and aneuploidy rates for each age group were compared.

Participants/materials, setting, methods: Paternal ages were grouped according to the following ranges: 25-30, 31-35, 36-40, 41-45, 46-50, 51-55, and > 55. Semen samples were prepared and processed for sperm aneuploidy

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by 9 chromosome FISH. The diploidy and disomy occurrences were recorded, as well as ICSI fertilization rates and pregnancy characteristics.

Main results and the role of chance: FISH assay was carried out on semen samples from a total of 87 men. We found that the average aneuploidy rate was highest for men in the >55 age group (9.6%). When comparing autosomal disomy rates among all age groups, we found that men in the 25-30 age group had the highest average disomy for chromosome 21 (1.2%). However, men in the >55 age group had the highest average disomy specifically for chromosomes 17 (1.2%) and 18 (1.3%), in addition to highest overall aneuploidy. Individual gonosomal aneuploidy was also assessed, in which disomy YY was found to be highest in the 51-55 age group (0.9%), disomy XX highest in the 46-50 age group (0.3%), and disomy XY highest in the >55 age group once again (1.2%).

A total of 157 ICSI cycles were also assessed and grouped according to paternal age. The fertilization rate of 87.7% in the youngest age group decreased to 46.0% in the >55 age group. Similarly, the clinical pregnancy rate was highest in the 25-30 age group (80.0%), but absent in the >55 age group. Furthermore, the rate of pregnancy loss was characterized by a steadily increasing trend, highest in the 51-55 age group (50.0%).

Limitations, reasons for caution: As it may be expected but not undeniably documented, advancing paternal age affected spermatogenic meiosis almost exclusively non-disjunction. While we could not fully control for an eventual female factor, increasing spermatogenic meiotic errors in aging men adversely affect their ability to fertilize and achieve a successful implantation.

Wider implications of the findings: Paternal age contributes to the functional health of the male gamete. With respect to male aging and chromatin integrity, we confirmed aging affects meiosis and increases aneuploidy except for chromosome 21, reported most in younger men. Men aging through the effect on autosomal aneuploidy, affects the success of ART outcome.

Trial registration number: Not applicable.

P-657 Is more better? A higher oocyte yield is independently associated with more day-3 euploid embryos

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Study question: Is the number of oocytes retrieved (COCs) after ovarian stimulation for IVF independently associated with the number of day-3 euploid embryos?

Summary answer: A higher oocyte yield is independently associated with more day-3 euploid embryos, although the expected benefit decreases significantly with advancing age

What is known already: Ovarian stimulation represents a key step for the success of in-vitro fertilization cycles. Although, traditionally, ovarian stimulation aims at collecting more than one oocytes in order to increase the chance of pregnancy, there is evidence suggesting that excessive ovarian response leads to lower live birth rates. Furthermore, there is evidence suggesting that mild stimulation is associated with embryos of higher reproductive potential. Whether indeed a higher oocyte yield after ovarian stimulation is associated with the genetic composition of the resulting embryos and therefore with their reproductive potential is still largely unproven.

Study design, size, duration: This is a multi-centered retrospective cohort study analysing all preimplantation genetic screening (PGS) cycles using day 3-biopsy and array-CGH (n = 724) between March 2011 and December 2016 in 3 laboratories. Overall, 464 patients underwent 724 cycles of PGS for advanced maternal age (n = 264), recurrent miscarriage (n = 137), recurrent implantation failure (n = 235), history of chromosomally abnormal children (n = 44) and patient request (n = 44). Cycles performed for genetic reasons (monogenic disease, parental chromosomal abnormality and gender selection) were excluded.

Participants/materials, setting, methods: The primary outcome measure was the number of euploid embryos (EE) on day-3. Cycles with no embryo available for biopsy on day-3 were classified as having 0 euploid embryos. Statistical analysis was performed using the generalized estimating equations framework and multivariate regression models to control for the clustering nature of the data while adjusting for potential confounders. Uncertainty around estimated effect sizes was expressed with either p-values or 95% CI.

Main results and the role of chance: The mean age of patients was 38.8 (38.5-39.2) years and the BMI was 23.7 (23.2-24.2) kg/m^2. The mean number of COCs was 11.7 (11.1-12.2) and that of EE was 1.01 (0.88-1.14).

The univariate analysis identified a significant negative association of EE with female age ($p < 0.001$), starting ($p<0.001$) and total dose of FSH ($p<0.001$) and duration of stimulation ($p = 0.018$). The number of COCs exhibited a positive association with EE (coeff:+0.10, 0.08-0.13). Also, the indication for PGS was significantly associated with EE, with advanced age leading to significantly less EE than all other indications ($p<0.001$). No association was present between EE and BMI ($p = 0.691$).

A multivariate regression GEE model including all the aforementioned variables as covariates as well as an interaction term between female age and number of oocytes revealed that the number of oocytes retrieved was still positively associated with EE (coeff:+0.40, 0.24-0.56). The interaction term was also highly significant (coeff:-0.01, $p<0.001$) indicating an effect modifying role of female age on the association of oocytes retrieved with EE. To produce one and two euploid embryos, 5 and 14 oocytes, respectively, are required at age 34, while 10 and 24 oocytes, respectively, are required at age 38.

Limitations, reasons for caution: This study is retrospective and although every effort has been made to control for potential confounders, residual unknown bias cannot be excluded. Furthermore, the population analyzed in this study might not be completely representative of the general population undergoing IVF.

Wider implications of the findings: These results provide an explanatory mechanism for the recently published positive association between the number of COCs and cumulative pregnancy. Also, they allow for a better understanding of the association between the number of COCs and EE in different age groups and can help clinicians optimise their ovarian stimulation strategy.

Trial registration number: not applicable.

P-658 Alterations in endocrine and paracrine regulation of oocyte maturation and folliculogenesis underlie the etiology of primary ovarian insufficiency

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Study question: We aimed to uncover the biological functions most often implicated in primary ovarian insufficiency (POI) through a systematic literature review and statistical validation analysis.

Summary answer: Genetic variants with the strongest effect-size across published studies for POI are implicated in endocrine and paracrine regulation of oocyte maturation and folliculogenesis.

What is known already: POI is thought to be a heterogeneous condition characterized by cessation of ovarian function before the age of 40. By the time of diagnosis, gonadotrophin levels in POI patients are close to the menopausal range, greatly limiting fertility treatment options. As with natural menopause, overt POI is preceded by a period of reduced fecundity coupled with reduced ovarian reserve. Although hundreds of studies have associated several genetic regions and biological processes with POI, a comprehensive literature review and statistical meta-analysis has not been performed across the entire body of work.

Study design, size, duration: Natural language processing algorithms were used to identify articles in NCBI PubMed pertaining to genetics and POI. Of the 3,259 articles identified algorithmically, 387 were found to be true positives after manual evaluation, which also uncovered articles cited in the references that were not identified algorithmically. Reported gene associations were ranked using the Clinical Genome Gene-Disease Clinical Validity Classification Framework. An in-house annotation database was used to assign a biological function to each gene.

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Participants/materials, setting, methods: Pathogenic variants residing in genes that met the criteria for "strong" evidence of clinical association with POI were statistically validated using random effects model meta-analyses using PRISMA guidelines. Variants were excluded from analysis if there were <2 published studies or overlapping cohorts, or if an article's presentation of information precluded the determination of the risk allele. Q value distribution was used to establish a false-discovery rate-adjusted p-value of 0.05.

Main results and the role of chance: Our analysis revealed that 14 genes currently satisfy ClinGen criteria for strong evidence of association with POI. These genes have well-established roles in hormone regulation, immune response regulation, steroidogenesis, ovarian follicle development, tissue remodeling, cell proliferation/differentiation, and glucose homeostasis. 28 variants within these genes have been studied across 80 case–control experiments, however, only 3 variants (within 3 of the 14 genes) were found to be significantly associated with POI across studies ($p<0.05$). Two of the variants that survived statistical validation across studies lie within genes involved in paracrine regulation of oocyte maturation and folliculogenesis (odds ratio (OR)=4.40, $p = 0.015$ and OR=1.54, $p = 0.002$, respectively). A third variant that demonstrated statistical significance after meta-analysis lies within a gene related to endocrine regulation of these same processes (OR of 4.01, $p = 0.027$). Genetic variants related to other biological processes implicated in POI do not yet demonstrate adequate clinical evidence across multiple studies.

Limitations, reasons for caution: This study relied upon study-specific aggregate data, not individual patient data, which may introduce bias from heterogeneity inherent in the study cohorts. The random-effects models, however, do take into account heterogeneity as one function of defining statistical significance.

Wider implications of the findings: Our analysis reveals three genetic variants strongly associated with POI. These variants implicate endocrine and paracrine ovarian factors not routinely assayed for in traditional fertility workups. Clinical use of these genetic markers may enable early identification of women at high risk for diminished ovarian reserve and POI.

Trial registration number: Not applicable.

P-659 A multivariate genome-wide DNA methylation analysis of placental villi from assisted reproductive technology and naturally conceived pregnancies

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Study question: Are genes differentially DNA methylated in the placenta between assisted reproductive technology and naturally conceived pregnancies?

Summary answer: A multivariate linear regression model identified no genes with significantly altered DNA methylation in the placenta that is associated with mode of conception.

What is known already: ART newborns are at a higher risk of being born intrauterine growth restricted, small for gestational age, and preterm. They are also at a higher risk for imprinting disorders and later life chronic diseases including diabetes and cancer, yet the causes and mechanisms are yet to be determined. Multiple studies have previously identified DNA methylation differences in the placenta associated with newborns conceived via ART, including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), compared to NC newborns; however, these studies focused on few genes or are small in sample size with little clinical information about the cohort.

Study design, size, duration: Cross-sectional case-control study: 16 NC, 16 IVF, and 16 ICSI pregnancies. ART pregnancies were recruited from fertility clinics, while NC pregnancies were recruited from hospitals across Vancouver, Canada. Consent was obtained by physicians and nurses prior to sample collection. Ethics was approved by the University of British Columbia ethics committee.

Participants/materials, setting, methods: Placentas were stored at 4 C for up to 2 days. Villi samples were stored at -80 C until DNA extraction and bisulfite conversion. Bisulfite converted DNA was sent to The Centre of Applied Genomics (Toronto, Canada) for the application of the Illumina Golden Gate Cancer Panel I. Raw idat files containing signal intensities for 1505 probes per sample were imported and analyzed in R (3.3.2). Clinical information was extracted from pregnancy outcome forms and self-reported questionnaires.

Main results and the role of chance: A total of 49 probes were excluded due to poor signal detection. There was significant clustering by sex, therefore an addition 82 probes targeting the X chromosome were excluded. A single sample was removed due to clustering uniquely from other samples due to > 5% of its probes having poor detection signal; this sample was excluded. Principal Variance Component Analysis (PVCA) revealed that the only known covariate associated with the top 5 principal components was batch (3% of the total variance). There were no significant differences in maternal age, gestational age, sex, mode of delivery, birth weight, placental weight and dimensions, and Apgar scores between conception modes ($P > 0.05$, ANOVA/Kruskal-Wallis/Fisher's exact test). Incidence of twins was significantly higher among ICSI newborns (7 ICSI, 3 IVF, 0 NC; $P = 0.002$). Using an empirical Bayes method in a univariate linear model, we identified 26 significant (adjusted $P < 0.05$) CpG sites with altered DNA methylation between IVF and ICSI pregnancies, where 3 CpG sites had a > 15% difference in DNA methylation between ICSI and IVF (22.9% TUCS3, 18.5% HOXA5, -15.6% HOXA11). A multivariate model adjusting for the batch effect and twin status identified no significant DNA methylation alterations.

Limitations, reasons for caution: The microarray only targets a small portion of the methylome; more CpG sites will need to be investigated in conjunction with more complete clinical information. Further inclusion of ART newborns with adverse neonatal outcomes will be needed to produce more accurate predictive models.

Wider implications of the findings: Our results suggest that adverse neonatal outcomes among ART pregnancies may not be associated with DNA methylation alterations in the placenta. It is also possible that DNA methylation alterations may not be linearly related to these adverse outcomes, suggesting that future studies should focus on poor outcome cases.

Trial registration number: not applicable.

P-660 Differences in aneuploidy survival to first trimester of aneuploid embryos

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Study question: Do different chromosome trisomies detected at blastocyst stage reach first trimester at different rates?

Summary answer: Most aneuploidies do not reach first trimester clinical detection, and those that do, survive at very different rates.

What is known already: Different chromosome susceptibilities to aneuploidy were observed from clinically recognized pregnancies and from embryos and gametes in FISH studies. POC studies are limited to those aneuplodies surviving to first trimester, FISH studies of embryos were limited in sample size and number of chromosomes analyzed. Those studies showed that some chromosomes are either more susceptible to aneuploidy or that are more likely to survive to first trimester. With the advent of comprehensive chromosome screening techniques such as next generation sequencing (NGS) and much higher use of PGS in clinical practice, all chromosomes and large sample size of embryos are being analyzed.

Study design, size, duration: This is a retrospective study of 5,238 cycles from over 100 fertility centers from January 2016 to December 2016 are included in this study involving 26,915 embryos sequenced by Next Generation Sequencing (NGS).

Participants/materials, setting, methods: All blastocyst stage embryos were biopsied and the samples were processed according to Illumina protocol for NGS. Embryos were classified as aneuploid, polyploid, complex abnormal, or mosaic. Mosaic samples were those that contained the equivalent of 20-80% abnormal cells. For this study single trisomies and monosomy X rates at blastocyst stage were compared with published POC data to determine the frequency of blastocyst aneuplodies that reach first trimester

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Main results and the role of chance: The results are summarized in table 1:

Chromosome	Trisomy in blastocyst stage embryos (%)*	Aneuploidies in spontaneous abortions in the 1st trimester**	Embryo Loss (%)
XY tris	1.85%	0.43%	76.76%
XY mono	1.72%	1.46%	15.12%
1	2.00%	0.00%	100.00%
2	2.03%	0.19%	90.64%
3	1.87%	0.04%	97.86%
4	1.97%	0.11%	94.42%
5	2.01%	0.01%	99.50%
6	2.00%	0.02%	99.00%
7	1.93%	0.15%	92.23%
8	2.00%	0.13%	93.50%
9	2.56%	0.12%	95.31%
10	1.79%	0.06%	96.65%
11	2.10%	0.01%	99.52%
12	2.02%	0.03%	98.51%
13	2.49%	0.18%	92.77%
14	2.48%	0.14%	94.35%
15	3.51%	0.29%	91.74%
16	4.90%	1.24%	74.69%
17	1.73%	0.03%	98.27%
18	2.20%	0.20%	90.91%
19	2.21%	0.00%	100.00%
20	2.28%	0.10%	95.61%
21	3.62%	0.36%	90.06%
22	4.36%	0.38%	91.28%

Limitations, reasons for caution: It is unknown if blastocyst mosaicism detected by NGS will result in clinically recognized first trimester full trisomies or even mosaicism, and if so if they should have been included in the first column.

Wider implications of the findings: Most trisomies are lost at a 90% rate, but only a 75% is lost for chromosome 16, and 15% for monosomy X. Therefore, even though trisomy 22 is more common than trisomy 16 in blastocyst, it seems that trisomy 16 and monosomy X survives to first trimester in higher proportions.

Trial registration number: N/A.

P-661 Preimplantation Genetic Diagnosis (PGD) for Multiple Indications: Patient Outcomes

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Study question: Do patients who pursue PGD for multiple indications undergo more IVF cycles, banking cycles, and have fewer embryos to transfer?

Summary answer: There is an increase in number of embryos available for transfer when couples pursue PGD for multiple indications undergo banking cycles.

What is known already: PGD testing for single gene disorders is performed effectively with linkage analysis via Karyomapping (Illumina, USA), a single nucleotide polymorphism (SNP) array technology. Comprehensive chromosome screening (CCS) using array comparative genomic hybridization (aCGH) (24sure, Illumina) or next generation sequencing (NGS) (VeriSeq, Illumina) are validated technologies utilized in PGD testing for translocations. With a trophectoderm biopsy, PGD testing for multiple indications, such as multiple single gene disorders, may opt to include CCS.

Study design, size, duration: This study documents outcomes from 1153 patients between 2014 through 2016 and identified 15 patients who pursued PGD for multiple indications. These indications include both a translocation and a single gene disorder or at least two single gene disorders; both dominant, both recessive, both X-linked, one dominant and one X-linked, one dominant and one recessive, one X-linked and one recessive.

Participants/materials, setting, methods: Patients must have undergone PGD testing for multiple indications; including two ore more single gene conditions or a single gene condition and translocation. PGD testing for the single gene disorder(s) for these cases was performed via Karyomapping. International patients were excluded from this study.

Main results and the role of chance: From January 2014 through December 2016, PGD testing for single gene conditions was performed for 1153 patients. Of these, 32 patients expressed interested in pursuing PGD for multiple indications and 15 underwent at least 1 in vitro fertilization (IVF) cycle with subsequent PGD results. These patients account for 1.3% (15/1153) of those pursuing PGD for single gene disorders. Of these patients, 20% (3/15) reported an underlying fertility issue. These 15 patients underwent 27 IVF cycles, which yielded 21 PGD reports. The majority, 95% (20/21), of these PGD reports included CCS via aCGH or NGS. Additionally, 5/15 (33%) of patients banked biopsied samples to pursue another IVF cycle prior to PGD testing. The percent of embryos available for transfer per the total number of embryos tested was 13/115 (11%). As a comparison, patients pursuing PGD for one single gene condition had 2129/7058 (30%) of the embryos available for transfer; which is not statistically significant.

Limitations, reasons for caution: This population is a small sample size compared to the total number of patients who pursue PGD for single gene disorders. As such, it demonstrates a strong indication of the expectations for cycles from these patients, but more data is needed to assess statistical significance.

Wider implications of the findings: With the increase in expanded carrier screening and patient awareness regarding availability of PGD, there may be an increase in patients wishing to pursue PGD for multiple indications. As such, this patient population may benefit from banking while undergoing multiple IVF cycles in order to increase their success rate.

Trial registration number: not applicable.

P-662 Incidental identification of copy number variations (CNV) in patients during preimplantation genetic diagnosis (PGD) – clinical implications and counseling issues when encountering secondary findings

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Study question: What is the clinical significance and implications of microdeletions and microduplications which are incidentally identified during test preparation for PGD via Karyomapping (Illumina, USA)?

Summary answer: Incidental findings identified during PGD can have clinical significance; awareness and implementation of standard procedures for reporting such findings to physicians/patients is of great importance.

What is known already: Karyomapping is found to provide comprehensive assessment of the region of interest and it has been proven to be an effective method of PGD for single gene disorders. The methodology utilizes a single

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nucleotide polymorphism (SNP) array which includes ~300,000 SNPs spread across the entire human genome. This allows for an advanced, genome-wide view of samples assessed which, similarly to other advanced methodologies utilized in the field of genetics, increases the chances for detection of incidental findings. Incidental findings are characterized as additional findings identified during a protocol in which the test is not designed to detect.

Study design, size, duration: This study records the incidence and significance of incidental findings identified among 624 couples undergoing PGD for gene disorders using Karyomapping. The findings were identified and reported during a 19 month period from July 2015 to January 2017.

Participants/materials, setting, methods: All PGD cases were prepared through utilization of Karyomapping SNP arrays. DNA samples were obtained from couples and family members for test preparation purposes. During evaluation of sample quality, review of the SNP array profiles was completed and revealed 17 CNVs; both, microduplications and microdeletions. All incidental findings were reported to the physician and patient and recommendation for follow-up chromosomal microarray testing via an outside diagnostic laboratory was provided.

Main results and the role of chance: The 17 incidental findings involved 9 unique chromosomes and consisted of 15 microduplications and 2 microdeletions. The size of microduplications detected ranged from 0.18 megabases (Mb) to 6 Mb. One microdeletion identified (located on chromosome X) was determined to be of considerable size at 28 Mb, potentially associated with health implications in the carrier female. Follow-up microarray analysis was pursued in 12 cases (60%), as 5 of the patients declined further evaluation. All microarray analyses confirmed the presence of the CNV identified via Karyomapping; however the significance of each varied. One CNV was classified as "possibly pathogenic" and another as a "possible susceptibility region". Five of the CNVs were reported as variants of uncertain significance (VUS) and 4 were reported as normal population variants. Results are pending on 1 case. Interestingly, 2 patients were found to have almost identical microduplications and follow-up microarray via 2 different labs revealed discordant classifications for the clinical significance. One patient elected to include their microduplication (classified as a VUS) in her PGD testing and results from 2 PGD cycles revealed 1 embryo available for transfer. This patient was expected to deliver in January 2017.

Limitations, reasons for caution: A few hundred couples were assessed as part of this study; 2.7% of these identified with incidental findings. Although this study provides a substantial view into the matter, a larger population needs to be assessed in order to definitively determine the general incidence, clinical implications and significance of such findings.

Wider implications of the findings: Incidental findings can potentially have significant health implications to the patient, other family members and/or future children. It is imperative to recognize this issue in the clinical laboratory setting and a standard procedure should be implemented to address cases in which these incidental findings are identified.

Trial registration number: not applicable.

P-663 Day of trophectoderm biopsy influences implantation and ongoing pregnancy rates of similarly-graded euploid blastocysts

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Study question: Does the day of trophectoderm biopsy (day 5 versus day 6) affect implantation rate (IR) and ongoing pregnancy rate (OPR) of similarly-graded euploid blastocysts?

Summary answer: Similarly-graded euploid blastocysts biopsied on day 5 are associated with a significantly higher IR and OPR compared to those biopsied on day 6.

What is known already: Trophectoderm biopsy for aneuploidy assessment is performed on embryos after reaching blastocyst stage, which preferentially occurs on day 5 or day 6 after fertilization. Studies have shown that transfer of day 5 blastocyst in a fresh IVF cycle is associated with a higher IR compared to day 6, which is attributed to a higher synchrony with the endometrium. However, there are inconsistent data on the IR of frozen/thawed embryos. Therefore, assessing for euploidy and controlling for the day of trophectoderm biopsy and blastocyst grading prior to biopsy may help identify the best embryos to replace.

Study design, size, duration: It is a retrospective cohort study at an academic medical center. All frozen/thawed embryo transfer (FET) cycles in which only euploid blastocysts were transferred between January 2013 and December 2016 were reviewed. Patients who had transfer of two blastocysts of different grading or timing of biopsy were excluded. A total of 779 FET cycles (972 embryos) were included. Preimplantation genetic screening was performed using array comparative genomic hybridization.

Participants/materials, setting, methods: Cycles were divided into two groups based on the day of trophectoderm biopsy: day 5 (n = 405) and day 6 (n = 374). The morphological grading of blastocysts was assessed immediately prior to trophectoderm biopsy allocating embryos into the following four groups: excellent (^{3}3AA), good (3-6AB, 3-6BA, 1-2AA), average (3-6BB, 3-6AC, 3-6CA, 1-2AB, 1-2BA), and poor (1-6BC, 1-6CB, 1-6CC, 1-2BB). χ^2, Fisher's exact test, odds ratio (OR) with 95% confidence intervals were calculated and adjusted for confounding factors.

Main results and the role of chance: Cycles in which blastocysts were biopsied on day 5 were associated with a significantly higher OPR compared to those biopsied on day 6 (60.9% vs. 46.7%; p = 0.009). The odds ratio remained significant after controlling for blastocyst grading prior to biopsy, number of available euploid embryos, age, type of FET cycle (natural vs. programmed), and peak endometrial thickness (aOR=1.5; 95%CI=0.9-1.1). Euploid blastocysts that were biopsied on day 5 were associated with a significantly higher IR compared to those biopsied on day 6 (64.8% vs. 49.7%; p<0.001). There was no significant difference in the SAB rate between the two groups (9.5% vs. 9.7%; p>0.05).

Within embryos biopsied on day 5, excellent-quality blastocysts (n = 49) were associated with a significantly higher IR (83.6%) compared to average-quality (n = 290) (63.1%; p = 0.007) and poor-quality (n = 78) (50%, p<0.001) blastocysts. The IR of good-quality blastocysts (n = 86) (73.2%) was significantly higher compared to poor-quality (50%, p = 0.002) counterparts. Similarly, within embryos biopsied on day 6, poor-quality blastocysts were associated with a significantly lower IR (39.4%) compared to excellent-quality (70.5%), good-quality (57.5%), and average-quality (52.5%) cohort. The odds ratios remained significant after adjusting for age, type of FET cycle, peak endometrial thickness, and number of available euploid embryos for each patient.

Limitations, reasons for caution: Although the large number of cycles in this study clearly demonstrates that the pace of embryo development significantly influences the outcomes of similarly-graded euploid blastocysts, it is limited by its retrospective nature. A prospective study would be valuable to confirm our findings.

Wider implications of the findings: In spite of the aneuploidy assessment, the day of trophectoderm biopsy and the evaluation of blastocyst morphometric parameters, which reflect the metabolic health of the blastocyst, are paramount to select the best embryo.

Trial registration number: N/A.

P-664 PGS is most beneficial for older women with many embryos

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Study question: Which strategy is better for AMA women with many embryos: eSET after PGS or freeze all embryos and transfer them one by one?

Summary answer: For this group, eSET after PGS resulted in 0.65 clinical pregnancy rate while the calculated chance to choose a euploid embryo for transfer is 0.23.

What is known already: Currently there are two opinions regarding PGS: a) It is beneficial because eSET after PGS improves the clinical pregnancy rate and reduces chances of twin pregnancy and miscarriage; b) It is harmful because

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some embryos are falsely diagnosed as aneuploid and rejected, thus reducing the chance of pregnancy, and it is better to freeze all embryos and transfer them one by one in consecutive cycles. When women of advanced maternal age have a choice of several blastocysts available for transfer, it is not clear which strategy is better.

Study design, size, duration: This is a retrospective cohort study of patients undergoing IVF coupled with frozen embryo transfer after PGS in a single clinic from October 2012 to July 2016. Calculated and actual rates for clinical pregnancy and live birth are compared for three groups of patients with different proportion of euploid embryos.

Participants/materials, setting, methods: The study cohort consisted of 169 women aged 25 to 46, implanted with thawed autologous embryos after PGS-aCGH. Total of 861 embryos were analyzed, and patients were divided into 3 groups by proportion of euploid embryos: 0.1-0.29 (n = 31, mean age 36.9), 0.3-0.57 (n = 77, mean age 35.3), 0.6-1.0 (n = 61, mean age 33.45). Real clinical pregnancy (CP) rates were compared to theoretical chance of picking the euploid embryo ('right choice') for transfer in the first cycle.

Main results and the role of chance: In the 0.1-0.29 group there were from 4 to 15 embryos (mean of 6 embryos) per patient, with average 1.38 euploid embryo per patient. Mean calculated "right choice" chance was 0.23, and the real CP rate for the first cycle was 0.65 ($p<0.05$), live birth (LB) rate 0.55. The majority of these patients had AMA as indication for PGS. In the 0.3-0.57 group there were from 2 to 16 embryos (mean of 5,6 embryos) per patient with average 2.5 euploid embryo per patient. Calculated chance and actual CP rate were equal, comprising 0.4, with 0.34 LB rate. For the 0.6-1.0 group there were from 1 to 10 embryos (mean of 3.9 embryos) per patient, with average 3 euploid embryo per patient. Real CP rate at 0.51 was lower than calculated chance of 0.82 ($p<0.05$). Since this is the youngest group, it is likely that outcomes of IVF cycles were influenced by uterine factors, in contrast to the first group where the success depended on the genetic component.

Limitations, reasons for caution: This study was performed on a relatively small number of patients, and the chance of picking the euploid embryo for the first transfer was calculated purely on a probability basis, while in clinical practice, of course, choice of non-screened embryos for transfer is based on their morphology.

Wider implications of the findings: Our results suggest that when AMA patients have a choice of blastocysts for transfer, PGS followed by eSET gives them a much higher chance of clinical pregnancy and live birth already after the first cycle than consecutive transfer of all available embryos.

Trial registration number: not applicable.

P-665 Intermediate number of CGG repeats in patients with Fragile X premutation is associated with poorer ovarian reserve

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Study question: Do Fragile X premutation carriers with intermediate number of CGG repeats have a lower ovarian reserve than those with lower and higher number of repeats?

Summary answer: Fragile X premutation carriers with intermediate number (70-90) of CGG repeats have lower ovarian reserve than those with lower and higher number of repeats

What is known already: FMR1 premutation carriers (56-200 CGG repeats within FMR1 gene on X chromosome) have diminished ovarian reserve and higher incidence of premature ovarian insufficiency [POI] when compared to healthy controls. One of the proposed mechanisms is gonadotoxicity of the transcription/translation product of the mutated gene. Patients with intermediate number of repeats (70-90) have a higher risk of POI then those with less (<70) and more (>90) repeats. Possible explanation is lower concentration of the gonadotoxic mRNA/protein in the lower repeat group, and decreased transcription of the FMR1 gene caused by epigenetic changes with the higher number of CGG repeats.

Study design, size, duration: Retrospective cohort study including all FMR1 premutation carriers who underwent controlled ovarian hyperstimulation [COH]and preimplantation genetic diagnosis [PGD] for FMR1 gene mutation in a 5-year period at one academic center. Man Whitney U was used for comparison of continuous variables and P value < 0.05 was considered statistically significant

Participants/materials, setting, methods: The electronic medical record system was searched for all patients undergoing COH and PGD for FMR1 gene mutation during the study period where the number of CGG repeats was available. Thirty-one patients were included. None had prior ovarian surgery. Primary outcomes included the Anti-mullerian hormone level [AMH] (ng/mL), antral follicle count [AFC] and the number of oocytes retrieved. If a patient had multiple COH cycles, the mean AFC and number of oocytes retrieved were used.

Main results and the role of chance: There were 12 patients with intermediate (70-90) number of CGG repeats (study group) and 19 patients with lower and higher (<70 and >90) number of CGG repeats (control group). All patients underwent antagonist protocol and were matched for body mass index. Even though the patients in the study group were younger (median age 32.5 vs. 34.5 years, P = 0.04), their median AMH levels were lower (0.5 (IQR 0.3-0.9) vs 0.8 (IQR 0.6-1.75) ng/mL, P = 0.05) as well as their median AFC (6 (IQR 4-8) vs 9 (IQR 6-13, P = 0.03). Number of retrieved oocytes was also significantly lower in the study group (6 (IQR 3-8) vs. 8 (IQR 6-14), P = 0.03).

Limitations, reasons for caution: Main limitations of our study represent its retrospective nature, small sample size and inability to control for possible cofounders.

Wider implications of the findings: Current study suggests that FMR1 gene premutation carriers with intermediate number of repeats have poorer ovarian reserve which should be taken into account when counseling such patients and setting expectations for COH outcome.

Trial registration number: N/A.

P-666 Refining embryo transfer strategies after PGD for translocations using comprehensive chromosome testing

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Study question: Does the genome-wide ploidy profile of PGD translocation embryos inferred from the blastomere status (balanced/euploid vs. balanced/aneuploid) predict implantation potential?

Summary answer: PGD translocation embryos with an inferred balanced/aneuploid genotype have a lower, but not zero implantation potential compared to embryos with a balanced/euploid genotype

What is known already: The scientific validity of comprehensive chromosome testing in preimplantation embryos to improve the live birth rate and reduce the miscarriage rate in IVF patients is still under debate. Healthy live births have recently been reported after transfer of blastocysts with an inferred mosaic aneuploid genotype, yet live birth rates seem to be lower and miscarriage rates higher compared to transfer of euploid blastocysts.

The use of NGS and array technology in couples undergoing PGD for chromosomal or monogenic disorders allows for simultaneous genome-wide ploidy analysis of all embryos. Whether this data can be used to optimise PGD treatment is unknown.

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Study design, size, duration: Observational, prospectively designed, multicentre study covering a 3-year period (2014-2016) during which 117 couples underwent 171 PGD cycles for structural chromosomal abnormalities.

The multicentre structure encompassed three IVF laboratories that had biopsied material tested by one and the same genetic centre. Comprehensive chromosome testing was performed by array CGH. The included study cohort comprised first attempt PGD cycles with day 3 single blastomere biopsy and fresh single embryo transfer (n = 73).

Participants/materials, setting, methods: In all three centres only embryos with a balanced karyotype for the chromosomes comprising the PGD indication were eligible for transfer. Two of the three centres received no ploidy information for non-indication chromosomes and ranked eligible embryos for transfer solely based on morphology. The third centre did receive this information and ranked embryos based on both chromosomal constitution and morphology, prioritising the balanced/euploid ones.

Main results and the role of chance: In all, 73 embryo transfers met the inclusion criteria. There were 38 transfers in the two centres that did not use ploidy information, resulting in 20 ongoing pregnancies. Twenty-seven transfers involved a balanced/euploid embryo, resulting in 19 ongoing pregnancies (70%), and 11 transfers involved a balanced/aneuploid embryo, resulting in one ongoing pregnancy (9%). There were 35 transfers in the center that did use ploidy status to rank embryos, resulting in 8 ongoing pregnanies. Twenty-seven transfers involved a balanced/euploid embryo, resulting in 7 ongoing pregnancies (26%), and 8 transfers involved a balanced/aneuploid embryo, resulting in one ongoing pregnancy (13%). All transfers in all centres taken together, 54 transfers involved a balanced/euploid embryo, resulting in 26 ongoing pregnancies (48%), and 19 transfers involved a balanced/aneuploid embryo, resulting in two ongoing pregnancies (11%) (p = 0.004). Aneuploidy of non-indication chromosomes involved a trisomy 19 and a trisomy X. Miscarriage rates, here defined as the percentage of cycles where a biochemical pregnancy did not result in an ongoing pregnancy, were 4% for balanced/euploid and 50% for balanced/aneuploid embryos.

Limitations, reasons for caution: The observed difference in implantation rate between balanced/euploid and balanced/aneuploid embryos is statistically different, but the number of cases is still small. To appraise the clinical effectiveness of both embryo transfer strategies, the cumulative live birth rate (result of fresh and cryo transfers) per woman should be assessed.

Wider implications of the findings: The biopsied blastomere-inferred embryo genotype of the non-indication chromosomes is predictive of the implantation potential. Whether ranking embryos for transfer accordingly improves PGD treatment outcome should be further investigated.

Trial registration number: Number?

POSTER VIEWING SESSION
REPRODUCTIVE ENDOCRINOLOGY

P-667 Involvement of local ovarian interleukin-1beta in insulin-resistance of granulosa cells in women with polycystic ovarian syndrome

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Study question: Does increased interleukin-1β abundance in the ovary play a role in local ovarian insulin resistance in PCOS women?

Summary answer: The increased abundance of Interleukin-1β abundance in PCOS ovary decreases insulin sensitivity by impairing its receptor-coupled signaling pathway in granulosa cells.

What is known already: The polycystic ovary syndrome (PCOS) is the most common disorder in women of reproductive age. Women with PCOS is more likely to develop insulin resistance (IR). However, the underlying cause of IR remains largely unknown. Low-grade inflammation is associated with IR in several major insulin-target tissues. We studied whether interlukin-1β (IL-1β), the best-studied pleiotropic proinflammatory cytokine, was increased in granulosa cells and follicular fluid in PCOS with IR, and whether these increases were associated with IR in PCOS women. The mechanism of IL-1β-induced IR in granulosa cells was also investigated.

Study design, size, duration: Ovarian granulosa cells and follicular fluid were collected from PCOS patients with IR (n = 21) or without IR (n = 23), who underwent in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). IR status was determined according to the homeostasis model of assessment for insulin resistance index (HOMA-IR) or fasting insulin level. Women of normal body mass index without IR but with tubal factor as the only cause of infertility were enrolled as control (n = 21).

Participants/materials, setting, methods: Follicular fluid and granulosa cells were collected. IL-1β abundance in the follicular fluid was determined with ELISA and IL-1β mRNA in the granulosa cells was measured with quantitative real time PCR. Cultured granulosa cells were used to study whether IL-1β treatment cause IR and to explore the underlying mechanism.

Main results and the role of chance: Higher IL-1β concentration in the follicular fluid and increased IL-1β mRNA abundance in the granulosa cells were detected in PCOS with IR than PCOS without IR or non-PCOS patients. The level of IL-1β was positively correlated with fasting insulin level and HOMA-IR, but negatively related with lactate concentration in the follicular fluid. Meanwhile, mRNA of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a lipid phosphatase which antagonizes the activity of PI3K thereby inhibiting Akt phosphorylation resulting in impaired insulin sensitivity, in granulosa cells was significantly elevated in PCOS with IR, and positively correlated with IL-1β concentration in the follicular fluid. IL-1β treatment of cultured granulosa cells up-regulated PTEN mRNA and protein abundance in a dose-dependent manner. Pretreatment with IL-1β attenuated not only Akt phosphorylation by insulin but also the shift of GLUT4 from cytoplasm to cell membrane. These data suggest that elevated IL-1β concentration in PCOS ovary is a causative factor of IR in granulosa cells.

Limitations, reasons for caution: IL-1β is only one of the factors that contribute to the development of IR in the PCOS ovary. Other contributing factors await to be identified.

Wider implications of the findings: Alleviation of the inflammatory process may ameliorate IR in the ovary so that the ovarian functions may be improved.

Trial registration number: Not applicable.

P-668 Effect of ovarian hyper-stimulation with follicular phase of progesterone on ovarian microenvironment in mice

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Study question: To investigate the effect of different doses of medroxyprogesterone acetate (MPA) combined with Human Menopausal Gonadotropin (HMG) on ovarian microenvironment during follicular phase in mice.

Summary answer: Administration of MPA and HMG achieved the double effect of promoting multiple follicular development and inhibiting premature luteinizing hormone (LH) surge in the follicular phase.

What is known already: Studies found that early administration of progesterone (P) in the follicle made endogenous ovarian LH could not be synthesized and secreted so as to achieve the purpose of controlling premature LH surge. Meanwhile, the production of endogenous P is not affected by exogenous P. And our clinical outcome suggested that a high retrieved oocyte number and clinical pregnancy rate achievedin the progestin primed ovarian stimulation (PPOS) protocol compared with short-term protocol.

Study design, size, duration: The BALB/C female mice were randomly divided into four groups: 1) control group: sodium carboxymethylcellulose

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(CMC-Na), HMG and MPA (4/10/20/40 mg/d) as control group; 2) study group: HMG+MPA (4/10/20/40 mg/d). Meanwhile, 3 week female mice were divided into 3 groups: CMC-Na, HMG and HMG+MPA groups. All animals were treated for 5 successive days.

Participants/materials, setting, methods: Vaginal smear was used to observe the sexual cycle, the levels of sex hormone were determined by enzyme-linked immunosorbent assay (ELISA) and the ovarian morphology and follicular development were observed by hematoxylin eosin (HE) staining. The expression of ovarian hormone receptor were observeed by immunohistochemistry, and the expression of PI3K/Akt/mTOR protein in follicular developmental signal pathway was determined by western blotting.

Main results and the role of chance: The ovary weight index in the HMG +MPA group was higher than that of control group. The levels of serum Follicle-Stimulating Hormone (FSH), Follicle Stimulating Hormone Receptor (FSHR) and the percentage of antral follicles in HMG and HMG+MPA groups were significantly higher than those in CMC-Na group and MPA group ($p<0.05$) while reduced levels of LH and LHR were observed. Western blotting showed that phosphorylation of PI3K/Akt/mTOR protein in the experimental group was lower than that in the HMG group and higher than in the MPA and CMC-Na groups.

Limitations, reasons for caution: The mechanism of PPOS protocol remain to be further explored and this study shown only in one species.

Wider implications of the findings: This animal study can provide guidance for optimizing clinical PPOS protocol.

Trial registration number: This study was registered with the Chinese Clinical Trial Registry (ChiCTR-ONRC-14004419).

P-669 Early serum oestradiol rise during ovulation induction in WHO Group II anovulatory infertility predicts the development of > 3 mature follicles

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Study question: Can the increments in serum oestradiol (E_2) levels during ovulation induction with gonadotrophins in WHO Group II anovulatory infertility predict the development of >3 mature follicles and thus cancellation of the cycle?

Summary answer: E_2 increments from stimulation Day 1 to stimulation Day 5 significantly predicted the development of an excessive response with >3 mature follicles.

What is known already: The prediction of hypo- or hyper-response to gonadotrophin stimulation during ovulation induction for anovulatory infertility is a clinical challenge. It has previously been shown that the number of intermediate follicles and the levels of E_2 on the day of human chorionic gonadotrophin (hCG) are correlated with high-order pregnancy rates in ovulation induction with gonadotrophins. However, parameters measured at this stage of stimulation may support the decision of withholding hCG but do not contribute to an individualised initial treatment approach.

Study design, size, duration: This prospective, clinical study performed between 2010 and 2012 included 71 normogonadotrophic, anovulatory women who were consecutively treated with an individualised, nomogram-based starting dose of highly purified human menopausal gonadotrophin (HP-hMG) (Nyboe Andersen *et al*, 2008). All women underwent one ovulation induction cycle according to a flexible, low-dose step-up regimen where it was also allowed to step-down in order to control for multiple follicular development.

Participants/materials, setting, methods: Serum levels of E_2 and follicle development were assessed at baseline, on stimulation Day 5, on the day of dominance, and on the day of hCG administration. Receiver operating characteristics (ROC) curves were used to evaluate the E_2 increments during stimulation as a predictor of the outcome of treatment.

Main results and the role of chance: E_2 increased significantly between all four time points ($P < 0.001$). On stimulation Day 5, a significantly higher E_2 increment was observed in women who developed > 3 mature follicles compared with the group of monofollicular development or 2–3 follicles (534.6%, 95% CI: 233.3–1227.4% and 237.1%, 95% CI: 184.5–304.1%, respectively, $P = 0.005$). The E_2 increment from stimulation Day 1 –5 could predict the development of > 3 mature follicles with an area under the curve (AUC) of 0.76 (95% CI: 0.60–0.92). Thus, a woman with more than at two-fold increase in E_2 from stimulation Day 1—5 had a 33% risk of developing >3 mature follicles. A woman with *less* than a two-fold increase in E_2 from stimulation Day 1 to stimulation Day 5 had an 88% chance of developing ≤3 mature follicles and fulfilling the hCG criteria.

Limitations, reasons for caution: The relatively small sample size of the study may reduce the power of the results.

Wider implications of the findings: Ultrasonography has to a large extent replaced E_2 measurements in the monitoring of follicular response to stimulation. However, our study underlines the fact that E_2 measurements may be valuable in the prediction of multifollicular growth that may not be immediately observed on ultrasound.

Trial registration number: EudraCT-number 2010-021459-16. ClinicalTrials.gov Identifier: NCT01250821.

P-670 Progesterone at baseline and during stimulation predict live birth rate after IVF and should be evaluated before and during ovarian stimulation

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Study question: Can progesterone at baseline of controlled ovarian stimulation and during stimulation anticipate progesterone at HCG day and predict Live birth (LB).

Summary answer: Progesterone history since baseline impacts LB rate following a non linear effect admitting an optimal value and decreasing at lower and higher values.

What is known already: Progesterone Elevation(PE) at HCG day (P_{HCG}) negatively impacts live birth. Previous studies suggest that baseline progesterone level P_b and during stimulation may predict PE. The double relationship $P_b \rightarrow P_{hcg} \rightarrow$LB allows us hypothesizing a direct impact of P_b on LB. However, previous results are not consistent between studies and were not based on the continuous values of P_{hcg} but dichotomized following the threshold 1.5 ng/ml. Besides, no studies provided evidence of the direct effect of P_b on LB.

Study design, size, duration: Non-interventional, retrospective, observational, single-centre cohort study. No inclusion or exclusion criteria were applied. A sample size of 4300 cycles was calculated to provide a power of 90% to detect an odds ratio (OR) on LB as large as .6/Log(P_b) at $P < .001$ level (type-I correction for multiple testing). Due to the expected correlation between cycles on the same patient, the analysis was conducted on the last recent 5447 IVF/ICSI cycles performed on 2192 patients during 2009-2015.

Participants/materials, setting, methods: Patients (median age 34y [IQ=31-37], BMI= 22.8 ± 4.8) underwent controlled ovarian stimulation (rFSH ± LH, HP-HMG) with either agonist or antagonist protocols. The effect of P_b on P_{hcg} was tested by a linear mixed model assessing the non linear effect of the log-transformed progesterone value at baseline and day 6 of stimulation on PE by polynomial regression. The effect of P_b on LB was tested by a non linear mixed model admitting the same factors and covariables.

Main results and the role of chance: Progesterone as baseline P_b was confirmed as the main predictor of PE(ratio RG of the geometric mean P_{hcg}/P_b RG= 1.58, (95%CI [1.55-1.62], $P < .001$, determination R^2=.29), followed by the total dose of gonadotropin FSH_{tot} (RG=1.11 per 10^3.iu/L [1.01,1.22], p=.023). A highly significant non-linear effect of P_b on LB was constituted by a

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linear (odds ratio OR=.86 [.61,.99], p=.04) and quadratic (OR=.22, [.10,.49], P < .001) components, resulting in an optimal LB value at .65 ng/ml ([.59,.75], bootstrapping) and decreasing LB rate for both lower values and higher values. According to our model, a mean decrease in LB of 28% (OR=.82, .68-.98) was found for patients out of the P_b interval [.20,.85] ng/ml (21.7% of the sample). Progesterone at day 6 of stimulation (P_6) was found more predictive than P_b with a similar non linear variation on LB admitting a maximum LB at P_6= .64 ng/ml [.5,.69], with a mean LB decrease of 24% (OR=.76, .63-.93) was found for patients out of the P_6 interval [.2,.9] ng/ml (18.7% of the sample).

Limitations, reasons for caution: Our results derive from a retrospective single-center study; the exact identification of the optimum value and its confidence interval is likely to depend of the lack of uniformity of progesterone assay kits.

Wider implications of the findings: Compared with progesterone measured at HCG, progesterone at baseline and day 6 also predict live birth. Measuring progesterone at baseline and during the stimulation are recommended. An optimal interval of progesterone value at baseline and day 6 is recommended for both fresh or differed embryo transfer.

Trial registration number: NA.

P-671 The effect of oil and water-soluble contrast medium in hysterosalpingography on thyroid function

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Study question: Is there a difference in thyroid function (thyroid stimulating hormone: TSH and free-T4: FT4) after hysterosalpingography (HSG) with an oil-soluble contrast medium (OSCM) and a water-soluble contrast medium (WSCM)?

Summary answer: Approximately 25% of the women in the OSCM-group developed subclinical hypothyroidism (SCH). Conversely, patients in the WSCM-group did not show a change in thyroid function.

What is known already: Several studies have already shown an adverse effect of OSCM on thyroid function after HSG. However, it is unknown whether WSCM also depresses thyroid function after HSG.

Study design, size, duration: This was a retrospective study. Thirty-one patients who underwent HSG with OSCM and 58 patients who had HSG with WSCM were included in this analysis, which took place between March 2013 and August 2014.

Participants/materials, setting, methods: The patients had undergone a thyroid function test at our clinic less than three months before HSG and all showed normal thyroid function. The subjects' thyroid function also was checked at least once following HSG. HSG was performed using the oil-soluble contrast medium Lipiodol® (an iodized poppy seed oil; Terumo, Japan) and the non-ionic water soluble contrast medium Isovist® (iotrolan, Bayer Schering Pharma AG Berlin, Germany).

Main results and the role of chance: TSH and FT4 levels did not differ between the patients before HSG with OSCM and WSCM (TSH: 1.46 ± 0.63 mIU/L in the OSCM group vs. 1.42 ± 0.65 mIU/L in the WSCM group; FT4: 1.20 ± 0.14 ng/dL in the OSCM group vs. 1.21 ± 0.14 ng/dL in the WSCM group). After HSG, TSH levels were significantly higher in the OSCM group (2.43 ± 0.94 mIU/L) compared to that of the WSCM group (1.64 ± 1.01 mIU/L). Patients in both the OSCM and WSCM groups showed unchanged FT4 levels after HSG. Some 22.5% of patients who underwent HSG with OSCM developed SCH within one month of the test. On the other hand, some patients who underwent HSG with WSCM also developed SCH (9.5%). In an interval-matched comparison, the proportion of SCH development following HSG was significantly higher in patients who underwent the test with OSCM than in those in which it was administered using WSCM.

Limitations, reasons for caution: The retrospective design is the limitation.

Wider implications of the findings: Our data clearly indicates that WSCM is safe for thyroid function in women who plan to get pregnant. SCH during pregnancy is a known risk factor for miscarriage. Therefore, we recommend that WSCM initially be used to detect tubal patency.

Trial registration number: none.

P-672 Endocrinological evidence why humans can hardly live above around 5.000 m altitude

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Study question: Is there any endocrinological evidence why humans can hardly live above around 5.000 m altitude?

Summary answer: Activation of stress axes and inhibition of reproductive axis at altitude above 5.000 m probably contribute to the incapability of humans to live at extreme altitudes.

What is known already: Humans cannot live for a long time above around 5.000 m altitude for reasons which have not been completely understood and which are not restricted to cardiorespiratory changes alone. Previous studies analysing the hormone system at high altitude were based on small study groups, limited to men, were only carried out at altitudes of up to 5.000 m or the study design was not ideal because the studies were carried out during mountaineering expeditions.

Study design, size, duration: The prospective observational cohort field study was performed in the context of the Swiss High Altitude Medical Research Expedition 2013 to Mount Himlung Himal (7126 m). 40 healthy subjects (21 male, 19 female) aged between 18 and 70 years had been included in the trial. Hormonal concentrations of all hypothalamus pituitary axes were measured between 550 and 7.050 altitude. No supplementary oxygen was used during the climb.

Participants/materials, setting, methods: Basal hormone concentrations (cortisol, TSH, fT3, fT4, LH, total testosterone, prolactin and GH) were analysed the next day in the morning after reaching the next altitude step at 550, 4.800, 6.025 and 7.025 m. Hormone concentrations were also measured after acclimatization at 4.800 m. Pulsatility of selected hormones (cortisol, TSH, prolactin, GH) was analysed following blood collections every 10-20 minutes over 8 hours in 8 females at 550, 4.800 and 6.025 m. Additionally cardiorespiratory parameters were evaluated.

Main results and the role of chance: Overall: Changes of hormonal concentrations correlated with altitude but not with oxygen saturation. Stress axes were activated and reproductive axes were inhibited at high altitude.

In detail: Cortisol and prolactin concentrations in males and females decreased at ascent to 4.800 m and then increased at 6.025 m and 7.050 m. Cortisol decrease at 4.800 m was normalized after acclimatization. fT4 increased with increasing altitude, increase at 4.800 m was also normalized after acclimatization. LH decreased in males with increasing altitude.

Pulsatility analysis: The increase of hormone concentrations such as cortisol and prolactin was due to an increase of pulse rate. Mass per pulse however decreased.

Limitations, reasons for caution: Effect of acclimatization was only analysed on 4.800 m altitude but not at higher altitudes.

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Wider implications of the findings: Activation of stress axes at high altitude and decrease of LH secretion might affect early and late pregnancy in women at high altitude. However, this negative effect might not be relevant in case of slow ascent allowing sufficient acclimatization.

Trial registration number: Clinicaltrials.gov: NCT01953198.

P-673 A randomised, assessor-blind, AMH-stratified, dose-response trial in Japanese IVF/ICSI patients undergoing controlled ovarian stimulation with follitropin delta

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Study question: To establish the relationship between follitropin delta dose (FE 999049; recombinant FSH produced from the human cell line PER.C6®) and ovarian response in Japanese IVF/ICSI patients.

Summary answer: A significant dose-response relationship was established between follitropin delta and number of oocytes retrieved in Japanese IVF/ICSI patients.

What is known already: A significant dose-response relationship has been established for follitropin delta with respect to number of oocytes retrieved as well as serum concentrations of estradiol, inhibin B, inhibin A and progesterone in a clinical trial conducted in European IVF/ICSI patients of whom 98% were Caucasians (Arce et al, Fertil Steril, 2014).

Study design, size, duration: Randomised, controlled, assessor-blind, multicentre, dose-response trial in 158 Japanese IVF/ICSI patients, 20-39 years (mean age 33.7 years), undergoing controlled ovarian stimulation with three dose levels of follitropin delta (Ferring Pharmaceuticals) and with follitropin beta (Follistim, MSD) included as a reference group for validity purposes. A single blastocyst was transferred in the fresh cycle. Clinical pregnancy was defined as a gestational sac.

Participants/materials, setting, methods: Patients were randomised to fixed doses of 6, 9 or 12 µg/day follitropin delta (N = 117) or 150 IU/day follitropin beta (N = 41). Randomisation was stratified by serum AMH (low: 5.0-14.9 pmol/L, high: 15.0-44.9 pmol/L; Elecsys® AMH, Roche Diagnostics). Gonadotropin dosing was initiated on day 2-3 of the menstrual cycle, ganirelix 0.25 mg/day added from day 6 of stimulation and triggering of final maturation done when observing ≥3 follicles ≥17 mm. OHSS was assessed using Golan's classification.

Main results and the role of chance: A significant ($p < 0.001$) dose-response relation between follitropin delta and number of oocytes retrieved was observed for the overall trial population ($p<0.001$), the high AMH stratum ($p<0.001$) and the low AMH stratum ($p = 0.004$). An average of 53% more oocytes were retrieved in the high AMH stratum compared to the low AMH stratum (6 µg: 7.9 vs 5.3; 9 µg: 11.2 vs 5.6; 12 µg: 12.9 vs 9.5; follitropin beta: 11.8 vs 9.3). Significant ($p<0.05$) dose-responses were also observed for serum estradiol, progesterone, inhibin B and inhibin A on day 6 and/or at end of stimulation with follitropin delta. The proportion of patients with excessive response (≥15 oocytes) after stimulation with follitropin delta increased from 6% with 6 µg to 15% with 9 µg and 28% with 12 µg, while it was 20% with follitropin beta. The rate of early moderate/severe OHSS for follitropin delta was 8% for 6 µg, 8% for 9 µg and 13% for 12 µg, and 20% for follitropin beta, with most cases (84%) in the high AMH stratum. The clinical pregnancy rate per started cycle with follitropin delta was 24% for 6 µg, 20% for 9 µg and 33% for 12 µg, and 20% for follitropin beta.

Limitations, reasons for caution: Patients with very low (<5 pmol/L) or very high (>45 pmol/L) AMH were not included in this dose-response trial.

Wider implications of the findings: The findings from this trial confirm the dose-response relationship of follitropin delta in Japanese IVF/ICSI patients in relation to number of oocytes retrieved and other pharmacodynamic parameters, and reinforce the concept that the gonadotropin dose should be guided by an ovarian marker such as initial serum levels of AMH.

Trial registration number: NCT02309671.

P-674 Telomere length is short in PCOS and oral contraceptive does not affect the telomerase activity of granulosa cells in patients with PCOS

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Study question: Are telomerase activity(TA) and telomere length(TL) associated with IVF outcome of PCOS patients? Does oral contraceptive affect the TA and TL in patients with PCOS?

Summary answer: Shorter TL was found in PCOS patients. No effect of oral contraceptive pill(OCP) pretreatment on the TA and TL either.

What is known already: Telomerase is the critical enzyme for maintaining TL. TA positively correlates with the proliferation activity of GCs. Our previous study showed that the TA of GCs in non-PCOS patients positively correlated with the pregnancy outcome of IVF. OCP could reduce the basal luteinizing hormone and androgen levels and improve IVF outcomes in PCOS patients.

Study design, size, duration: 163 infertile women were enrolled and divided into a PCOS group(n = 65) and a non-PCOS group(n = 98). The PCOS group were further divided into a TA<0.070 group(n = 34) and a TA≥0.070 group(n = 31), a TL<1 group(n = 41) and a TL≥1 group(n = 24), an OCP pretreatment group(n = 35) and a non-OCP pretreatment group(n = 30), respectively.

Participants/materials, setting, methods: The TA of GCs was determined using the telomeric repeat amplification protocol enzyme-linked immunosorbent assay(TRAP-ELISA). The TL was analyzed using real-time fluorescence quantitative PCR.

Main results and the role of chance: No obvious differences were observed in TA between these groups. The TL was 0.971 in PCOS group and 1.118 in non-PCOS group($P = 0.005$). The patients with TL≥1 accounted for 36.9%(24/65) in PCOS group and 54.1%(53/98) in non-PCOS group ($\chi^2 = 0.441$, $P = 0.032$). The infertility duration of PCOS patients was 5 years in TA<0.070 group and 4 years in TA≥0.070 group($P = 0.038$), 5 years in TL<1 group and 3 years in TL≥1 group($P = 0.006$), respectively. No obvious differences were observed in IVF outcome between these groups. No obvious differences were observed in TA, TL and IVF outcome between OCP pretreatment group and non-OCP pretreatment group in PCOS patients.

Limitations, reasons for caution: Whether extension of the OCP pretreatment time affects the TA and TL of GCs in PCOS patients still needs further exploration.

Wider implications of the findings: These findings suggest that impaired TL is associated with PCOS. PCOS patients with a lower TA and shorter telomeres have an earlier onset of infertility symptoms. OCP does not affect the TA of granulose cells in patients with PCOS.

Trial registration number: N/A.

P-675 increased follicular expression of growth differentiation factor 8 and its receptors in PCOS ovaries

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Study question: Do human ovarian cells express growth differentiation factor 8 (GDF8)?

Summary answer: GDF8 and its functional receptors are expressed in human ovarian tissues and with an increased follicular expression in PCOS ovaries than normal ovaries.

What is known already: Our recent studies have shown that GDF8 plays a critical role in the regulation of ovarian functions, including steroidogenesis,

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gonadotropin responsiveness, cell proliferation, lysyl oxidase activity and cumulus expansion.

Study design, size, duration: This is a laboratory study conducted over a 1-year period, and 48 samples was included.

Participants/materials, setting, methods: A total of 34 normal menstrual cycle women and 14 PCOS patients undergoing ovariectomy or partial ovariectomy were recruited at a University hospital. Immunohistochemical staining of ovarian tissue sections using microscopic evaluation to assess the presence, distribution, and cellular localization of GDF8 and its functional receptors.

Main results and the role of chance: The immunostaining of GDF8, ACVR2A, ACVR2B and ALK5 was detected in a few granulosa cells (GCs) of primary follicles, but not in primordial follicles in normal and PCOS ovaries. The antigens for these proteins were expressed in the oocytes regardless of the developmental stage. Additionally, all these proteins were localized in the GCs and thecal cells (TCs) of different sizes (0-2, 2-5 and 5-10 mm) of antral follicles in normal and PCOS ovaries with a progressively increasing expression pattern ($P < 0.05$). A significantly higher expression of GDF8 was detected in the GCs than in the matched TCs of 2-5 mm ($P < 0.01$) and 5-10 mm ($P < 0.01$) antral follicles in normal and PCOS ovaries. Furthermore, these proteins were also expressed in the luteal cells of the corpus luteum of normal ovaries, with a higher expression in the large luteal cells ($P < 0.05$). Compared to the normal ovaries, the GCs and TCs of large antral follicles (2-5 mm and 5-10 mm) in PCOS ovaries display a higher expression of GDF8, ACVR2A, ACVR2B and ALK5 ($P < 0.05$).

Limitations, reasons for caution: This was mainly a descriptive study with no functional studies on the target proteins found.

Wider implications of the findings: The distinct expression of GDF8 and its functional receptors in human ovarian tissues suggest their roles in the growth of oocytes, GCs, TCs and luteal cells. The different expression of GDF8 in antral follicle cells between normal and PCOS ovaries suggest the possible involvement ofGDF8 in the pathogenesis of PCOS.

Trial registration number: null.

P-676 Epiregulin (EPI) promotes the maturation of human denuded germinal vesicle (GV) stage oocyte taken from ≥38 aged women undergoing IVF

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Study question: Epiregulin was known as mediator of LH action for oocyte maturation. Can epiregulin practically trigger maturation of human denuded oocyte?

Summary answer: mRNA levels of epiregulin were 5 times higher in cumulus-cells of young-age-women than old-age-women. Addition of epiregulin in IVM could significantly increase maturation-rate of denuded-oocytes

What is known already: Epiregulin is a recently described member of the EGF family, which also includes amphiregulin (AR), epiregulin (EPI), and betacellulin (BTC). In animal models, epiregulin show higher expression in younger ovary and matured COC than older ovary and immature COC. EGFs including EPI are paracrine mediators that propagate the LH signal throughout the follicles. EGSs promote oocyte maturation by signaling through the EGFR. EGFR are expressed in granulosa ell, cumulus cell, 8 cell embryos, and blastocyst as well as oocyte.

Study design, size, duration: A total of 1224 human germinal vesicle (GV) oocytes obtained from 411 IVF patients were investigated in this study between January 1, 2016 and January 10, 2017. The spontaneous nuclear maturation and cytoplasmic maturation in IVM was investigated in two groups [young-age-group (YG); ≤ 35 years old and old-age-group (OG); ≥ 38 years old)]. Individual healthy GV oocytes were randomly divided into two epiregulin treatment groups (10 ng/ml group and 20 ng/ml group)

Participants/materials, setting, methods: Epiregulin mRNA expression in cumulus cells was investigated by real-time RT-PCR. The epiregulin protein was localized by immunohistochemistry of human ovarian tissues. The cumulus cells were totally removed for confirming of the presence of GV and nuclear maturation (NM) was determined by examining the oocytes at 24 h for GVBD and polar body extrusion. Oocytes reaching into MII stage were fertilized by ICSI for examining cytoplasmic maturation (CM).

Main results and the role of chance: Epiregulin protein was founded to be localized in the granulosa cells of primary follicles, secondary follicles and preovulatory follicles. mRNA levels of epiregulin were 5 times higher in cumulus cells of younger aged women than cumulus cells of older aged women. When comparing maturation rates from results of younger and older group, higher maturation rate was observed in younger group than older group [nuclear maturation rate(NMR); 37.79% vs 23.99%, cytoplasmic maturation rate (CMR) 55.5% vs 49.2%, $p<0.01$)]. When comparing maturation rates in studies treated with 20 ng/ml concentration, more GV oocytes could reach to MII oocytes and then could be fertilized in both of younger and older group than no treatment control [NMR (younger control vs younger treatment: 37.79% vs 51.82% and older control vs older treatment: 23.99% vs 51.3%), CMR (younger control vs younger treatment: 55.5% vs 60.2% and older control vs older treatment: 49.2% vs 67.2%).

	control	10 ng/ml	20 ng/ml
Young-age-group			
NMR (%)	37.79	41.67	51.82
Old-age-group			
NMR (%)	23.99	48.92	51.30
Young-age-group			
CMR (%)	55.51	60.00	60.22
Old-age-group			
CMR (%)	55.51	61.20	72.02

Limitations, reasons for caution: Our study was restricted to patients treated by GnRH antagonist protocol. After fertilization, the quality of the embryo was not confirmed.

Wider implications of the findings: Epiregulin mediated oocyte maturation may be important in disorder of androgen excess, such as polycystic ovarian syndrome which is characterized by anovulation, unregulated follicle growth, and the absence of dominant follicles.

Trial registration number: none.

P-677 Prospective randomized study comparing two short IVF protocols for low ovarian reserve

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Study question: Differences in the effectiveness of short GnRH antagonist cycles with corifollitropin alfa and GnRH agonist cycles with follitropin alfa in low ovarian reserve.

Summary answer: The number of MII, good quality and frozen embryos, and pregnancy rate are slightly higher with GnRH antagonist and corifollitropin alfa. Differences aren't statistically significant.

What is known already: Low ovarian reserve is one of the most difficult challenges in reproduction, related to the poor prognosis and the increasing number of patients with this condition. The combination of GnRH antagonist and corifollitropin alfa is a patient friendly and efficient treatment due to corifollitropin alfa´s extended half-life of 7 days. There is still little literature comparing the efficacy of this protocol against the short agonist protocol with follitropin alpha in patients with low ovarian reserve.

Study design, size, duration: This is a prospective randomized study between September 2015 and January 2017 in patients undergoing their first IVF cycle after their diagnosis of low ovarian reserve (Bologna criteria). All

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patients were treated with estradiol valerate and norgestrel (Progyluton®) for a month before ovarian stimulation. Patients (82) were then randomized for treatment with GnRH antagonist (Orgalutran®) with corifollitropin alfa 150 mcg (Elonva®) (group A) or GnRH agonist (Synarel®) with follitropin alfa 300 IU/day (Gonal®) (group B).

Participants/materials, setting, methods: IVF patients in a private clinic were randomized in group A (43) or group B (39). Ovarian stimulation started on day 3. HMG (HMG Lepori®) was added when needed after day 8. Primary endpoint was the number of MII obtained. Secondary variables were days of stimulation, dose of additional HMG, endometrial thickness, number of follicles>13 mm, total oocytes, developing, top quality and frozen embryos, pregnancy rate per cycle and transfer, and cumulative pregnancy rate.

Main results and the role of chance: There were no differences in demographic characteristics of both groups. Mean age was 38.8, BMI 22.4, antral follicle count 6.95, baseline FSH 3.22 mUI/ml and basal estradiol 57.63 pg/ml.

The stimulation in group A lasted 7.85 days vs. 7.28 in B. Additional dose of HMG required was 382.84 UI in group A vs 215.38 IU in B. Two cycles were cancelled in group B (low response) and none in A. The number of follicles>13 mm was 6.19 in group A vs 5.51 in B. Total number of oocytes and MII retrieved was 5.7 and 4.72 in group A vs. 5.05 and 3.66 in B respectively. Number of developing embryos was 2.04 vs 1.49 in group A and B respectively. Top quality embryos (A and B) on day +3 were 0.53 and 0.63 in group A vs. 0.33 and 0.43 in group B respectively. The number of frozen embryos was 0.79 in group A and 0.66 in group B. Pregnancy rate per cycle and per transfer was 25.9% in group A vs. 17.9% and 19.9% in group B. Cumulative pregnancy rate (fresh plus frozen embryo transfers) were 27.9% in group A vs. 20.5% in group B. Differences were not statistically significant.

Limitations, reasons for caution: The sample size of this study limits the conclusions. Studies with larger sample of patients are needed to reach consolidated conclusions in this area.

Wider implications of the findings: Stimulation with GnRH antagonist and corifollitropin alfa might increase the number of mature oocytes, developing embryos, good quality embryos (A/B), frozen embryos and pregnancy rate. Differences found with the use of GnRH agonist and follitropin alpha were not statistically significant, but might be clinically important.

Trial registration number: Pending.

P-678 Circulating irisin and GIP, but not asprosin, underscore the manifestation of polycystic ovary syndrome

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Study question: We hypothesized the newly identified white adipose tissue-derived hepatic glucose release hormone, asprosin, may play a role in the manifestation of polycystic ovary syndrome (PCOS).

Summary answer: PCOS patients have an overtly elevated irisin and glucose-dependent insulinotropic polypeptide (GIP) levels; however, serum asprosin level is not associated with the risk of PCOS.

What is known already: PCOS is characterized by oligo/anovulation, polycystic ovary, and hyperandrogenism. In addition, we have recently reported that aberrant regulation of the muscle-derived irisin and gut-derived GIP is associated with the risk of PCOS, and hyperinsulinism/dyslipidemia in PCOS patients. Asprosin is a glucogenic adipokine derived from the C-terminus of profibrillin, and its level is elevated in humans and animals with insulin resistance. On the other hand, the blockage of asprosin functions has a profound glucose- and insulin-lowering effect. However, its role in the development of PCOS remains to be investigated.

Study design, size, duration: A total of 792 patients diagnosed with PCOS according to the Rotterdam criteria and 138 healthy women were recruited in a period of 4 years.

Participants/materials, setting, methods: Healthy women and PCOS patients were recruited for a metabolic syndrome test at a teaching hospital. Serum levels of asprosin, irisin, GIP, androgens, lipids, insulin, and glucose were measured. HOMA-IR, beta cell function, QUICKI, and ISI $_{Matsuda}$ were calculated to measure insulin sensitivity and insulin resistance.

Main results and the role of chance: As a group, PCOS patients exhibited hyperandrogenism, glucose intolerance, hyperinsulinism, and dyslipidemia. Levels of asprosin, irisin, and GIP were significantly correlated with levels of steroid hormones, glucose, insulin, and lipids in patients ($p < .01$). As reported earlier, we found that serum irisin and GIP levels were significantly elevated in PCOS patients when compared with control women ($p < .001$). However, the serum level of asprosin in PCOS patients was similar to that of healthy volunteers ($p = 0.959$). This finding hinted that PCOS can be partly attributed to abnormal irisin and GIP metabolism, whereas the asprosin-mediated glucose metabolism does not contribute to the risk of PCOS or the presentation of PCOS-associated metabolic syndrome.

Limitations, reasons for caution: Although we did not observe an aberrant regulation of asprosin in PCOS patients, a role of asprosin in the development of glucose intolerance in select PCOS patients cannot be ruled out.

Wider implications of the findings: While serum levels of the white adipose tissue-derived asprosin correlated with a variety of metabolic biomarkers, asprosin's glucogenic action does not play a critical role in the development of PCOS, or the manifestation of hyperandrogenism, glucose intolerance, hyperinsulinism, and dyslipidemia in PCOS patients.

Trial registration number: Not applicable.

P-679 Agonist trigger is associated with defective progesterone production and decreased LH receptor and VEGF expression in luteal granulosa cells that are partially reversed by hCG

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Study question: Is there any difference in luteal function of the granulosa cells of the antagonist IVF cycles triggered with a GnRH agonist vs. hCG?

Summary answer: Yes. Luteal function is defective and luteolysis is enhanced in the granulosa cells of IVF cycles triggered with a GnRH agonist.

What is known already: Pregnancy rate declines in antagonist IVF cycles when ovulation is triggered with a GnRH agonist without hCG support and fresh embryo transfer is performed. But it is understudied at molecular level if luteal function is defective that can explain lower pregnancy rates in these IVF cycles and if hCG administration improves it. We therefore analyzed and compared molecular characteristics of the luteal granulosa cells obtained from good responder patients undergoing antagonist IVF cycles triggered with a GnRH agonist vs. hCG.

Study design, size, duration: A translation research study utilizing ex vivo models of human luteal granulosa cells obtained during oocyte retrieval procedure from IVF patients (IRB approval #: 2016.262.IRB2.123).

Participants/materials, setting, methods: Luteal granulosa cells were obtained from 42 antagonist IVF cycles triggered with recombinant hCG ($n = 24$) or GnRH agonist leuprolide acetate ($n = 18$). Steroidogenic enzyme and receptor expressions were analyzed by qRT-PCR. Autophagy, the predominant form of granulosa cell death during luteolysis was investigated with specific markers (LC3B-I/II) on western blot. A subset of the cells from agonist triggered cycles were treated with recombinant hCG (10 IU/mL) to investigate if hCG administration improves their luteal function.

Main results and the role of chance: There was a significant reduction in the viability (56% vs. 94% respectively, $p<0.001$) along with increased autophagic death of the luteal granulosa cells of agonist triggered IVF cycles compared to hCG triggered ones. The expression of LH receptor, 3β-HSD, progesterone receptor, VEGF-A/B/C and E2/P productions were significantly lower in these cells compared to hCG triggered ones. hCG (10 IU/mL) treatment for 24 h partially reversed these findings and improved progesterone production from these cells compared to their counterparts not treated with hCG.

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IVF characteristics

	hCG trigger	Agonist trigger	P
Age	33.7 ± 2.5	33.4 ± 1.8	0.32
FSH day3	6.7 ± 1.4	5.8 ± 1.7	0.33
E2 day 3	47.7 ± 14	51.3 ± 12	0.27
E2 hCG day	2082 ± 730	3783 ± 1177	**0.0014**
P hCG day	0.8 ± 0.3	1.2 ± 0.5	**0.03**
Total oocyte number	10.2 ± 4.2	15.6 ± 3.2	**0.0012**
Mature oocyte number	8.7 ± 2.6	12.6 ± 2.3	**0.0056**

Molecular luteal characteristics	hCG trigger (reference)	Agonist trigger before hCG treatment	Agonist trigger after hCG treatment
LH receptor	1[a,c]	0.43 ± 0.01[a,b]	0.81 ± 0.04[b,c]
P receptor	1[a,c]	0.51 ± 0.02[a,b]	0.91 ± 0.05[b,c]
3b-HSD	1[a,c]	0.34 ± 0.01[a,b]	0.88 ± 0.05[b,c]
Aromatase	1[a,c]	0.67 ± 0.04[a,b]	0.28 ± 0.03[b,c]
VEGF	1[a,c]	0.2 ± 0.01[a,b]	0.6 ± 0.03[b,c]
In vitro P production (ng/mL)	1581 ± 186[a,c]	164 ± 16[a,b]	466 ± 35[b,c]
In vitro E2 production (pg/mL)	2328 ± 147[a,c]	1477 ± 106[a,b]	978 ± 106[b,c]

a:p<0.05, b:p<0.01, c:p<0.01 for aromatase.
a:p<0.001, b:p<0.01, c:p<0.05 for LH and P receptor,3β-HSD, VEGF, in vitro P and E2.

Limitations, reasons for caution: Reduced IVF success after agonist trigger may not be solely attributed to defective luteal function as some other mechanisms might be involved in the process.

Wider implications of the findings: Increased luteolysis along with reduced VEGF expression and in vitro estradiol and progesterone production in luteal granulosa cells after agonist trigger not only provide molecular evidence for lower pregnancy rates in these cycles but also explain why this strategy should be adopted to reduce the risk of OHSS in hyper-responders.

Trial registration number: None

P-680 The usage of Daphaston and hMG protocol in normalovulatory women undergoing controlled ovarian hyperstimulation during IVF/ICSI treatments in combination with embryo cryopreservation

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Study question: Was the delivery of dydrogestrone (Daphaston) from the early-follicular phase feasible to suppress the premature luteinizing hormone (LH) surge in normalovulatory women undergoing in vitro fertilization(IVF)/intracytoplasmic sperm injection(ICSI) treatments?

Summary answer: Daphaston can effectively block the premature LH surge in normalovulatory women undergoing controlled ovarian hyperstimulation (COH).

What is known already: Thanks to vitrification techniques, the concept of progesterone blocking estrogen(E_2) -induced positive feedback was recently extended to the the early follicular phase. It was known that the bioavailability and the clinical effects differ with different types of progesterone formulation. Hitherto, there has been no investigation evaluating the efficacy of dydrogesterone (brand name: Daphaston), a kind of synthetic progesterone, for the inhibition of premature LH surge during COH.

Study design, size, duration: 250 women were recruited from October 2015 to July 2016 in this prospective trial and participants were allocated randomly to the Daphaston group and the Utrogestan group.

Participants/materials, setting, methods: Normalovulatory patients with 25-40years old were enrolled. Daphaston 20 mg per day or Utrogestan 100 mg per day was taken orally from cycle day3 until the trigger day, with hMG 150/225IU was injected alternatively. When the dominant follicles reached mature, GnRH-a 0.1 mg was used for trigger. Viable embryos were cryopreserved in both protocols for later transfer. The hormone profile, embryo results and pregnant outcomes were analysed exhaustively to discriminate the differences between the two groups.

Main results and the role of chance: Consistent LH suppression was achieved during COH in most patients, none of the participants experienced a premature LH surge. No significant between-group differences were observed in the number of oocytes retrieved (8.22 ± 5.46 vs. 8.8 ± 5.62, P > 0.05), mature oocytes (7.2 ± 4.72vs. 6.98 ± 4.68, P > 0.05), fertilized oocytes (6.16 ± 4.34 vs. 6.32 ± 4.23, P > 0.05), viable embryos (2.96 ± 2.22 vs. 3.4 ± 2.54, P > 0.05). Furthermore, the clinical pregnancy rate(53.04% vs. 51.7%, P > 0.05), early miscarriage rate(8.2% vs. 11.84%, P > 0.05), implantation rate(38.68% vs. 35.71%, P > 0.05) and cumulative pregnancy rate per woman(66.67% vs. 69.47%, P > 0.05)were also comparable.

Limitations, reasons for caution: A major limitation of our study is the limited number of participants enrolled. In addition, the unfinished FET cycles as well as the limited data on neonatal outcomes also contribute to decrease the power of the study.

Wider implications of the findings: Our findings corroborated the validity of Daphaston in the prevention of premature LH surges. It is of great significance not only in the application, optimization and popularization of progesterone protocol, but also in the establishment of a more patient-friendly stimulation protocol in combination with embryo cryopreservation.

Trial registration number: The trial was registered with the Chinese Clinical Trial Registry (ChiCTR-IOR-15007265).

P-681 Ontogeny of newly described hypo-androgenic PCOS phenotype in infertile women from younger through older ages

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Study question: What is the ontogeny over advancing age of women with hypoandrogenic polycystic ovary syndrome (hPCOS)?

Summary answer: hPCOS can already be identified in young women in age-stratum <35 years, and changes in phenotype with age.

What is known already: hPCOS is a surprisingly frequent PCOS phenotype in older infertile women, characterized by disproportionally high anti-Müllerian hormone (AMH) for age and/or follicle stimulating hormone (FSH) levels. It if

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further characterized by age-specific hypo-androgenism of adrenal origin and is strongly associated with autoimmunity, especially anti-thyroid autoimmunity.

Study design, size, duration: We extracted data on 708 consecutive infertility patients, and separated them into three age-strata, <35, 36-42, and >42 years. In each stratum, we investigated how levels of AMH and testosterone (T) interrelate between high-AMH (AMH $\geq 75^{th}$ quantile) and normal AMH (25th - 75th quantile) and low-T (total testosterone $\leq$ 19.0 ng/dL), normal-T (19.0-29.0 ng/dL) and high-T (>29.0 ng/dL).

Participants/materials, setting, methods: Routine in vitro fertilization (IVF) cycle outcomes and clinical phenotypes of patients were then compared between groups with AMH and T as variables.

Main results and the role of chance: The hPCOS phenotype already exists in age stratum <35 years. It likely arises from a lean, initially hyper-androgenic PCOS phenotype that develops autoimmune-induced insufficiency of the adrenal zona reticularis (low-T and low-DHEAS) as well as zona fasciculata (low-C) in comparison to controls. Adrenal insufficiency in these patients, thus, concomitantly affects adrenal androgen and glucocorticoid production (mineralocorticoid were not investigated).

Limitations, reasons for caution: Data are based on a relatively small cohort of patients treated at a single fertility center.

Wider implications of the findings: At least partially, this phenotype represents under Rotterdam Criteria PCOS Phenotype-D. The ontogeny of at least some Phenotype-Ds at young ages is, therefore, likely driven by adrenal autoimmunity, supporting the Androgen Excess and PCOS Society which claims that Phenotype-D varies in etiology from classical hyper-androgenic PCOS.

Trial registration number: n/a.

P-682 Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials

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Study question: Is inositol, a nutritional supplement, associated with improved ovulation, clinical pregnancy and live birth in women with polycystic ovary syndrome?

Summary answer: Inositol is associated with significantly improved ovulation rate and regularised menstrual cycles; however data is limited on clinical pregnancy and no data on live birth.

What is known already: Polycystic ovary syndrome is a common cause of anovulation and infertility, and a risk factor for development of metabolic syndrome and endometrial cancer. Inositol has been reported to improve glycaemic and hormonal factors in women with PCOS.

Study design, size, duration: Systematic review and meta-analysis of randomised controlled trials that evaluated the effects of inositol as an ovulation-induction agent.

Participants/materials, setting, methods: We searched MEDLINE, EMBASE, Cochrane library, ISI conference proceedings, Randomised Controlled Trial Number, Register and Meta-register for RCTs and WHO trials' search portal for relevant studies. We included studies that compared inositol with placebo or other ovulation induction agents. Quality of studies was assessed for risk of bias. We pooled the results using random effects meta-analysis and reported the findings as relative risk (RR) for dichotomous variables, and standardized mean differences (SMD) for continuous estimates.

Main results and the role of chance: We included 10 randomised trials which enrolled a total of 601 women; 8 studies compared myo-inositol or di-chiro-inositol with placebo, one study compared Myo-inositol with Di-chiro-inositol with placebo, one study compared Myo-inositol with metformin, and none compared myo-inositol with clomiphene.

Inositol was associated with significantly improved ovulation rate (RR 2.3; 95% CI 1.1, 4.7; $I^2 = 75\%$) and regularised menstrual cycles (RR 6.8; 95% CI 2.8, 16.6; $I^2 = 0\%$) compared with placebo. Only one study reported on clinical pregnancy rate with inositol compared with placebo (RR 3.3; 95% CI 0.4, 27.1), and one study compared with metformin (RR 1.5; 95% CI 0.7, 3.1). No studies evaluated live birth rates and miscarriage rates as an outcome.

There was a significant reduction in levels of biomarkers such as total androgens, total testosterone, free testosterone and serum DHEA levels with inositol than placebo. The glycaemic parameters of serum fasting insulin, fasting glucose, HOMA and insulin area under the curve and Glucose/Insulin ratio were significantly improved with inositol.

Limitations, reasons for caution: Data on clinical pregnancy rate is limited to only one study. There is no data available on live birth and miscarriage rate. There is a lack of data on long term outcomes.

Wider implications of the findings: Inositol appears to regulate menstrual cycles, improve ovulation and induce metabolic changes in PCOS. If found to be effective with well-designed multicentre trial, inositol supplementation, alongside lifestyle advice could become a first line treatment to improve fertility, and reduce the burden of endometrial hyperplasia and malignancy in women with PCOS.

Trial registration number: NA.

P-683 Krüppel-like factor12 induced granulosa cell apoptosis by repressing SPHK1 expression

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Study question: Does KLF12 regulate granulosa cell apoptosis and follicular atresia?

Summary answer: KLF12 induces granulosa cell apoptosis by transcriptionally repressing SPHK1, which decreases S1P production and the post-translational modification of FOXO1.

What is known already: S1P, formed by the phosphorylation of the sphingisine catalyzed by sphingosine kinase, significantly inhibits granulosa cell apoptosis via PI3K/AKT signaling pathway. Forkhead box Os (FOXOs), a subfamily of forkhead box transcriptional factors, are well known to play essential roles in many cellular processes, including proliferation, differentiation, and apoptosis. It has been reported that oxidative stress induces FOXO1 nuclear translocation and activation in granulosa cells, resulting in increased atretic follicles in mouse ovaries.

Study design, size, duration: KGN and mouse granulosa cells (mGCs) were used to investigate the molecular mechanisms in vitro. We further address the mechanism of granulosa cell apoptosis and follicular atresia through KLF12-mediated SPHK1/S1P pathway in Klf12 knock-in mice model. This is an experiment study lasted one year.

Participants/materials, setting, methods: KGN and mGCs were used as study models. Western blotting, quantitative PCR, cell death detection assay and human S1P ELISA were used to determine the mechanism of granulosa cell apoptosis induced by KLF12.

Main results and the role of chance: We found that the pro-apoptotic stimuli H_2O_2 efficiently promoted KLF12 expressioninKGN cells.KLF12 significantly induced granulosa cell apoptosis and caspase-3 activation. Moreover, Western Blot analysis revealed that the levels of AKT(S473) and FOXO1(S256) phosphorylation decreased and FOXO1 acetylation increased in KLF12-overexpressingKGN cells.In addition, the expression of SPHK1 and the intracellular levels of pro-survival S1Pwere negatively regulated by H_2O_2 and KLF12 in KGN cells.Furthermore, FOXO1 acetylation and pro-apoptotic gene activation induced by H_2O_2 and KLF12 were also reversed by SPHK1 overexpression in KGN cells.

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Limitations, reasons for caution: This is an in vitro study utilizing KGN and mGCs. The relationship between KLF12 and SPHK1 should be further evaluated. Furthermore, the results obtained should be confirmed by using ovarian granulosa cell conditional Klf12 knock-in mice in vivo.

Wider implications of the findings: This is an in vitro study utilizing KGN and mGCs. The relationship between KLF12 and SPHK1 should be further evaluated. Furthermore, the results obtained should be confirmed by using ovarian granulosa cell conditional Klf12 knock-in mice in vivo.

Trial registration number: None.

P-684 Study of Foxo3a, FoxL2, p27 and PTEN gene expression in ovarian tissue in women with premature ovarian insufficiency (POI) and polycystic ovary syndrome (PCOS)

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Study question: Is the expression of Foxo3a, FoxL2, p27 and PTEN altered in ovarian tissue in women with premature ovarian insufficiency (POI) and polycystic ovary syndrome (PCOS)?

Summary answer: Foxo3a and FoxL2 are under-expressed in ovarian tissue in women with POI compared to controls; however, no such pattern was identified in women with PCOS.

What is known already: Studies in mutant mouse models have revealed that loss of Foxo3a, FoxL2, p27 and PTEN (suppressors of follicular activation) function may cause global activation of the primordial follicle pool and depletion of the follicle reserve, resulting in POI. Likewise, alterations of follicle function in PCOS ovaries include increased primordial follicle activation, suggesting a possible similar genetic background for these disorders. FoxL2 is the only one of the four investigated genes, whose mutations is linked with POI in humans (Blepharophimosis-ptosis-epicanthus syndrome and cases of non-syndromic POI).

Study design, size, duration: The expression of Foxo3a, FoxL2, p27, PTEN genes was studied in ovarian tissue obtained from women diagnosed with POI, versus women with PCOS and versus healthy control women with normal menstruation. The patients were recruited in the Gynaecology Department of Patras University Hospital between the years 2014 and 2016 (the study is ongoing). The ovarian biopsy was performed during planned or acute laparoscopic pelvic surgery for benign disorders. All participants gave written informed consent.

Participants/materials, setting, methods: Ovarian tissue was obtained from five women with POI, three women with PCOS and six age matched healthy controls. The gene expression of Foxo3a, FoxL2, p27, PTEN and beta-Actin (housekeeping gene) was studied using reverse transcription PCR and real time PCR. The quality of the PCR reactions was confirmed by melting curve analysis and the $2^{-\Delta\Delta Ct}$ algorithm was used to analyze the relative expression of the target genes in comparison to the housekeeping gene beta-Actin.

Main results and the role of chance: The preliminary results of our study revealed a statistically significant 2.66-fold decrease in FoxL2 gene expression in ovarian tissue in women with POI compared to healthy controls ($p = 0.029$). Moreover, statistically significant under-expression of Foxo3a was demonstrated in the POI group compared to both PCOS patients ($p = 0.0436$) and controls ($p = 0.0131$). Our study did not identify a statistically significant difference in FoxL2 and Foxo3a expression between PCOS patients and controls ($p > 0.05$ for both). As far as PTEN and p27 gene expression is concerned, no statistically significant difference between POI patients and control patients was shown; however, a statistically significant decrease in PTEN and p27 gene expression was revealed in POI patients compared to PCOS patients ($p = 0.0286$ and $p = 0.0083$ for PTEN and p27 expression respectively).

Limitations, reasons for caution: Although we managed to achieve a ratio of POI patients and healthy controls of nearby 1/1, the study size was small. The current presentation contains preliminary data, as the study is still ongoing.

Wider implications of the findings: Our findings support literature data regarding the role of FoxL2 and Foxo3a in pathogenesis of POI not only in mice but also in humans. As suppressors of primordial follicle activation and genetic causes of POI, FoxL2 and Foxo3a may prove to be of great diagnostic-therapeutic value in the future.

Trial registration number: Not Applicable.

P-685 L-carnitine supplementation increases the expression of SDHA gene in granulosa cells and improves oocyte maturation and embryo quality in aging women undergoing IVF

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Study question: Could the supplementation of L-carnitine improve controlled ovarian stimulation (COS) and IVF results and mitochondrial function in granulosa cells in aging women undergoing IVF?

Summary answer: The supplementation of L-carnitine Increases the numbers of mature oocytes and good quality embryos and *SDHA* expression in granulosa cells in aging women undergoing IVF.

What is known already: Female fertility decreases rapidly after age 37. Diminished ovarian reserve is the major contributing factor for infertility with aging. Ovarian aging has shown to be accompanied by mitochondrial dysfunction. The processes of oocyte maturation require energy, which is provided by mitochondria. Carnitine plays a critical role in energy production. It transports fatty acids into the mitochondria so they are oxidized to produce energy.

Study design, size, duration: This retrospective cohort study included 102 consecutive IVF/ICSI cycles in 102 infertile patients over 37 years who were given L-carnitine at a dose of 330 mg twice a day orally for 60-90 days before and during COS period (study group, n = 53) or were not given L-carnitine (control group, n = 49) in our center between January 2015 and March 2016.

Participants/materials, setting, methods: If patients underwent two or more cycles of IVF/ICSI during the study period, charts corresponding to the 1st IVF/ICSI cycle were reviewed and data of other IVF/ICSI cycles except 1st cycle were excluded from this analysis. The expression of *SDHA* mRNA in granulosa cells obtained during oocyte pick-up was analyzed by realtime RT-PCR. Chi-square test and Fisher's exact test were used to compare fraction. Statistical significance was defined as $P < 0.05$.

Main results and the role of chance: There were no significant differences in patient's characteristics between the study and control groups. Total dose and days of gonadotropins used for COS were similar between the two groups. The number of oocytes retrieved was also comparable. However, the numbers of mature oocytes, fertilized oocytes and grade I or II embryos were significantly higher in the study ($p < 0.001$, $p < 0.001$, $p = 0.003$, respectively). Relative amount of *SDHA* mRNA in the ganulosa cells was significantly higher in the study group of 9.44 ± 3.94 compared with 4.10 ± 2.36 in the control group ($p < 0.001$). Clinical pregnancy rate seemed to be higher in the study, but the difference did not achieve the statistical significance.

Limitations, reasons for caution: This study may have a limitation to assess the efficacy of L-carnitine due to a small number of sample available and its retrospective nature. In addition, no study has been conducted on the appropriate dose of L-carnitine for infertile women with advanced age.

Wider implications of the findings: L-carnitine supplementation before and during COS period is a feasible and effective adjuvant treatment to improve the oocyte and embryo quality through the mitochondrial activation in aging women undergoing IVF.

Trial registration number: None.

P-686 Deficiency of adrenomedullin 2/intermedin (ADM2/IMD) in oocytes leads to reduced fertility in mice

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Study question: We hypothesized that oocyte-derived adrenomedullin 2/intermedin (ADM2 or IMD) could play a critical role in the regulation of ovarian folliculogenesis and fertility.

Summary answer: Although oocyte-specific knockout of *Adm2* significantly increased ovulated oocytes after superovulation treatment, ADM2 deficiency led to significant reduction of fertility.

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What is known already: ADM2 and related adrenomedullin (ADM) peptides signal through the heterodimeric CLR/RAMP1, 2, and 3 receptors. These peptides have been shown to play a role in the maintenance of uterine blood flow and cardiovascular adaptation during pregnancy. In addition, ADM is essential for normal embryo implantation and embryogenesis. On the other hand, ADM2 has been shown to represent an oocyte-derived ligand that regulates cell interactions in cumulus-oocyte complexes (COCs), and the suppression of ADM2 signaling in rat ovaries *in vivo* leads to oocyte atresia and aberrant cell cycle progression in follicular cells.

Study design, size, duration: To test the hypothesis that ADM2 plays a role in the regulation of ovarian folliculogenesis and fertility, we generated mice with oocyte-specific knockout of *Adm2*. These mice were generated by crossing mice carry a LoxP-flanked *Adm2* exon 3 transgene cassette and mice with a zona pellucida 3 (Zp3) promoter-Cre recombinase transgene. The Zp3-Cre transgene allows specific deletion of *Adm2* exon 3 in growing oocytes prior to the completion of the first meiotic division.

Participants/materials, setting, methods: Mice were bred to generate animals that have heterozygous or homozygous deletion of the *Adm2* gene in oocytes. The expression of *Adm2* transcript was determined by quantitative PCR. Female mice with wild-type, heterozygous, or homozygous genotypes were stimulated with gonadotropins at 28 days of age to induce synchronized ovulation. The number of fertilized and unfertilized oocytes in oviducts was determined after manual dissection.

Main results and the role of chance: The heterozygous female mice are fertile and appear to have no reproductive abnormality. On the other hand, mice with homozygous deletion have significantly reduced fertility, perhaps due to aberrant embryogenesis ($p < .01$). Stimulation with gonadotropins led to superovulation in all genotypes of animals. Interestingly, the number of ovulated oocytes from heterozygous and homozygous animals was significantly higher than that of wild-type littermates ($p < .01$), and this increase appeared to be gene copy-dependent. In addition, we found that a large fraction of fertilized eggs from homozygous animals were arrested at early stages of development.

Limitations, reasons for caution: Although mice with ADM2 deficiency in oocytes have increased number of ovulated oocytes following superovulation, they actually have reduced fertility. Therefore, the exact roles of oocyte-derived ADM2 in the process of folliculogenesis and embryogenesis remain to be fully vetted.

Wider implications of the findings: Our data strongly implicate that, in addition to functioning as an anti-apoptosis factor in COCs, ADM2 may play a follicular compartment-specific regulatory role in the regulation of follicle recruitment, and act as a survival factor during early embryo development.

Trial registration number: Not applicable.

P-687 Coasting revisited: Duration and drop in Estradiol levels affects IVF outcome

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Study question: To assess the effect of percentage drop in Estradiol (E_2) level and duration of coasting on IVF outcomes.

Summary answer: The overall LBR was 44%. However, this is compromised when coasting is prolonged (>7days) and the percentage of E2 drops is >60%.

What is known already: Ovarian hyperstimulation syndrome (OHSS) is a complication of fertility treatment with varying severity. The combined incidence of moderate or severe OHSS ranges from 3.1 to 8%. Serum estradiol (E2) measurement remains an integral part of cycle monitoring in most IVF treatments.The pattern of rise in E2 is a useful tool in the identification of both women at risk of OHSS and poor responders. Coasting is a strategy to minimise severe OHSS. It involves withdrawing exogenous gonadotrophins and withholding the hCG trigger until the serum estradiol level decreases to a 'safer' level.

Study design, size, duration: Data was collected prospectively on 97 IVF cycles where coasting occurred for 2 or more days between February 2010 to March 2016. The decision to coast was based on E2 levels,and the hCG trigger was administered when the serum E_2 level was <15000 pmol/l. Data was analysed based on the numbers of days of coasting and the percentage drop in E_2 levels. The primary outcome was Live Birth Rate (LBR).

Participants/materials, setting, methods: 97 coasted cycles were analysed. The primary outcome measure was Live birth rate and secondary outcomes were the mean number of oocytes retrieved, fertilised embryos, overall fertilisation and implantation rate. Outcome measures were analysed based on the number of days of coasting and the percentage drop in E_2 levels.Statistical analysis was performed using Microsoft Excel. Mann-Whitney test and Fisher exact test or chi-squared test were used for analysis of effect respectively.

Main results and the role of chance: The mean age, E_2 at coasting, peak E_2 and E_2 at HCG trigger were 34.82 ± 3.79, 18722 ± 6406 pmol/l, 26071 ± 7582 pmol/l and 13299 ± 4750 pmol/l respectively.Coasting ranged between 2-11 days(mean 3.64 ± 1 .68). The mean number of oocytes retrieved, fertilised embryos, overall fertilisation, implantation and LBR were 10.62 ± 5.53, 6.80 ± 4.47, 94%, 56% and 44% respectively. Coasting days divided into three groups: group I (n = 54) –coasting <3 days only, group II (n = 37) coasting ≥4–6 days and group III (n = 6) ≥7 days. LBR in group I, II and III were 51.85%, 37.82% and 16.67% (P = 0.04) respectively. Data was further analysed to determine the effect of percentage change in E_2. Group A (1-50%), Group B (51-60%), Group C(>61%). LBR were 47.06%, 53.33% and 35.48% respectively (P = 0.02).

Limitations, reasons for caution: None

Wider implications of the findings: Several papers have suggested a significant compromise with duration of coasting beyond 2.6 days. However, these studies are limited by the varying methods and outcome measure. Our findings are in keeping with an earlier retrospective study which showed that coasting for up to 8 days did not affect LBR.

Trial registration number: Not applicable.

P-688 Existence and physiological significance of osteopontin in human follicular fluid and blood during the in vitro fertilization cycle

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Study question: This study was aimed at investigating the presence and physiological significance of osteopontin (OPN) in follicular fluid and blood in the human ovarian cycle.

Summary answer: The blood OPN concentration was positively correlated with OPN and VEGF concentration in follicular fluid irrespective of the blood sampling period.

What is known already: OPN is a secreted glycosylated phosphoprotein that is distributed in a wide variety of tissues and involved in various physiological and pathological processes. Recently, we have reported that Opn expression is markedly upregulated in mouse ovarian granulosa cells (GCs) in response to a gonadotropin surge through epidermal growth factor receptor signaling. By using cultured GCs, we further demonstrated experimental evidence showing that OPN promotes progesterone synthesis and VEGF production through PI3K/AKT signaling during the early luteal phase (Kuwabara et al., Journal of Endocrinology, 2015).

Study design, size, duration: Twenty-two women undergoing in vitro fertilization with a minimal stimulation protocol with clomiphene from February to May 2016 participated in this study. Sample collection was performed on the third day of withdrawal bleeding (a), 2 days before oocyte retrieval (b), and on the day of oocyte retrieval (c).

Participants/materials, setting, methods: Blood was collected in Spitz tubes containing EDTA and centrifuged at 2500 rpm for 15 min. The supernatant was transferred to a separate container and stored at -70°C until measurement. The follicular fluid collected during oocyte retrieval was also prepared in the same way. The OPN concentration in each specimen and the VEGF concentration in follicular fluid were measured by using ELISA, and the statistical correlation between each specimen was examined.

Main results and the role of chance: The plasma OPN concentrations were as follows: (a) 372.3 ± 69.3 ng/mL (median: 287.9 ng/mL), (b) 357.2 ± 63.0 ng/mL (median: 288.5 ng/mL), and (c) 381.5 ± 73.3 ng/mL (median: 278.4 ng/mL), with no significant difference between groups. The OPN concentration in follicular fluid was 106.2 ± 13.4 ng/mL (median: 70.94 ng/mL), and a positive correlation was found between plasma samples

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(a: r = 0.48 p < 0.01, b: r = 0.51 p<0.02, c: r = 0.49 p<0.05). No correlation was found between VEGF concentration in follicular fluid and predictive factors of ovarian hyperstimulation syndrome (OHSS), including anti-Müllerian hormone, estradiol, and luteinizing hormone/follicle-stimulating hormone in serum; however, the follicular fluid VEGF concentration was positively correlated with the plasma OPN concentration (a: r = 0.65 p<0.01, b: r = 0.79 p<0.01, c: r = 0.77 p<0.01).

Limitations, reasons for caution: Because the sample size of this study is small, further investigation with more patients is necessary to confirm our preliminary findings.

Wider implications of the findings: OPN is considered to be involved in ovarian VEGF production, as suggested by the mouse experiment. Because the serum OPN concentration showed a positive correlation with the follicular fluid VEGF concentration at oocyte retrieval, OPN is suggested to be an independent clinical marker for evaluating the risk of OHSS.

Trial registration number: Not appliacable.

P-689 Ovarian reserve, as assessed by measuring serum anti-Müllerian hormone levels, declines more rapidly than expected in a longitudinal cohort of rheumatoid arthritis patients

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Study question: Does ovarian function, as measured longitudinally by serum anti-Müllerian hormone (AMH) levels, show a faster decline in women with chronic inflammation, like rheumatoid arthritis (RA)?

Summary answer: In RA, serum AMH levels declined faster over time than in controls, indicating that chronic inflammation compromises the ovarian function.

What is known already: A compromised ovarian function has been described in patients with type II diabetes mellitus and in young girls with cancer, suggesting that an unhealthy soma results in an early decline of ovarian reserve. It is unknown whether the same holds true for chronic inflammation. RA is an example of a chronic inflammatory disease, which is known to compromise fertility, and often affects women in their fertile age. Serum AMH levels are a proxy for the size of the ovarian follicle pool, and as such are the most reliable predictor of the age at which menopause sets in.

Study design, size, duration: In a prospective nationwide cohort on reproduction in RA (PARA study, 2002 – 2008, N = 297), 128 (43%) women were re-assessed in 2015-2016, 10.7 ± 1.8 years after the first study visit. From the original PARA study only serum samples from the preconception period and 6 months postpartum were used.

Participants/materials, setting, methods: All women had established RA according to the 1987 revised American College for Rheumatology (ACR) criteria. Serum AMH levels were measured using the pico AMH assay (provided by Ansh Labs, Texas, USA). AMH levels were compared to a cross-sectional healthy control group (n = 554) (Lie Fong 2012). A linear mixed model was built to assess the effect of RA related clinical factors on the decline of serum AMH levels over time.

Main results and the role of chance: 128 women were re-assessed at a mean age of 42.6 ± 4.4 years, with a median disease duration of 15.8 (IQR 12.7 – 21.5) years. The mean age at baseline was 31.8 ± 3.8 years. The participants appeared to be a more fertile selection of the original PARA cohort, with less participants (4.7%) than non-participants (22%) being nulliparous at the end of the original PARA study, and less smokers in participants (7%) than in non-participants (17%). Nonetheless, at follow-up, more patients had AMH levels below the 10th percentile of controls (39%; 95%CI 31–48%), compared to their AMH levels at baseline (16% (95%CI 9.3–22%) under the 10th percentile of controls). The linear mixed model showed a significant effect of age, but no significant effect of RA related factors on the decline of serum AMH levels over time.

Limitations, reasons for caution: This study was performed in a selected population (response rate 43%). Since participants appeared to be more fertile, the steeper AMH decline may be even more pronounced in the total RA population. The use of a conversion factor for AMH levels in the healthy control group should be interpreted cautiously.

Wider implications of the findings: The findings in this study support the idea that also in chronic inflammatory conditions, the body is less fit for reproduction, resulting in a faster decline of ovarian function and probably an earlier age at menopause. Optimal treatment of chronic inflammation in an early phase may improve long-term women's health.

Trial registration number: NA.

P-690 The effect of 6 months weight -loss/maintenance on anthropometric, biochemical and psychological profile in Lebanese PCOS women: A prospective randomised control study

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Study question: Does a 6 months weight -loss/maintenance improves anthropometric, biochemical and psychological profile in Lebanese PCOS women?

Summary answer: Conclusion: A 6-month hypo-caloric weight-loss/maintenance program has shown to be effective in Lebanese PCOS women by decreasing their weight and improving their health status.

What is known already: Polycystic ovarian syndrome (PCOS), the most common cause of anovulatory infertility, is a disorder of the endocrine system affecting 5-10% worldwide of women in their reproductive age. Nowadays, PCOS is a major public health concern accompanied with different metabolic and endocrine abnormalities. Obesity usually co-exists with PCOS and leads to complications such as increased androgen level, hirsutism, infertility, anovulation, type 2 diabetes, and cardiovascular diseases. Research for possible treatments and intervention options leading to disease improvement can be beneficial to treat these complications.

Study design, size, duration: A randomized prospective public health nutrition intervention was conducted. Seventy eight PCOS patients were subject to food frequency, physical and psychological activity (BDI-II and GAD-7) questionnaires, two 24-hour-recalls on weekdays and weekends, and biochemical analysis at baseline, 3 and 6 months from baseline. The duration for this study is 6 months.

Participants/materials, setting, methods: Setting: Patients were recruited from obstetrics and gynecology clinics at the American University of Beirut Medical Center (AUB-MC), Lebanon. **Subjects:** PCOS women grouped as: Group1: overweight/obese PCOS patients receiving weight-loss program and lifestyle modification, Group2: overweight/obese PCOS controls, Group3: Lean PCOS controls and Group4: lean PCOS patients receiving weight maintenance nutritional program and lifestyle modification.

Main results and the role of chance: After six months, 7% weight-loss was achieved in overweight/obese intervention groups and weight maintenance in lean intervention groups. There was a significant reduction in waist (-4.2 cm (±5.6)) and hip circumference (-3.1 cm (±3.5)) with P< 0.001.There was no significant (P > 0.001) improvement in biochemical analysis (fasting blood sugar, CRP (C-reactive protein), LDL-C (Low density lipoprotein cholesterol), HDL-C(High density lipoprotein cholesterol), TG (Triglycerides), total cholesterol, fasting insulin, total testosterone, Vitamin D). Physical activity increased (3.1 hours/week (±1.5)). Anxiety and depression scores decreased (BDI-II (Beck depression inventory) and GAD-7 (Generalized anxiety disorder assessment)); -0.8 (±0.8) and -0.7 (±0.7) with P < 0,001 compared to controls.

Limitations, reasons for caution: This is a single centered (one medical center as a setting of the study) study done at the capital of Lebanon, which does not cover patients with different socio-economic and cultural status.

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Wider implications of the findings: Those who significantly lost weight demonstrated improvement in their reproductive and psychological abnormalities. The weight loss in this study Intervention groups depicted a deviation to a healthy lifestyle that affected their PCOS features. All PCOS patients should seek nutritional intervention in order to prevent further metabolic and reproductive complications.

Trial registration number: N/A.

P-691 New additional biomarker proposal for the diagnosis of polycystic ovary syndrome through serum midkine cut-off value with anti-Müllerian hormone in Turkish population: A prospective study

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Study question: Can midkine (MK) be used as a new additional biomarker with an old conventional biomarker anti-Müllerian hormone (AMH) for the diagnosis of polycystic ovary syndrome in Turkish population?

Summary answer: MK levels were increased in concomitant with AMH levels at the PCOS cases, thus it can be a accepted as a new additional biomarker.

What is known already: The abnormally elevated AMH levels in serum and follicle fluid of PCOS patients participate in the major steps of the anovulation, and are related to pathogenesis and pathophysiological characteristic of PCOS. MK, a heparin-binding growth factor/cytokine, promotes growth, survival, migration and gene expression of various target cells and play roles in many diseases. In normal adult tissues, MK expression is highly restricted. MK has a mitogenic effect on primordial germ cells and retionic acid promotes cytoplasmic maturation of oocytes through MK promoter in vivo models. The role of MK in infertility is still understudied.

Study design, size, duration: A prospective study of 220 women suffered from PCOS who were scheduled for intracytoplasmic sperm injection (ICSI) at hospital infertilityclinic during 2011-2016.

Participants/materials, setting, methods: This study included 130 PCOS patients and 90 proven fertile women aged 24-41 years. Serum levels of folicule stimulating hormone (FSH) and luteinizing hormone (LH) with the radioimmunoassay, estrodiol 2 (E2), prolactin (PRL) with the immunoradiometric test, AMH and MK levels with the enzyme-linked immunosorbent assay (ELISA) on cycle day 3 and body mass index (BMI), MII oocyte and fertilisation rates were all evaluated by using

ANOVA test and $p<0.05$ was considered statistically significant.

Main results and the role of chance: Mean values of hormone and MK levels for the control group (fertile women) were FSH 5.7 mIU/ml, LH 3.2 mIU/ml, E2 39.1 pg/ml, PRL 15.67 ng/ml, AMH 3 ng/ml and MK 250 pg/ml. For the PCOS group, these were FSH 5.4 mIU/ml ($p<0.05$), LH 5.6 mIU/ml ($p<0.0001$), E2 41.9 pg/ml ($p<0.05$), PRL 15.44 ng/ml ($p>0.05$), AMH 5.83 ng/ml ($p<0.00001$) and MK 420 pg/ml ($p<0.000001$). These data can be summarized as follows that LH, E2, AMH and MK levels were increased at the PCOS group, but FSH and PRL levels were found decreased. Consequently, the serum MK cut-off value was determined as 420 pg/ml for PCOS. BMI was similar between groups as 26.8 kg/m^2 and 27.3 kg/m2 for the control group and the PCOS group, respectively ($p>0.05$). MII oocyte rate was lower at the PCOS group (45 %) than at the control group (74 %) ($p< <0.00001$). The lowest fertilisation rate was determined at the PCOS group as 50 %, where it was 93 % at the control group ($p<0.000001$).

Limitations, reasons for caution: Only patients enrolled for ICSI were included in this study and the number of the study participants were also low. These may limit the generalizability of these findings.

Wider implications of the findings: The results of this study are in agreement with most previously published studies about the PCOS and AMH. No study was found about the role of MK in PCOS cases as well as its' mechanism of action with AMH. This data can be used in clinical practice internationally.

Trial registration number: This study with a trial registration number of 3/2011 was approved by ethical committee of Istanbul University, Cerrahpasa Faculty of Medicine.

P-692 FP receptor antagonist, OBE002, inhibits both PGF2α- and OT-induced contractions of human pregnant myometrium in vitro

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Study question: To demonstrate the effect of the FP receptor antagonist, OBE002, on suppressing $PGF_{2\alpha}$-induced myometrial contractions, and to investigate its potential effect on OT-induced contractions.

Summary answer: OBE002 showed significant, dose-dependent inhibition of both $PGF_{2\alpha}$- and OT-induced contractions of human myometrium. These findings suggest a possible interaction between FP and OT receptors.

What is known already: Prostaglandins play key roles in the onset of labour. $PGF_{2\alpha}$ has been shown to exert uterotonic effect via the FP receptor, and the contractile effects of OT have been reported to be partially regulated by $PGF_{2\alpha}$ by augmenting receptor expression. Antagonism of FP has been suggested to inhibit contractions in isolated sheep and human myometrium and to open BK Ca^{2+} channels. To demonstrate the tocolytic ability of OBE002, a novel FP receptor antagonist, it is critical to evaluate its effects *in vitro*.

Study design, size, duration: The inhibitory effects of OBE002 in both $PGF_{2\alpha}$- and OT-induced myometrial contractions were investigated in human pregnant myometrial strips. Myometrial tissues from 6 different patients were used for each experimental condition. All experiments were performed within 24 hours of tissue collection to ensure tissue viability.

Participants/materials, setting, methods: Myometrial strips were obtained from non-labouring women undergoing caesarean section at term. Baseline measurement of contraction frequency, peak, duration, work per contraction and total work were made using a DMT Myograph 800 MS. OBE002 was added (6, 60, 600 or 6000 nM) and effects upon spontaneous contractility measured in the next 10 min. Then agonists ($PGF_{2\alpha}$/OT) were added at 10, 100, and 1000 nM or 1, 10, and 100 nM, respectively, at 10 min intervals and contractility was recorded.

Main results and the role of chance: OBE002 suppressed the effect of $PGF_{2\alpha}$ on myometrial contraction ratio and peak amplitude in a dose-dependent manner and had a significant overall effect on the total work done at 600 nM ($p<0.05$ vs DMSO, ANOVA) and 6000 nM ($p<0.01$ vs DMSO, ANOVA). OBE002 also exerted a dose-dependent uterorelaxant effect on OT-induced contractions, affecting the contraction ratio and peak amplitude. A significant relaxant effect on OT-induced contractility was observed on addition of 6000 nM OBE002 ($p<0.05$ vs DMSO, ANOVA). Spontaneous myometrial contractility was reduced in presence of OBE002, however, this did not reach significance.

Limitations, reasons for caution: The mechanisms involved in the inhibition of $PGF_{2\alpha}$-induced myometrial contractions by OBE002 remains to be determined. In order to elucidate the exact link between FP and OT receptors, further experiments are required.

Wider implications of the findings: These studies confirm the effect of OBE002 as an FP antagonist in human tissues and provide evidence for receptor crosstalk between OT and FP receptors. Our findings raise a potential combinational therapeutic target for the management of term and preterm labour via the manipulation of the differential GPCR interactions/crosstalk.

Trial registration number: N/A.

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P-693 GnRH agonist (GnRHa) trigger for final oocyte maturation in fresh embryo transfer IVF/ICSI cycles – a systematic PRISMA review and meta-analysis

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Study question: Compared to hCG trigger, does GnRHa trigger and modified luteal LH activity support (LLS) reduce live birth rate (LBR) in IVF patients undergoing fresh transfer?

Summary answer: There was no significant difference in LBR when GnRHa trigger followed by modified LLS was compared to hCG trigger in fresh transfer cycles.

What is known already: The use of GnRHa as a final oocyte maturation trigger in oocyte donation and elective frozen embryo transfer cycles is well established due to lower OHSS rates than hCG trigger. Notwithstanding, a recent Cochrane meta-analysis concluded that GnRHa trigger is associated with reduced LBRs and increased miscarriage rates in fresh autologous IVF cycles compared to hCG. However, the evidence is not unequivocal, and some studies have found promising reproductive outcomes among couples undergoing GnRHa trigger and LLS. Due to the clinical implications of such intervention, the role of GnRHa trigger and LLS in fresh IVF cycles needs to be clarified.

Study design, size, duration: We conducted a systematic review and meta-analysis of randomized clinical studies published in English, as of December 14th 2016. An electronic search was performed within the PubMed and EMBASE databases, including a search within the references of relevant articles. The PRISMA statements for systematic reviews and meta-analysis were followed. We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) for assessing the quality of the evidence of all outcome measures.

Participants/materials, setting, methods: Participants were patients submitted to IVF/ICSI cycles using GnRH antagonist co-treatment undergoing fresh embryo transfer. The intervention was GnRH agonist trigger followed by modified LLS. The comparator was hCG trigger. The primary outcome measures were LBR and OHSS rate. The secondary outcome measures included the number of oocytes retrieved, M2 oocytes, the number of good quality embryos, clinical and ongoing pregnancy rates, and miscarriage rates. We conducted a thorough bias evaluation of the included studies.

Main results and the role of chance: A total of five publications met the selection criteria and were included in the qualitative / quantitative analysis, with a total of 859 patients. There was no statistically difference in live birth rate when comparing GnRHa to hCG group (Odds Ratio [OR] 0.77, 95% confidence interval [CI] 0.56, 1.05). Both ongoing and clinical pregnancy rates were close to unity, with an OR 0.96 (95% CI 0.64, 1.34; I^2 = 50%) and OR 0.98 (95% CI 0.73, 1.30; I^2 = 25%), respectively. OHSS were seen in a total of 4/413 cases in the GnRHa group compared to 7/413 in the hCG group (OR 0.48, 95% CI 0.15, 1.60). For miscarriage rate, a non-significant difference was observed between groups (OR 1.28; 95% CI 0.73-2.22). No significant differences were seen regarding number of oocytes retrieved, M2 oocytes and number of good quality embryos.

Limitations, reasons for caution: GRADE evidence was low regarding OHSS and very low regarding LBR. Caution should be applied when examining studies concerning LLS after GnRHa trigger in fresh transfer cycles. While earlier studies were designed as 'proof of concept', recent trials have optimized the method, albeit not adequately powered to draw firm conclusions

Wider implications of the findings: The GnRHa trigger group seems to have a slight, albeit non-significant reduction in the ORs for LBR; however, the most recent RCTs show ORs close to unity as regards the LBRs. The evidence suggests that GnRHa trigger is non-inferior to hCG regarding reproductive outcomes in fresh transfer antagonist co-treated cycles.

Trial registration number: Prospero registration number: CRD42016051091

P-694 Serum testosterone level is not a marker of ovarian reserve nor ART outcome for poor ovarian responders

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Study question: Is there a correlation between serum testosterone level and ovarian reserve tests, or assisted reproductive technology (ART) outcomes for poor ovarian responders?

Summary answer: No significant correlation was recognized between serum testosterone level and ovarian reserve tests, nor ART outcomes for poor ovarian responders.

What is known already: Testosterone, an androgen, has been shown to play an important role in follicular recruitment or development. In a recent meta-analysis, testosterone pretreatment during ART cycle resulted in an increased number of retrieved oocytes. Some researchers suggested an increased chance of live birth with testosterone supplementation, however more supporting data is still required to make such a conclusion. If supplementation of testosterone improves the outcome of ART and contributes to successful implantation, serum testosterone level would have a positive correlation with the number of retrieved oocytes and pregnancy rate in ART cycles.

Study design, size, duration: The present prospective control study is designed to identify correlation between serum testosterone level and ART outcome. Four hundred and forty-one women had blood test in order to record testosterone and anti-mullerian hormone (AMH) from January 2015 until December 2016. One hundred and fourteen poor responders fulfilled Bologna criteria, and they had IVF-ETs with ovarian stimulation in the same period.

Participants/materials, setting, methods: This study was performed at a private ART clinic located in Japanese urban area. Hormone data was available from all infertile women with various causes after written informed consent. Hormone testing methods were electro-chemiluminescence immunoassay for testosterone, and enzyme-linked immunosorbent assay for AMH gen II. The ART data was collected from medical records on the women who had IVF-ET with conventional or mild ovarian stimulation.

Main results and the role of chance: The average age of the participants was 39.2 (SD = 4.51, 25-49). The average testosterone was 0.16 ng/mL (SD = 0.12, 0-2.25). Testosterone from 151 women (34.2%) showed a below normal limit (0.11 ng/mL), and that of 46 women (10.4%) was below a detection sensitivity limit (<0.03 ng/mL). The coefficient of determination were R2 = 0.0036 (vs. age) and 0.047 (vs. AMH). The numbers of poor responders with lower testosterone (group A) and those with normal to high testosterone (group B) were 38 and 76, respectively. The profiles of the two groups on age and AMH were identical. The average number of retrieved oocytes in group A was 3.27 ± 2.29, and 3.21 ± 2.39 in group B. The clinical pregnancy rate (PR) in group A was 36.8%, and 25.0% in group B. The miscarriage rate (MR) in group A was 14.2%, and 26.3% in group B. There were no significant difference in the number of retrieved oocytes (p = 0.45), PR (p = 0.07) or MR (p = 0.67).

Limitations, reasons for caution: Small sample size, possible dispersion of the cause of their infertility or the patients' back ground.

Wider implications of the findings: One third of infertile women showed decreased serum testosterone level. Testosterone is independent from ovarian reserve markers. ART outcomes such as the number of retrieved oocytes, pregnancy rate and miscarriage rate are identical despite testosterone levels. The findings raise questions regarding the effect of testosterone supplementation on ART treatment.

Trial registration number: none.

P-695 GnRH agonist compared with hCG trigger for the induction of final oocyte maturation results in decreased oocyte competence in women aged 35-40 years

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Study question: Does the use of gonadotropin releasing hormone agonist (GnRHa) to trigger final oocyte maturation results in decreased oocyte and/or embryo competence compared with human chorionic gonadotropin (hCG)?

Summary answer: The results of this study suggest that GnRHa when used in hyper-responder women, aged 35-40 years, has a negative effect on oocyte and embryo competence.

What is known already: A single bolus of GnRHa can stimulate the release of LH and FSH that mimic the natural mid-cycle surge required for final oocyte maturation. However, this gonadotropin release is of shorter duration and results in significantly reduced amounts of gonadotropins released from the pituitary gland compared with natural surge. This results in limited exposure of the cumulus-oocyte-complex to endogenous gonadotropins. LH surge is important in keeping the activity of the gap junctions between the oocyte and granulosa cells in the final oocyte maturation stage. Whether the limited LH exposure following GnRHa trigger result in suboptimal oocyte maturation is still unknown.

Study design, size, duration: 272 hyper-responder women aged 35-40 years who underwent GnRH antagonist IVF cycles in which final oocyte maturation was achieved either by GnRHa (n = 168) or hCG (n = 104), and freeze-all embryos was performed due to increased risk of ovarian hyperstimulation syndrome (OHSS), from 2012-2014 were studied retrospectively. Embryo transfer was performed in subsequent warming cycles (n = 542). All the warming cycles per woman were included in the analysis until a live birth was achieved.

Participants/materials, setting, methods: Increased risk for OHSS was considered when peaked stimulated E2 levels >12000 pmol/l or the presence of >15 follicles of 12 mm in diameter. In these cases, cycle segmentation with freezing all embryos at the blastocyst stage was performed. Exclusion criteria included: cycles in which subsequent warming cycles was not performed and cycles in which embryo culture was not extended to the blastocyst stage. The primary outcome was the cumulative live birth rate.

Main results and the role of chance: Maternal age was similar (median 37 years, p = 0.88). Women in the GnRHa group required lower total gonadotropin dose (1795 vs. 2318 IU, p < 0.001), had a higher level of stimulated E_2 (13674 vs. 11499 pmol/l, p = 0.001), had a higher number of oocytes retrieved (22 vs. 21, p = 0.002) and a higher proportion of MII oocytes (78 vs. 74%, p = 0.045). Despite higher number of MII oocytes available in the GnRHa group, the number of 2pn embryos and the number of cryopreserved blastocysts (Median of 5 blastocysts in both groups) were similar. The GnRHa group underwent 370 frozen blastocyst transfer (FBT) cycles and the hCG group underwent 172 FBT cycles. The number of cycles per woman (2.2 vs. 1.65, p < 0.001) and the proportion of transferred blastocysts (68 vs. 53%, p = 0.01) were higher in the GnRHa group. When comparing the clinical outcomes, the hCG group had a higher implantation rate (48 vs. 39%, p = 0.008), needed a lower number of cycles to achieve live birth (1.32 vs. 2.12, p < 0.001) and the proportion of embryos thawed and transferred was significantly lower (33 vs. 57%, p < 0.001). The cumulative live birth rate was similar between the groups (48.15 vs. 48.08%, p = 0.90)

Limitations, reasons for caution: The retrospective nature of the study was a major limitation, the study included women aged 35-40 years and studies examining this effect in younger patients are needed.

Wider implications of the findings: Although the GnRHa induced surge is sufficient to yield adequate number of mature oocytes, the limited exposure of the cumulus-oocyte-complex result in suboptimal development which manifest in limited embryo formation and decreased implantation rates. The use of GnRHa should be limited to cases where increased risk of OHSS is present.

Trial registration number: N/A.

P-696 Clinical utility of the novel automated AMH assay as a diagnostic test for PCOS in women of Indian origin

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Study question: Can Anti-müllerian hormone (AMH) facilitate the diagnosis of PCOS in women of Indian origin?

Summary answer: AMH can facilitate the diagnosis of PCOS in women of Indian descent, with adequate sensitivity and specificity to enable its routine clinical use.

What is known already: Women of white descent with PCOS have higher serum AMH level than non-PCOS women. AMH has been proposed as a diagnostic test for PCOS in white women.

Study design, size, duration: This is a single centered prospective study of 441 Indian women undergoing infertility evaluation from January 2016 to January 2017.

Participants/materials, setting, methods: The study included women with polycystic ovarian morphology (PCOM) who met the Rotterdam criteria for PCOS (n = 75), women with PCOM only (n = 72) and controls (n = 294). AMH was measured with the Elecsys automated assay. We estimated the performance characteristics of AMH (sensitivity, specificity, optimal cut-off and area under the ROC (AUROC) for diagnosing PCOS.

Main results and the role of chance: Women with PCOS had substantially higher AMH (7.4 (IQR: 5.2 to 10.8) ng/ml) than women with PCOM (4.7 (IQR: 3.2 to 7.2) ng/ml) or women without PCOS (1.6 (IQR: 0.9 to 2.7) ng/ml) (p < 0.001). AMH greater than 4.3 ng/ml can discriminate women with PCOS from women without (controls and PCOM) with a sensitivity of 88% and specificity of 82% (AUROC of 0.92). Sensitivity analysis where we compared PCOS with women with non-PCOS male factor infertility (non-infertile) did not materially change this cut-off. AMH greater than 6.9 ng/ml discriminates PCOS from PCOM with a sensitivity of 60% and specificity of 74% (AUROC of 0.73).

Limitations, reasons for caution: Single centre study that used the Rotterdam Consensus PCOS criteria, alternative PCOS diagnostic criteria may require differential threshold values.

Wider implications of the findings: A serum AMH > 4.3 ng/ml is very sensitive to diagnose PCOS on women of Indian origin. This can facilitate diagnosis of PCOS in primary care when sonography is not available.

Trial registration number: N/A.

P-697 Prediction models for ovulation, conception, pregnancy and live birth in infertile women with polycystic ovary syndrome

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Study question: Can we predict ovulation, conception, pregnancy and pregnancy outcome in Chinese women with WHO type II anovulatory infertility?

Summary answer: In Chinese women with WHO type II, ovulation, pregnancy and live birth could be predicted from demographic, clinical and biochemical variables.

What is known already: Prediction models for pregnancy outcomes in infertile women with PCOS have been reported. And there are some models with good predictive performance that can be used reliably as a guide for making decisions about fertility treatment, in patients similar to the development population. However, there are no papers on prediction models for pregnancy in

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Chinese women with PCOS, in particular with the different stages of pregnancy.

Study design, size, duration: From July 2012 to October 2015 we performed a multicenter randomized clinical trial in 1000 Chinese women with PCOS (Rotterdam criteria). In a factorial design women were randomized to active/control acupuncture and clomiphene citrate (CC)/placebo up to four months. Here, we evaluate whether ovulation, pregnancy and live birth could be predicted.

Participants/materials, setting, methods: We used multivariate logistic regression analysis using significance level of 0.10 among 43 candidate variables. Interaction between selected variable and treatment were assessed. We constructed receiver operating characteristic (ROC) curves and calculated the areas under them (AUC) to assess the predictive power of models.

Main results and the role of chance: Among the 1000 women, 780 ovulated, 320 conceived, 218 were pregnant, of which 205 resulted in a live birth. Previous conception (OR = 1.70, 95%CI = 1.07-2.69) increased the probability of ovulation, while high free androgen index (FAI) level (OR = 0.94, 95% CI = 0.90-0.98) anti-Mullerian hormone level (OR = 0.95, 95%CI = 0.91-0.98), a longer menstrual cycle (OR = 0.99, 95%CI = 0.99-1.00) and smoking (OR = 0.38, 95%CI = 0.16-0.89) decreased ovulation chances. Among women who ovulated, higher difference between systolic and diastolic blood pressure (OR = 1.03, 95%CI = 1.00-1.06), E2 level (OR = 1.001, 95%CI=1.000-1.001) and previous pregnancy loss (OR=1.86, 95%CI=1.29-2.76) were predictors for conception. Among women who conceived, CC treatment (OR=0.53, 95% CI=0.27-1.00), higher sex hormone-binding globulin (SHBG) level (OR=0.99, 95%CI=0.98-1.00), FAI level (OR=0.85, 95%CI=0.77-0.94) and cholesterol level (OR=0.70, 95%CI=0.51-0.95) were predictive of lower chances of pregnancy. Among women who were pregnant, acupuncture treatment (OR=0.10, 95%CI=0.01-0.75), lower low density lipoprotein (OR=4.78, 95% CI=1.32-17.37), higher mean arterial pressure (OR=0.66, 95%CI=0.51-0.85), previous pregnancy loss (OR=0.08, 95%CI=0.01 = 0.61), and concurrent diagnosis of metabolic syndrome (OR=0.10, 95%CI=0.01-1.00) were significantly negatively associated with live birth. AUCs were 0.81 (95%CI=0.77-0.85) for the ovulation model, 0.66 (95%CI=0.61-0.71) for the conception model, 0.69 (95% CI=0.61-0.76) for the pregnancy model.

Limitations, reasons for caution: There are limitations to the generalizability of our findings to clinical practice and other populations. Our participants were all anovulatory and our interventions only included CC and acupuncture. Our collection of information may not include potential predictors that could impact on clinical outcome.

Wider implications of the findings: Overall, most of our predictors overlap with those found in other studies. Expected predictors such as age and BMI were not statistically significant in our assessment. The proposed models could be applied to anovulatory patients with PCOS after confirmation through a further validation study.

Trial registration number: The trial was registered at clinicaltrials.gov (NCT-01573858) and Chinese clinical trial registry (ChiCTR-TRC-12002081).

P-698 Brain-derived neurotrophic factor promotes human granulosa-like tumor cell steroidogenesis through activating FSH receptor-mediated signaling pathway

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Study question: How brain-derived neurotrophic factor (BDNF) promotes steroidogenesis and proliferation of human granulosa-like tumor cell (KGN cell)?

Summary answer: BDNF treatment can regulate FSH receptor expression and modifications and activate FSHR-mediated signaling pathway in KGN cell.

What is known already: BDNF and FSH receptor are expressed in ovarian granulosa cells, and play important roles in regulating ovarian development and functions. BDNF functions as a regulator in follicle growth, oocyte maturation, accelerating the extrusion of polar bodies and maintaining the corpus luteum. FSHR plays essential roles in the regulation of steroidogenesis and follicle proliferation during ovary maturation. FSHR-induced signaling is involved in the modulation of nuclear events in granulosa cells. Studies have linked the BDNF-associated signaling pathway to FSHR mRNA expression in the regulation of follicle development, suggesting that BDNF may potentially affect granulosa cells through FSHR.

Study design, size, duration: This study was designed to investigate the steroidogenesis in human granulosa-like tumor cell line KGN treated by BDNF and the underlying mechanism.

Participants/materials, setting, methods: KGN cells were exposed to different treatments: FSH (100 ng/ml), BDNF (5 ng/ml) and combined FSH (100 ng/ml) and BDNF (5 ng/ml). Steroidogenesis (estradiol and progesterone) were determined on a chemiluminescent immunoassay system. Cell proliferation was assessed with EdU assay. BDNF and cAMP levels, and PKA kinase activity were analyzed by ELISA. FSHR, CREB and phospho-CREB levels were determined by Western blotting. Phosphorylation and ubiquitination of FSHR was examined by immunofluorescence and immunoprecipitation.

Main results and the role of chance: KGN cells products and secrets BDNF. After exposing to different treatment, the secretion of estradiol ($p < 0.01$) and progesterone ($p < 0.01$) were stimulated in KGN cells by combination of BDNF and FSH, also the cell proliferation ($p < 0.01$). BDNF plus FSH treatment decreased BDNF level and enhanced FSHR phosphorylation and ubiquitination. Combined treatment also increased the cAMP level, and PKA and CREB activity, suggesting the activated cAMP/PKA/CREB signaling pathway. Moreover, inhibition of BDNF expression by siRNA markedly reduced the estradiol secretion and down-regulated FSHR, aromatase and phosphorylated CREB; meanwhile, FSH treatment partly alleviated the effects of BDNF siRNA on KGN cells. These findings suggested that BDNF modulates graunlosa cell functions and the action probably mediated by FSHR-coupled signaling pathway, to affect aromatase-mediated steroidogenesis.

Limitations, reasons for caution: Although KGN cells have common characteristics and are functionally similar to primary human ovarian granulosa cells and widely used to study the steroidogenesis of granulosa cell in vitro, there are many different between the primary granulosa cell and KGN cell such as karyotype and the response to the stimulating factors.

Wider implications of the findings: The present study has established a molecular link between BDNF and FSHR function, partly revealing the mechanisms by which neurotrophic factors modulate hormone secretion in granulosa cells. Overall, these findings concerning in KGN cells may be applied for a further understanding of the functional regulation of granulosa cells.

Trial registration number: None.

P-699 A pharmacogenetic approach to improve low ovarian response by androgens pre-treatment

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Study question: Does the length in the polymorphism of androgen receptor (AR) gene affect the ovarian response when using androgen in a previous cycle?

Summary answer: Androgen receptor genotyping could help us to identify poor ovarian responder (POR) patients that will be benefited of transdermal testosterone pre-treatment.

What is known already: Androgens and their receptors have been shown to play an important role in ovarian physiology. Recently, clinicians have attempted to improve the ovarian response in POR by using androgens or androgen modulators prior to IVF treatment. However, there is still some controversy about the evidence that pre-treatment with transdermal testosterone may improve the clinical outcomes for POR. The human androgen receptor (AR) gene contains a highly polymorphic CAG repeat. The aim of this work was to investigate if AR polymorphism could be used for selection patients that they will benefit of androgen treatment in previous cycle.

Study design, size, duration: A retrospective study was performed. We included 88 ovarian stimulation cycles performed by 44 patients diagnosed as POR and genotyped for AR polymorphism. All patients carried out two cycles: one without androgens pre-treatment and the second one with androgen

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preparation. We compare the results in pair from each. The main outcomes were the number of retrieved oocytes and MII.

Participants/materials, setting, methods: We included 44 patients diagnosed as POR according to the Bologna criteria. All patients were genotyped for AR polymorphism using fluorescent PCR. For androgen pre-treatment 25 mg testosterone were applied for 28 days in the cycle preceding the ovarian stimulation. The ovarian stimulation protocol was adjusted according to patient characteristics.

Main results and the role of chance: No significant differences were reported when we compared the cycles for each patient in total according to oocyte yield, MII, days of stimulation and gonadotrophin dosages. However, according to AR polymorphism statistical differences were shown in oocyte yield and MII. Patients that carried CAG repeats in AR gene between 22 and 24 showed an increased in the number of oocytes when they were pre-treated with androgens, from 2.61 oocytes yielded in the cycle without androgens to 5.11 in the cycle with androgens ($p<0.05$). For the patients that carried a number repeats lower than 22 and higher than 24 no significance differences were reported in the number of oocytes obtained in the cycle with or without androgens (2.94 vs 2.56; $p = 0.876$). Similar result was obtained for mature oocytes. More MII oocytes were obtained in the pre-treated cycle in patients that carry a number of CAG repeats between 22 and 24 (1.86 vs 4.04; $p<0.05$). No significant differences in the number of MII oocytes were found in patients that get out of 22 and 24 repeats between the two cycles (2.31 vs 2.13; $p = 0.878$).

Limitations, reasons for caution: The study is limited by its retrospective nature. A higher sample size or a prospective randomized design should be used in future studies to corroborate the current findings. In addition, research into the contribution of the effect of the polymorphism in the AR function will be of a great interest.

Wider implications of the findings: The use of androgens as strategy to increase the number of retrieved oocyte in POR remains controversial. Our data suggest that the AR genotype could clarify the effectiveness of the androgen pre-treatment. Androgen receptor genotyping could help us to identify POR patients that will be benefited of transdermal testosterone pre-treatment.

Trial registration number: No trial.

P-700 The potential mechanisms involved in the intervention of Electro-acupuncture on decreasing the progression of OHSS in a rat model

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Study question: How electroacupuncture (EA) improve the characteristics of ovarian hyperstimulation syndrome (OHSS) in rats?

Summary answer: EA reduce ovarian size and serum hormones of OHSS, manifested by negative regulation of ovarian corpus luteum formation and ovarian enzymes expression of steroidogenesis.

What is known already: OHSS is a potentially life-threatening complication of ART, characterized by enlarged ovarian size of a large number of corpus luteum, high sex hormones synthesized and released. However, there was no direct evidence implicating the modulators during the occurrence. Recently, we have shown that EA, as a treatment can efficiently reduce the ovarian size and decline serum sex hormones level of OHSS rats, which signified the signs of OHSS were improved. However, the underlying mechanisms of EA on changing ovarian structure and steroidogenesis of OHSS still unknown, in this study we will explore that in a rat model.

Study design, size, duration: OHSS was induced in 22 days (D22) female Sprague–Dawley rats by four consecutive daily injections of 50IU PMSG from D25 to D28, sacrificed 48 h later by 150IU hCG at D29; regular-stimulation with 10IU PMSG at D27 and 10IU hCG at D29; control rats injected with saline ($n = 8$ per group); EA (Sanyinjiao (SP6) and Guanyuan (CV4), 15 min/day, 2/15 Hz) applied for OHSS rats from D22 to D31; Serum and ovary were collected for further experiments.

Participants/materials, setting, methods: Ovarian sections with HE staining to assess the histomorphology changes, modulators in serum and ovarian that PGE2, PGF2α detected by ELISA. Serum E2, P, T and FSH, LH measured by chemiluminescence or ELISA. Expressions of crucial ovarian steroidogenesis enzymes and upstream PKA pathway were examined by Western blot and qPCR. Neuropeptide Y (NPY), target of EA, and its regulated cell proliferation-related molecules PCNA evaluated to interpret the original control of EA on OHSS.

Main results and the role of chance: The ovary/body weight ratio in EAO rats was significantly lower than that in OHSS ($p<0.01$), the average number of corpus luteum ($p<0.05$) and expressions of luteotrophic regulator PGE2 in ovary and serum (both $p<0.001$) were synchronous reduced in EAO rats compared with OHSS, EA increased the ovarian luteolytic factor PGE2α expression ($p<0.05$) than OHSS, but not serum. In addition to serum sex hormones, the gonadotropins FSH, LH also declined after EA intervention. Ovarian steroidogenesis enzymes StAR, Cyp11a1, 3β-HSD, 17β-HSD, aromatase Cyp19 mRNA and protein expression were all down regulated in EAO group, the upstream FSHR, LHR and they actived PKA/CREB pathway were attenuated consistently with the enzymes expression. NPY expression in EAO ovary was up-regulated, which can inhibit the ovarian cell proliferation, we found that ovarian PCNA was reduced but cell cycle inhibit molecule P27 and phosphorylated P27 were enhanced. Immunohistochemical staining of ovarian sections also showed that PCNA in EAO rats was weaker than in OHSS.

Limitations, reasons for caution: The *in vivo* experiments were performed in a rat model.

Wider implications of the findings: Our findings provide underlying mechanisms of electroacupuncture on OHSS treatment: EA can efficiently alleviate the signs of OHSS by reducing the corpus luteum formation and attenuating the steroid hormone synthesis pathway. Clarifying the inner properties of EA could open new therapeutic avenues for OHSS patients in ART.

Trial registration number: None.

P-701 Non-equivalence of anti-müllerian hormone automated assays - clinical implications for use as a companion diagnostic for individualised gonadotrophin dosing

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Study question: Do Anti-müllerian hormone automated immunoassays (Elecsys® and Access) exhibit similar performance and can they be used interchangeably as a companion diagnostic for individualisation of follitropin delta dosing?

Summary answer: The Access assay gives systematically higher AMH values than the Elecsys® assay. This discordance misclassifies over 49% of women to an inappropriate follitropin delta dose.

What is known already: Personalised medicine encompasses in-vitro companion diagnostics for the safe and effective use of a corresponding therapeutic product. The use of a companion diagnostic with a particular therapeutic product is stipulated in the instructions for use in the labelling of both the diagnostic device and the corresponding therapeutic product. Follitropin delta is the first gonadotrophin to be licenced with a companion diagnostic, the Roche Elecsys® AMH Plus assay. Alternative automated AMH assays including the Beckman Coulter Access immunoassay are considered to provide similar results, but clarification of their suitability as an off licence companion diagnostic for follitropin delta is required.

Study design, size, duration: We systematically searched the existing literature for studies that had measured AMH using both automated assays in the

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same cohort of women. Individual paired patient data were acquired from each author and combined with unpublished data.

Participants/materials, setting, methods: We identified 5 eligible prospective published studies and one additional unpublished study. 100% response from the authors was achieved. We collected paired AMH data on 848 women. Passing-Bablok regression and Bland-Altman plots were used to compare the analytical performance of the two assays. The degree of misclassification to different treatment categories was estimated should the Access AMH be used as a companion diagnostic instead of the Elecsys AMH in determining the dosing of follitropin delta.

Main results and the role of chance: The Passing-Bablok regression shows a linear relationship (Access = -0.05 + 1.10*Elecsys). The Access assay systematically gave higher values by an average of 10% compared with the Elecsys assay (slope =1.10, 95% CI: 1.09 to 1.12). The average bias between the two assays was 2.7 pmol/L. The 95% limits of agreement were -11.7 to 6.0. A substantial proportion of women (ranging from 49 to 90% depending on the AMH category) would receive a lower dose of follitropin delta based on the Access AMH assay. Up to 10% (ranging from 2.5 to 10%) of women with high ovarian reserve would have been misclassified to a greater dose of follitropin delta based on the Access AMH assay.

Limitations, reasons for caution: We compared the values of the two principal automated assays, extrapolation of our findings to other automated AMH assays would require replication.

Wider implications of the findings: An international standard for the calibration of the automated AMH assays is warranted to facilitate efficient use of AMH as a companion diagnostic. For optimal performance of the follitropin delta dosing algorithm it should be used in conjunction with its licensed companion diagnostic the Elecsys® AMH Plus immunoassay.

Trial registration number: N/A.

P-702 Ovarian volume as a surrogate marker of ovarian recovery following ovarian stimulation with different triggers of oocyte maturation: kisspeptin, GnRH agonist and human chorionic gonadotropin

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Study question: Is ovarian volume at early OHSS screening (day 2-5 following oocyte retrieval) altered when using different triggers of oocyte maturation (human chorionic gonadotropin(hCG), gonadotropin-releasing hormone (GnRH) agonist or kisspeptin)?

Summary answer: *Ovaries recovered most rapidly following kisspeptin triggering:* Mean ovarian volume (± SD) was 192 ± 97mls following hCG, 53 ± 37mls following GnRH agonist and 9 ± 4mls following kisspeptin; P < 0.0001.

What is known already: Ovarian hyperstimulation syndrome (OHSS) is a serious iatrogenic condition that is predominantly related to the mode of triggering oocyte maturation during IVF treatment. Kisspeptin is a novel trigger which stimulates the physiological release of GnRH from the hypothalamus. Kisspeptin has recently been shown to safely trigger oocyte maturation in a population at high risk of OHSS, but has yet to be directly compared with other triggers. Ovarian volume and ascitic fluid are commonly used to categorise the severity of OHSS in diagnostic guidelines.

Study design, size, duration: Women at high risk of OHSS (antral follicle count ≥23), aged <35yrs, BMI <30 kg/m^2 with both ovaries intact, were screened sonographically and for OHSS symptoms during early OHSS screening (2-5 days following oocyte retrieval) at Hammersmith Hospital, London, UK (2013-2016).

Participants/materials, setting, methods: Ovarian volume on ultrasound, ascitic volume and OHSS symptoms were determined when patients were triggered with (hCG) (n = 29), GnRH agonist (GnRHa) (n = 94) or kisspeptin (n = 115) at time of early OHSS screening. Statistical analysis was performed using One-way ANOVA with post-hoc Bonferroni correction.

Main results and the role of chance: Mean ovarian volume (MOV) (±SD) following GnRH agonist trigger (53 ± 37mls) was significantly lower than in patients triggered with hCG (192 ± 97mls; p <0.0001). MOV following kisspeptin trigger (9 ± 4mls) was significantly lower still when compared with GnRH agonist trigger (p <0.0001). Compared to baseline ovarian volumes prior to commencing ovarian stimulation, ovarian volumes at early OHSS screening remained enlarged 28-fold following hCG, 10-fold following GnRH agonist and 5.8-fold following kisspeptin triggering. Mean total ascitic volumes were lower after GnRH agonist (5.8 ± 22mls) and kisspeptin (4.6 ± 7.8mls) when compared with hCG (102 ± 150mls; p<0.0001).

Symptoms of OHSS were more frequently reported following GnRH agonist use than kisspeptin and more frequently still following hCG:

Abdominal pain was reported in 80% following hCG, 22% following GnRH agonist and 12% following kisspeptin. Abdominal bloating was reported in 90% following hCG, 30% following GnRH agonist and 11% following kisspeptin. Nausea was reported in 43% following hCG, 14% following GnRH agonist and 2% following kisspeptin. Vomiting was reported in 10% following hCG, 5% following GnRH agonist and 1% following kisspeptin.

Limitations, reasons for caution: Further research studies are warranted to directly compare kisspeptin to more established triggers of oocyte maturation in prospective randomized controlled trials.

Wider implications of the findings: Kisspeptin may present a safer alternative to GnRHa or hCG triggering in patient at high risk of OHSS undergoing IVF treatment.

Trial registration number: Clinical Trials Registration Number: NCT01667406

P-703 A modified natural protocol for frozen-thawed embryo transfer – Don't mind the LH surge (It's the progesterone that matters). A proof of concept study

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Study question: Does a FET protocol where progesterone is started during the late follicular phase disregarding the LH surge for scheduling embryo transfer provide satisfactory results?

Summary answer: The tested protocol is simple to implement, flexible and can potentially increase the clinical pregnancy rate after FET for ovulating women.

What is known already: The best protocol for FET cycles has yet to be determined. Natural cycle and modified natural cycle FET protocols achieve synchronization between endometrium development and embryo age by timing the embryo transfer relative to the LH surge or an exogenous hCG injection. The former requires meticulous monitoring of the cycle and requires the IVF unit to be available daily for transfer, while the latter has yielded conflicting results.

Study design, size, duration: Candidates for FET with a history of regular menstrual cycles were recruited for the study prospectively. This proof of concept trial was approved by the local ethics committee and the Ministry of Health for 20 patients completing the protocol. The patients were recruited from May to December 2016. Results were compared retrospectively to a cohort of 40 patients with similar clinical attributes with an artificial cycle protocol.

Participants/materials, setting, methods: Good prognosis patients scheduled for FET with 48 hour embryos were included. Endometrial thickness, follicle size and estradiol, progesterone and LH levels were monitored during the follicular phase. Progesterone suppositories were started 48 hours prior to scheduled embryo transfer regardless of the timing of the LH surge provided there was a leading follicle, endometrial thickness of at least 7 mm and any elevated progesterone began no sooner than 72 hours before transfer.

Main results and the role of chance: Twenty patients completed the study protocol. Baseline parameters between the study group and the control group were similar. There were 12/20 (60%) clinical pregnancies in the study group versus 8/40 (20%, p<0.01) clinical pregnancies in the control group. The

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implantation rate was 45% and 13.24% respectively (p<0.001). The multifetal pregnancy rate was 33% versus 12.5% (NS). The differences in pregnancy rates were more pronounced when considering only those cycles where at least one high quality embryo was available for transfer: 12/15 (80%) clinical pregnancies versus 7/33 (21%, p<0.001) and an implantation rate of 56% versus 13.8% respectively (p<0.0001). Most of the cycles in the study group required two visits before the transfer, a baseline visit during menstruation and one monitoring visit during the late follicular phase. Weekend transfers were completely avoided.

Limitations, reasons for caution: The sample size is small and the groups were not randomly selected. The results should be analyzed in the context of being a proof of concept trial. Larger prospective trials should be conducted.

Wider implications of the findings: Progesterone administered during the late follicular phase does not hinder the chances of a clinical pregnancy. Synchronization of endometrium and embryo by controlling the timing of progesterone administration is attained. By disconnecting the process from the LH surge, cycle monitoring is simplified with more flexibility in transfer scheduling.

Trial registration number: NCT02749344.

P-704 Effect on endometrial histology and pharmacokinetics of different dose regimens of progesterone vaginal pessaries, in comparison with progesterone vaginal gel and placebo

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Study question: Which progesterone vaginal pessary (Cyclogest®) dose regimen induces adequate secretory transformation of the endometrium, in comparison with progesterone vaginal gel (Crinone®) and placebo?

Summary answer: The most adequate secretory transformation of the endometrium was observed during treatment with 400 mg progesterone vaginal pessaries, administered twice daily.

What is known already: In clinical practice, progesterone via the vaginal route is widely used for luteal phase support in assisted reproduction technique (ART) cycles. Although few studies have investigated the endometrial effect of vaginal progesterone, no clinical trials have been performed yet to determine the optimal dose of progesterone pessaries in this indication.

Study design, size, duration: The study consisted of two randomized, observer-blind crossover parts. Part 1 (3-way crossover) investigated 200 mg progesterone vaginal pessaries twice daily (bid), 400 mg pessaries bid, and 90 mg progesterone vaginal gel once daily (od) including 61 treated subjects. Part 2 investigated 100 mg pessaries bid and 400 mg pessaries od in a 2-way crossover including 64 treated subjects. Of these, 22 subjects additionally received placebo vaginal pessaries bid in a third study period.

Participants/materials, setting, methods: The study was performed in healthy female volunteers of reproductive age. The subjects used 2 mg estradiol bid for 24 days in each treatment cycle. Progesterone or placebo was administered vaginally from cycle day 15 onwards during 10 days. An endometrial biopsy for histological evaluation was performed on cycle day 23. Pharmacokinetic parameters were determined after the first progesterone dose on day 15 and after the last dose on day 24.

Main results and the role of chance: Frequencies of secretory transformation of the endometrium, i.e. adequate responses, during treatment with Cyclogest® 200 mg and 400 mg bid were comparable with those during Crinone® treatment (90-94%), whereas lower secretory transformation rates were observed during treatment with 100 mg bid and 400 mg vaginal pessaries od (64% and 75% respectively). No adequate response was observed after placebo demonstrating the assay sensitivity of the trial concept. The late secretory state of the endometrium, which is physiologically closer to the endometrium receptivity conditions on cycle day 23 than the early secretory state, was observed in 90%, 82% and 78% with Cyclogest® 400 mg bid, Crinone® od and Cyclogest® 200 mg bid respectively. Pharmacokinetic parameters after multiple dosing of progesterone vaginal pessaries showed a dose-dependent, but not dose-proportional, increase of plasma progesterone levels, with 400 mg bid presenting a consistently higher progesterone plasma level over the full dosing period in comparison to the vaginal gel and all other pessary dose regimens. The lowest incidence of bleeding and spotting was reported during treatment with 400 mg vaginal pessaries bid. Progesterone treatments were safe and well tolerated. Drug-related adverse events were not dependent on the absolute amount of progesterone administered.

Limitations, reasons for caution: The primary outcome parameter, endometrial histology, is a surrogate for the actual clinical endpoint, endometrial receptivity, which is investigated in a subsequent efficacy study in patients undergoing assisted reproduction treatment.

Wider implications of the findings: Based on the results of this study, the 400 mg bid vaginal pessary seems to be optimal for luteal phase support and as effective as 90 mg vaginal gel od to induce adequate endometrial transformation. The efficacy of this dose regimen is confirmed by evaluating pregnancy rates in ART cycles.

Trial registration number: EudraCT number 2012-001726-95

P-705 Effects of TSH levels on pregnancy outcomes and neonatal birth weights for patients undergoing IVF in China

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Study question: The objective of the investigation was to study the effects of TSH levels on pregnancy outcomes of IVF and the clinical characteristics of their neonates.

Summary answer: Pregnancy outcomes and neonatal birth weights in IVF patients appear to have no relation to TSH levels.

What is known already: The upper limit of normal TSH has been revised from 5 mIU/L to 2.5 mIU/L for pregnant women.The ideal thyroid-stimulating hormone (TSH) range for infertile women undergoing IVF has not been determined.

Study design, size, duration: Design: Retrospective cohort study.

Setting: University-affiliated reproductive medicine center.

Patient(s): A total of 10259 first fresh IVF cycles with autologous oocytes from June 2011 to June 2015.

Participants/materials, setting, methods: All the patients were divided into polycystic ovary syndrome (PCOS) group and non-PCOS group, and then according to TSH levels, each group was subdivided into 5 subgroups[(0–0.5), [0.5–2.5), [2.5–3.5), [3.5-4.5)and [4.5-12] mIU/L]. The live birth rates, miscarriage rates and neonatal birth weights among the TSH groups were compared.

Main results and the role of chance: There were no difference in live birth rates and miscarriage rates between TSH groups.Live birth rates and miscarriage rates were not associated with TSH level after adjusting for age. No significant differences were observed in the NBWs and premature birth conceived via IVF cycles between TSH groups.

Limitations, reasons for caution: Owing to the limited sample size in our center, the sample size of neonates was small, especially in groups 1 and 5. Additionally, there is little research on the relationship between NBW and TSH levels in ART at present. Therefore, more NBW studies must be performed in the future.

Wider implications of the findings: There is a lack of evidence that revised the upper limit of normal TSH from 5 mIU/L to 2.5 mIU/L is beneficial. Pregnancy outcomes and neonatal birth weights in IVF patients appear to have no relation to TSH levels.

Trial registration number: not applicable.

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P-706 The effect of storage conditions on stability of AMH measurement in serum and blood using the automated AMH assay

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Study question: Does the automated AMH assay acquire the validation concerning time-dependent stability in serum, and the stability of AMH values after exposure and storage in whole-blood?

Summary answer: The automated AMH assay exhibits very high reproducibility and stability in serum and whole blood for a week, comparable to the revised Gen II assay.

What is known already: Recent studies suggested that AMH measurement may be prone to preanalytical instability depending on sample storage conditions. In clinical setting, blood samples are not processed within the recommended time scales. The original Gen II assay provided time-dependent reduced AMH value of serum samples, but provided significantly increased AMH values when measured in whole-blood samples storaged at room temperature subsequently separated serum.

Study design, size, duration: This was a prospective study in which 386 samples from volunteers were stored at three different temperatures and assayed for AMH at times 0, 48 hours and 1 week after collection using the automated AMH (Access) and revised Gen II assay.

Participants/materials, setting, methods: Volunteers (n = 76) were healthy non-pregnant women aged 25–45 years. We divided Serum group 1(centrifuged within 2 hours of collection and stored at fridge temperature), Serum group 2(centrifuged within 2 hours of collection and stored at -20°C) and Whole-blood group (stored at room temperature before centrifugation and assay). Samples were evaluated simultaneously with the revised Gen II and Access assay. Comparisons of sample concentrations at each time point were undertaken as paired t-tests.

Main results and the role of chance: AMH concentrations measured using the Access assay remained unchanged in serum for up to 1 week at fridge temperature and -20°C (correlation coefficient > 0.99). The time-dependent stability of Access assay in stored serum was comparable to that of the revised Gen II assay (correlation coefficient > 0.99). The concordance between log-transformed values measured using the Access and Gen II assay was rho = 0.92 (95% CI 0.84–0.95). The Passing–Bablok regression equation was y (Access assay in ng/ml) = 0.82 × Gen II (correlation coefficient = 0.99, *p*-value < 0.01). Testing the effects of storage in whole-blood samples at room temperature showed a consistent AMH values for up to 1 week with both the Access and revised Gen II assay (correlation coefficient > 0.99).

Limitations, reasons for caution: The reproducibility and stability may vary depending on basal AMH levels. The observed stability of AMH in stored serum and whole-blood samples requires further larger investigations.

Wider implications of the findings: In clinical setting, protracted storage of whole-blood at room temperature has little impact on the AMH concentration measured using the automated AMH assay. The results indicate that minor variations of AMH levels due to storage issues in the automated AMH assay will have little impact on the clinical decision.

Trial registration number: not applicable.

P-707 Growth differentiation factor-8: A new clinical biomarker and treatment target for the improvement of pregnancy outcome for polycystic ovary syndrome patients

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Study question: To evaluate the expression pattern and predictive value of serum growth differentiation factor-8 (GDF-8) for the pregnancy outcome in polycystic ovary syndrome (PCOS) patients

Summary answer: Serum GDF-8 levels are dysregulated during the process of controlled ovarian hyperstimulation (COH) and affect the pregnancy outcome in PCOS patients.

What is known already: We have shown that GDF-8 down-regulates StAR expression and decreases progesterone production in human granulosa cells. In addition, in non-PCOS patients, serum GDF-8 level on the hCG administration day and the level of GDF-8 decrease from hCG administration day to oocyte pick-up (OPU) day are valuable predictors of pregnancy during IVF/ICSI-ET treatment. Our findings suggest that before hCG administration day, higher serum GDF-8 may be a beneficial factor for pregnancy by keeping a lower progesterone level. After hCG administration, decrease of GDF-8 level may be critical for early embryo implantation and successful pregnancy by maintaining the high level of progesterone.

Study design, size, duration: Human serum and follicular fluid samples were obtained from 24 control and 20 PCOS patients (pregnant: n = 9 and non-pregnant: n = 11) undergoing IVF/ICSI-ET at the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University in China. Informed consent was obtained from all patients. The diagnosis of PCOS was based on the Rotterdam-PCOS criteria in which at least two of the following features were present: oligo/amenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasonography.

Participants/materials, setting, methods: All patients were treated with a standard long GnRH-agonist protocol. Blood samples were obtained by venipuncture at GnRH-agonist day, gonadotropin day, hCG administration day, 12 h after hCG administration, OPU day, 48 h after OPU and 14 days after ET. Serum GDF-8 levels were measured by ELISA at different time points and were correlated with the pregnancy results. GDF-8 levels in the follicular fluid were also measured. RT-qPCR was used to measure mRNA level.

Main results and the role of chance: Serum GDF-8 levels on gonadotropin day and OPU day were significantly higher in the PCOS patients than that in the non-PCOS patients (7.0 ± 4.0 vs 4.5 ± 1.3, p = 0.007; 2.8 ± 1.5 vs 1.9 ± 0.8, p = 0.035). GDF-8 levels in follicle fluid were also significantly higher in PCOS patients than that in non-PCOS patients (2.2 ± 1.6 vs 1.2 ± 0.8, p < 0.001). Interestingly, unlike the results that obtained from control patients, serum GDF-8 levels at all the time points during the COH procedure as well as follicular fluid GDF-8 levels of PCOS patients were significantly lower in the pregnant group than in non-pregnant group. RT-qPCR analyses showed that the basal mRNA levels of GDF-8 receptor, ALK5 (also known as TGFβR1), in the primary culture of granulosa cells did not vary significantly between control and PCOS patients. Interestingly, treatment with hCG up-regulated ALK5 mRNA levels in the granulosa cells of PCOS patients, while this effect did not observed in the granulosa cells of control patients. In addition, treatment granulosa cells of PCOS patients with human recombinant GDF-8 increased aromatase and FSH receptor mRNA levels but decreased StAR and LH receptor mRNA levels. Moreover, the regulatory effects of GDF-8 on these genes were similar in between granulosa cells of PCOS and control patients.

Limitations, reasons for caution: Despite the interesting results that serum and follicular fluid GDF-8 levels were lower in the pregnant than non-pregnant of PCOS patients, the underlying mechanisms remain unknown and need to be further defined. In addition, further studies using more clinical samples to confirm the current findings are warranted.

Wider implications of the findings: This work, for the first time, demonstrated the GDF-8 expression pattern in PCOS patients during COH procedure which increased our understanding of the roles of GDF-8 in the pathogenesis of PCOS and unraveled that maybe GDF-8 can be therapeutic targeted to improve the pregnancy rate for PCOS patients.

Trial registration number: Not applicable.

P-708 Pregnancy Outcomes Study in euthyroid women with Thyroid Autoimmunity after Levothyroxine intervention—an open labelled RCT study (The POSTAL study)

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Study question: What is in euthyroid infertile women with thyroid autoimmunity (TAI) undergoing IVF-ET, the effect of levothyroxine treatment on pregnant outcomes.

Summary answer: Among euthyroid TAI infertile women undergoing IVF levothyroxine treatment does not improve the pregnant outcomes.

What is known already: It is known that TAI is associated with spontaneous pregnancy loss and poor pregnancy outcomes. In an IVF program, women with thyroid autoantibodies had lower fertilization, implantation and pregnancy rates, but higher abortion rates than women without such antibodies. Whether to give adjuvant therapy to regulate TAI before and during IVF is still controversial. Here, we investigate whether suppletion with levothyroxine in women with TAI undergoing IVF improves outcomes.

Study design, size, duration: The POSTAL study is a randomized, open-labelled, parallel two-group, single-centered clinical trial (ChiCTR-TRC-13004097). After informed consent, participating women were randomly assigned (1:1) to the levothyroxine (25-50ug/d) treatment group or the non-treatment controls. Levothyroxine treatment started two weeks before the IVF cycle. In women who conceived, levothyroxine was continued till delivery. We planned to recruit 600 women. A subgroup analyses was planned for women with TSH <2.5 or ≥2.5 mIU/L.

Participants/materials, setting, methods: Eligible women were euthyroid women with thyroid antibodies (Ab) (thyroperoxidase (TPO) and/or thyroglobulin (TG) Ab positive), aged 23-40 years, who were scheduled for their 1st or 2nd IVF cycle with fresh embryo transfer. Women with autoimmune diseases other than TAI, recurrent spontaneous miscarriage, diabetes mellitus or diagnosed endocrinological diseases were excluded. The primary endpoint was miscarriage rate. Secondary endpoints were, among others, clinical pregnancy rate and the live birth rate.

Main results and the role of chance: Between July 1st, 2012, and Nov 30th, 2016, 600 women were randomized, 300 to the levothyroxine group and 300 to controls. Baseline TSH levels were higher in the levothyroxine group than in the control group (2.891.02 vs. 2.210.99 mIU/L). Other baseline characteristics were comparable. In the levothyroxine group, 282 women had a fresh transfer, 15 did not complete their cycle, and 3 did not start their IVF cycles. In the control group, 285 women has a fresh transfer, 11 did not complete their cycle, and 4 did not start their IVF cycle.

In the intervention group, the average levothyroxine dosage during COH was 30.012.2 ug/d. The miscarriage rate was not different between the levothyroxine group and the controls (9.1% vs. 9.1%, P = 0.81). The clinical pregnancy rates (44% vs. 48.7%, P = 0.38) and live birth rates (36.9% vs. 37.6%, P = 0.94) were also both not different between the groups. Comparing the pregnant outcomes between individuals with TSH < 2.5 mIU/L and TSH ≥2.5 mIU/L, no significant difference were found between the groups and within the groups.

Limitations, reasons for caution: Thus was an open labelled RCT which might have confounded our findings effects. The lower levothyroxine dosage in the study might also minimize the effect of levothyroxine on pregnant outcomes to some extent. The trial was registered 12 months after start of the study.

Wider implications of the findings: In euthyroid infertile women undergoing IVF with TAI, levothyroxine treatment did neither decrease miscarriage rates, nor improve clinical pregnancy and live birth rates.

Trial registration number: The trial is registered at http://chictr.org.cn, the trial number ChiCTR-TRC-13004097.

P-709 Effectiveness and safety of follitropin alfa in infertile women using assisted reproductive technology in real-world clinical practice: a multicenter, prospective, open, non-interventional study

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Study question: To examine number of oocytes retrieved and clinical pregnancy rate following controlled ovarian stimulation therapy with Ovaleap® in infertile women treated in real-world clinical practice.

Summary answer: Effectiveness and safety of controlled ovarian stimulation with Ovaleap® were supported during real-world assisted reproductive technology treatment using a gonadotropin releasing hormone (GnRH) antagonist protocol.

What is known already: Ovaleap® (follitropin alfa), a recombinant human follicle-stimulating hormone authorized by the European Medicines Agency in 2013, showed therapeutic equivalence in efficacy and safety to Gonal-f® for stimulation of follicular development in a multinational, multicenter, randomized, active-controlled, assessor-blind, comparative study of infertile women using assisted reproductive technology (ART). Using a GnRH agonist protocol, number of retrieved oocytes following controlled ovarian stimulation (COS) was equivalent in Ovaleap® and Gonal-f® patients. Safety of Ovaleap® treatment was further demonstrated in an open-label, uncontrolled, follow-up study of these patients with up to 2 additional Ovaleap® treatment cycles.

Study design, size, duration: 400 participants are planned for this multicenter, prospective, non-interventional study of women undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) following COS using Ovaleap® in a GnRH antagonist protocol during routine ART in approximately 40 specialized reproductive medicine centers in Germany. Study enrollment began in March 2016, with participant recruitment planned for 1 year. This planned interim analysis examined women who received ≥1 Ovaleap® dose and had Visit 3 (clinical pregnancy evaluation) data.

Participants/materials, setting, methods: Eligible women were 18-40 years old, undergoing first COS for IVF/ICSI, and were treated with Ovaleap® using a GnRH antagonist protocol. Study visits included baseline, Visit 2 (embryo transfer day), and Visit 3 (clinical pregnancy examination). Ovaleap® treatment duration was 1 stimulation cycle. Primary endpoints included number of oocytes retrieved and clinical pregnancy rate. Safety was examined by frequency and intensity (mild, moderate, severe) of adverse drug reactions (ADRs) and serious ADRs.

Main results and the role of chance: Of 192 patients who screened eligible, the 155 included in this interim analysis received ≥1 Ovaleap® dose and had Visit 3 data. Patient mean age was 32.8 ± 4.1 years, mean body mass index was 23.5 ± 3.2 kg/m^2, and mean anti-Müllerian hormone level was 3.6 ± 2.5 µg/L. Mean duration of the GnRH antagonist administration was 5.7 ± 1.9 (median = 6.0) days. Mean duration of Ovaleap® administration was 9.8 ± 1.7 (median = 10.0) days, and mean total Ovaleap® dose was 1752.7 ± 539.0 (median = 1650.0) IU. In women with oocyte retrieval (n = 152), mean number of retrieved oocytes was 11.7 ± 8.0 (median = 10.0; range 1-61). Oocyte retrieval was cancelled in 3 women due to low follicle number (1), premature increase in progesterone level and bleeding (1), and not reported (1). The overall clinical pregnancy rate was 33.5% (52/155) and by embryo transfer (n = 132; 1 excluded due to missing day of embryo transfer) was 39.4% (52/132). Of the 13 patients (8.4%) reporting ≥1 ADR, 9 (5.8%) reported ovarian hyperstimulation syndrome (OHSS), including 5 rated mild, 3 moderate, and 1 severe and 2 patients (1.3%) reported miscarriage. Six ADRs were rated serious (4=OHSS, 2=miscarriage). Of the 9 OHSS ADRs, 3 (including the severe OHSS) occurred during pregnancy (Week 5, late-onset OHSS) and 3 (all mild) resulted in egg/embryo freezing.

Limitations, reasons for caution: This study presents the planned interim analysis of the ongoing multicenter, prospective, open, non-interventional study. Caution should be used in the interpretation of results pending final analysis of the completed study.

Wider implications of the findings: The effectiveness (number of oocytes retrieved, clinical pregnancy rate) and safety of controlled ovarian stimulation with Ovaleap® appear to extend to real-world ART clinical practice, including IVF and ICSI treatment using a GnRH antagonist protocol. These interim real-world outcomes are consistent with those previously reported in randomized controlled clinical trials.

Trial registration number: Not applicable.

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P-710 Ovulation induction (OI) or intrauterine insemination (IUI) with gonadotropins in patients with polycystic ovary syndrome (PCOS): description of patients and triggering rates. The GLOBALE-SOPK study

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Study question: Describe the triggering rates, the profiles and the management of PCOS patients undergoing OI/IUI having been prescribed follitropin alfa in a second gonadotropin stimulation attempt

Summary answer: The study highlights the great wealth of PCOS symptoms.

Real trigger rate is 93.3% [89.8; 96.8] with most patients (84.3%) receiving human chorionic gonadotropin alpha injection.

What is known already: PCOS is the most common endocrine disorder in infertile women (15-20% of prevalence), leading to anovulation and fertility disorders.

The diagnosis is complicated due to the multiplicity of criteria which are all rarely present in a single patient.

By consensus, PCOS is defined by the presence of at least two of the following criteria:

(1) Oligo and/or anovulation,
(2) Clinical and/or biochemical hyperandrogenism,
(3) Polycystic ovaries;

Treatment of infertility due to PCOS is in first-line clomiphene citrate. In case of failure or resistance, a personalized treatment by gonadotropins is recommended.

The mean triggering rate in literature is 74.1% in PCOS patients.

Study design, size, duration: It was a longitudinal observational national multicenter study with a maximum of 14 weeks follow-up. PCOS adult patients receiving follitropin alpha in a second stimulation attempt according to usual medical practices were included.

To demonstrate a triggering rate ≥ 70%, providing a maximum error of 5%, inclusion of 320 patients in 64 centers was needed. Participating physicians were asked to include the first 7-15 patients meeting inclusion/exclusion criteria and agreeing to participate in the study.

Participants/materials, setting, methods: Physicians were selected from an exhaustive listing and randomized until obtaining 64 French active participants. Their data were recorded using a registry.

Patients were selected at first or second attempt, but included only during the second attempt. Inclusion took place at the first monitoring visit (about one week after initiation of stimulation).

Follow-up was based on usual clinical practices until the 12th week of amenorrhea if pregnancy was achieved.

CRFs were completed by participating physicians.

Main results and the role of chance: Out of 172 selected physicians, 51 were active and recruited 202 patients. The sample was characterized by a mean age of 29.9 ± 4.3 years, mean infertility duration of 2.9 ± 1.7 years and mean BMI of 23.9 ± 4.4 kg/m2. Cycles averaged ≥ 35 days in 52.2% of patients (amenorrhea in 19.4%). Mean number of follicles was 40.8 ± 18.6.

35.1% of patients presented hyperandrogenism. Although NIH and Rotterdam definitions were not inclusion criteria, patients' disease matched with those definitions in 38.9% and 46.4% respectively.

72.8% of patients were previously treated by clomiphene citrate during an average of 3.8 ± 1.2 cycles at a mean dose of 93.5 ± 33.9 mg/day.

At second attempt, mean starting dose of FSH was 61.5 ± 23.7 UI, increased or stabilized since first attempt in 34.5% and 60.5% respectively. Mean stimulation duration was 9 ± 6.4 days with 55.7% of patients managed by OI (44.3% by IUI). Real triggering rate was 93.3% [89.8; 96.8] with most patients (84.3%) receiving a human chorionic gonadotropin alpha injection. B-hCG test revealed pregnancy in 25.9% of patients following triggering while periods occurred in 70.9%. Clinical pregnancy (mainly single) was confirmed in 20.1% of patients.

Limitations, reasons for caution: This study demonstrates the difficulty of finding a real definition for PCOS patients given the heterogeneity of patient profiles due to various criteria.

No triggering factors were identified because of the limited number of patients without a trigger not allowing the comparison based on the presence or absence of trigger.

Wider implications of the findings: A broad description of PCOS population was provided in this study but the great wealth of PCOS symptoms was also highlighted. However the real triggering rate reported at the second cycle of stimulation cycle by gonadotropins in those patients was high: 93.3% [89.8; 96.8] compared to the literature (74.1%).

Trial registration number: NA.

P-711 Effectiveness of a monoclonal antibody against human chorionic gonadotropin (hCG) to prevent reproductive and metabolic dysfunctions in transgenic female mice

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Study question: Is the monoclonal antibody against human chorionic gonadotropin (hCG) effective in preventing the phenotypic alterations of transgenic mice hypersecreting hCG?

Summary answer: The treatment was more effective in reversing the phenotype of transgenic female mice when it was administered at the juvenil stage as compared to adulthood

What is known already: hCG is involved in many functions during placentation and fetal development. Its levels are increased during pregnancy and postmenopause, and in pathological conditions, such as trophoblastic and non-trophoblastic tumors. Our previous studies demonstrated that transgenic female mice overexpressing the hCGβ-subunit (hCGβ+ mice) are infertile, exhibit increased levels of hCG, prolactin, progesterone and testosterone. Later in life, they develop prolactinomas and mammary gland tumors. In addition, these animals present insulin resistance and glucose intolerance. A monoclonal antibody developed by Dr. Talwar's group has high affinity for the hCG β-subunit, without cross-reaction with pituitary hormones.

Study design, size, duration: hCGβ+ females were injected i.p. with the monoclonal antibody (300 µg/mouse/dose) at 5 weeks (hCGβ+ mAB5w: every other day for one week, followed by one dose weekly; n = 10) and 16 weeks of age (hCGβ+ mAB16w: one dose weekly; n = 4) during two months. These were compared with females hCGβ+ injected with vehicle as controls (n = 5). Fertility studies, body weight, carbohydrate metabolism and the presence of tumors at 3-6 months of age were conducted.

Participants/materials, setting, methods: For fertility studies, hCGβ+ treated females were mated with wild type males two days after the last injection. Body weight was registered for 12 days. Blood glucose was measured in fasted female mice at 0, 30, 60 and 90 min after glucose administration (2 g/kg; i.p) for intraperitoneal glucose tolerance test (IGTT). The same procedure was used for insulin tolerance test (ITT; 0,75 IU/kg of insulin).

Main results and the role of chance: The effectiveness of the treatment would depend on the stage in which it is administered. During the juvenil stage, (hCGβ+ mAB5w) female mice normalized the estrous cycle and recovered fertility, but not at adulthood (hCGβ+ mAB16w). No differences in body weight between the groups were observed. The glucose tolerance test was normalized in both treated groups ($p<0.05$). All groups maintained the insulin resistance ($p<0.05$). Pituitary tumor development was prevented in the hCGβ+ mAB5w group.

Limitations, reasons for caution: Complementary studies will be needed for a better understanding of the mechanisms involved during the treatment with the monoclonal antibody against hCG. Other doses should be tested in order to get all the phenotypic alterations normalized.

Wider implications of the findings: This treatment was able to immunoneutralize the effects of hCG, which could be a useful tool for therapies related with cancer and reproduction/infertility.

Trial registration number: not applicable.

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P-712 Are there differences in Anti-Müllerian Hormone (AMH) values between populations? Usefulness of AMH reference values in a large Spanish population to predict ovarian reserve

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Study question: Are there differences in the distribution of AMH values in Spanish women compared with other studied populations?

Summary answer: AMH values in Spain are higher than those reported for other European countries and the USA, but lower than those reported for China.

What is known already: There is evidence in the world medical literature that infertility is a growing phenomenon; prevention and treatment strategies are therefore needed. One of the most reliable tests of a woman's ovarian reserve and her potential fertility is the Anti-Müllerian Hormone level. To date, the distribution of AMH values has not been reported for Spanish women. The aim in this study was therefore to define normality data of AMH by age and age groups in Spanish women, and to compare these results with those from other studied populations.

Study design, size, duration: This is a cross-sectional study with 10443 healthy women aged between 20 and 45 years from eleven different communities in Spain. We determined AMH values for each age and in groups of 5 years. Linear regression analyses were used to calculate ovarian age.

Participants/materials, setting, methods: All AMH values were assessed using an ELISA assay (AMH Gen II ELISA assay; Beckman Coulter, Brea, CA, USA). All samples were processed in the same central laboratory.

Main results and the role of chance: The mean age of the women was 36.6 years ± 4.3 years. Reference values for AMH, expressed as 25th, 50th, and 75th centiles by age and five year groups, were obtained. There were significant differences in AMH values between groups of women aged 30-<35 years, 35-<40 years, and 40-45 years. No significant differences were observed in AMH values in the first two age groups (20-<25 years, and 25-<30 years). The 50th centiles of mean AMH ranged from 3.45 to 0.72 ng/ml. AMH values were found to be significantly, and inversely, correlated to age (r= 0.35; p<0.001). From the regression equation, the estimated yearly decrease in AMH was 0.2 ng/ml. The range of AMH values in Spanish women were higher than those reported for other European countries and the USA, and lower than those reported for China.

Limitations, reasons for caution: The weakness of our study is the use of cross-sectional data, making it difficult to distinguish the behaviour of different variables over time. Thus, longitudinal values are needed to validate our nomogram. A further limitation is that it was not possible to correlate clinical data with the AMH values.

Wider implications of the findings: Each population may use its AMH reference values to define the ovarian reserve in addition to the patient's chronological age. AMH values in Spain are different from those reported for other populations.

Trial registration number: Not applicable.

P-713 Comparison of pretreatments with leupron stop, oral contraceptive, estrogen priming and estrogen primed antagonist in short antagonist ivf cycles

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Study question: Do pretreatments pretreatments with leupron stop, oral contraceptive, estrogen priming and estrogen primed antagonist have difference in the outcome of short antagonist ivf cycles?

Summary answer: Collected and fertilized oocyte numbers, and live birth rates are lower with estrogen priming in comparison with leupron stop, oral contraceptive and estrogen primed antagonist.

What is known already: Non-homogenous follicular growth is one of the frequent problems of short antagonist ivf cycles and leads less mature and fertilized oocyte rates among the collected cumulus oophorus complexes (COC), thus it decreases the IVF success rates. Pretreatments aiming to lower pre and early follicular FSH levels are used to develop follicles with homogenous sizes during the initial phase of controlled ovarian stimulation. Although, there are numerous studies suggesting the benefits of the modalities, a through comparison of the efficacy of them with each other is lacking.

Study design, size, duration: Our retrospective, case controlled study included 655 short antagonist protocol ivf cycles between 2011 and 2015 in İstanbul Memorial Hospital. Following age, body mass index and Anti-Müllerian Hormone (AMH) level matching, four pretreatment modality groups including leupron stop (LS; n = 128), oral contraceptive (OC; n = 228), estrogen priming (EP; n = 128) and estrogen primed antagonist (EPA; n = 171) were formed.

Participants/materials, setting, methods: Infertile women with previous multiple unsuccessful IVF cycles with non-homogenous follicular growth were included. Ivf trial number, infertility duration, oocyte pick up characteristics including cumulus oophorus complex, mature oocyte and fertilized oocyte numbers; and ivf outcome measures including biochemical, clinical and ongoing pregnancy rates and live birth rates of the four groups were compared using one way analysis of variance and Bonferroni correction tests. A p value <0.05 was considered significant.

Main results and the role of chance: Daily and total dose of used gonadotropins and stimulation length in EP and OC groups were significantly lower than LS and EPA groups (p<0.05). Lack of FSH suppression after the last dose of OC till the follicular phase and mild suppression of FSH with EP may be the explanation. Long stop group, because of the most powerful suppression, had the longest stimulation period.

Cumulus oophorus, mature oocyte and fertilized oocyte numbers of EP group (6.8 ± 4.9, 5.2 ± 3.8, 4.1 ± 2.9, respectively) were significantly lower than LS (10.0 ± 6, 8.0 ± 4.8, 6.8 ± 4.1, respectively), OC (8.7 ± 6.8, 6.7 ± 5.2, 6.0 ± 4.6, respectively) and EPA (8.8 ± 6.0, 6.0 ± 4.9, 5.9 ± 4.4, respectively) groups' (p<0.05). Higher estrogen levels with EP may suppress some follicles at the recruitment stage.

Although, biochemical, clinical and ongoing pregnancy rates did not differ among groups (p > 0.05), live birth rate of EP group (17%) was significantly lower than LS (30%), OC (32%) and EPA (31%) groups' (p<0.05).

Used daily/total gonadotropin dose was significantly lower in OC group (p<0.05) and followed by estrogen priming group.

Estrogen priming group with the least oocytes, mature oocytes and fertilized oocytes had the lowest live birth rates (p<0.05), although the biochemical, clinical and ongoing pregnancy rates did not differ among groups (p>0.05).

Limitations, reasons for caution: The study was retrospective and presented the results of age, BMI and AMH matched cases. In clinical practice, presence of endometriosis, adenomyosis and cysts may urge to select certain pretreatment modality. Doses were adjusted depending the experience and preferences of the clinicians and randomization lacked.

Wider implications of the findings: Pretreatment modalities may be more effective in clearly defined specific groups. Higher gonadotropin doses in EP may improve the group specific IVF outcomes. However, until more definitive results EP should not be the first choice pretreatment modality to constitute homogenous follicular growth.

Trial registration number: None.

P-714 Fetal sex affects the maternal level of plasma anti-Mullerian hormone during pregnancy in cattle

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Study question: The aim of this study was to investigate the association between the plasma anti-Mullerian hormone (PI AMH) levels in cows carrying male vs. female fetuses.

Summary answer: Cows carrying a male compared to a female fetus showed a significantly different change in PI AMH between day 35 and 135 of pregnancy.

What is known already: AMH has recently gained attention as a potential early pregnancy sex-determining marker due to its markedly different expression in male vs. female fetuses. Only a few studies to date have investigated human maternal levels of AMH during gestation, with only one having reference to fetal sex. All of these studies showed a decline of maternal AMH levels during pregnancy, presumably due to inactivation of follicle growth, except of the single study in humans considering the impact of fetal sex on maternal AMH revealed a significantly higher PI AMH levels in mothers carrying a male fetus.

Study design, size, duration: PI AMH was measured in cows (n = 8) carrying female and male fetuses throughout gestation day 0, 35, 135 and 275 for each animal, as well as measured at single time points in the period between 85 and 155 days of gestation in cows (n = 21) and their fetuses. Expression of AMH receptor 2 (AMHR2) was measured in the placenta membranes and cotyledons of male and female fetus pregnancies (n = 13) between 38 and 80 days of gestation.

Participants/materials, setting, methods: Cows were subjected to peripheral or post-mortem blood collection coupled with a matching fetal blood collected via heart puncture in EDTA tubes and centrifuged at 1.400 rpm. The top plasma layer was aspirated and the Bovine AMH ELISA kit AL-114 was used to measure concentration of PI AMH. Fetal sex was determined by the measurement of the annogenital distance. Cotyledon and placenta membranes from each reproductive tract were used to measure AMHR2 mRNA expression via qPCR.

Main results and the role of chance: PI AMH in the multiple time point experiment between cows carrying a male vs. a female fetus between day 35 and 135 was significantly different, ($p = 0.036$; 255.4 ± 178.5 pg/ml vs. -181.3 ± 146.1 pg/ml, respectively); however, PI AMH between days 135 and 275 was not significantly different ($p = 0.786$; 87.6 ± 161.2 pg/ml vs. 59.5 ± 54.3 pg/ml, respectively). PI AMH in the single time point experiment in cows carrying a male or female fetus for the period of 85 to 155 days of gestation was not significantly different ($p = 0.557$; 947.3 ± 146.6 pg/ml vs. 809.4 ± 180.5 pg/ml, respectively). Significant differences were also observed between the average male and female fetus PI AMH ($p<0.05$; 175,309.9 ± 16,903.7 pg/ml vs. 154.1 ± 22.3 pg/ml, respectively), and between PI AMH of pregnant cows and their corresponding fetuses of both sexes ($p<0.05$). AMHR2 mRNA expression in cotyledon and placenta membrane samples were evaluated and found not to be significantly different in opposite sex fetuses ($p = 0.553$, and $p = 0.660$ respectively). When comparing within each sex samples, results were not significantly different for the male ($p = 0.381$), or the female ($p = 0.927$). The relative AMHR2 mRNA expression average in male cotyledon and placenta were 0.015 ± 0.004 and 0.050 ± 0.030 respectively, and in female cotyledon and placenta were 0.017 ± 0.001 and 0.081 ± 0.060 respectively.

Limitations, reasons for caution: Larger studies with additional sampling time points should be completed to verify these findings.

Wider implications of the findings: The evidence of thousand times higher fetal PI AMH levels in male vs. female fetuses and a linked significant increase in the maternal PI AMH levels between day 35 and 135 supports the hypothesis that ovarian function and maternal physiology can be affected by the sex of the fetus.

Trial registration number: 0.

P-715 Severe vitamin D deficiency is negatively associated with antral follicle count in infertile women

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Study question: Is severe vitamin D deficiency associated with anti-Mullerian Hormone (AMH) and antral follicle count (AFC) in infertile patients?

Summary answer: Severe vitamin D deficiency is negatively associated with antral follicle count in infertile women.

What is known already: Vitamin D receptor has been identified in many reproductive tissues, while vitamin D serum levels have been negatively associated with female infertility. However, it remains unclear what levels of vitamin D are normal and what levels indicate severe deficiency in infertile women. Moreover, it is not known what are the implications of severe vitamin D deficiency regarding female reproductive potential, although there is evidence to support an association between vitamin D and ovarian reserve, as assessed by AMH and AFC.

Study design, size, duration: This is a prospective, cross-sectional study, performed between January 2016 and January 2017, in 157 consecutive Caucasian women, attending an IVF Unit. Cause of infertility varied among women and was due to age, endometriosis, fibroids, tubal factor or was idiopathic. Ovarian reserve was evaluated by assessment of serum AMH and AFC.

Participants/materials, setting, methods: AMH and vitamin D were assessed in the same blood sample using the automated Elecsys-AMH and Elecsys-Vitamin D total assay (Roche-Diagnostics), respectively. Severe vitamin D deficiency was defined as serum vitamin D <10 ng/mL.The time of blood samples collection was recorded to account for seasonal variation of vitamin D levels. Statistical analysis was performed using bivariate and multivariable logistic regression. Continuous variables are presented as mean (95% CI) and binary variables as proportion (95% CI).

Main results and the role of chance: Female age was 38.5 (37.7-39.2) years, BMI was 25.1 (24.5-25.8) kg/m^2 and infertility duration was 5.1 (4.6-5.6) years. Baseline serum FSH and AMH levels were 10.9 (9.8-12.2) IU/mL and 12.5 (10.4-14.5) pmol/L, respectively. AFC was 11.7 (10.4-12.9), while serum vitamin D levels were 19.5 (17.9-21.2) ng/mL. Out of the 157 patients analysed, 31 (19.6%, 13.7-26.7) had severe vitamin D deficiency. No significant association was present in bivariate analyses between severe vitamin D deficiency and age (coefficient: +0.04, 95%CI: -0.05 to +0.12), BMI (coefficient: +0.04, 95%CI: -0.04 to +0.12), duration of infertility (coefficient: +0.03, 95% CI: -0.08 to +0.14), AMH (coefficient: -0.03, 95%CI: -0.07 to +0.02), FSH (coefficient: +0.01, 95%CI: -0.04 to +0.06) and smoking (odds ratio: 1.28, 95% CI: 0.57 to 2.89). On the other hand, a significant negative association was present between severe vitamin D deficiency and AFC (coefficient: -0.07, 95%CI: -0.14 to -0.01, $p = 0.03$). This negative association remained significant ($p = 0.04$) in multivariable logistic regression analysis, controlling for age, BMI, smoking, duration of infertility, AMH, FSH and seasonal variation of vitamin D levels. AFC was significantly lower in patients with (mean: 8.58, 95%CI: 6.73 to 10.43) as compared to those without (mean: 12.41, 95%CI: 11.28 to 13.53) severe vitamin D deficiency.

Limitations, reasons for caution: The sample size in the current study might have limited the establishment of a significant association between severe vitamin D deficiency and AMH. Moreover, the results presented do not necessarily imply the presence of a causative association between severe vitamin D deficiency and AFC.

Wider implications of the findings: Confirmation of the present results in further prospective studies may justify the conduction of clinical trials evaluating vitamin D supplementation in infertile patients with severe vitamin D deficiency.

Trial registration number: -.

P-716 Differential incorporation of estrogen receptor β (ERβ) into promoter regions and its relevence to aromatase gene expression in women with polycystic ovary syndrome

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Study question: Is there any correlation between differential binding of ERβ into promoter regions of *CYP19A1* and its gene expression in cumulus cell of PCOS?

Summary answer: Differential occupancy of regulatory promoters of *CYP19A1* gene with ERβ protein was observed in cumulus cells (CCs) of PCOS patients compared to non-PCOS women.

What is known already: Estrogen biosynthesis by aromatase coding gene (*CYP19A1*) expression is a hallmark of several aspects of human reproductive health, ovarian functions and is also implicated in the development or progression of numerous diseases, including PCOS. Human ovarian cell aromatase is controlled by locally produced estradiol hormone through its receptor (i.e., Estrogen Receptor β). ER β is a transcription factor which directs the expression of the aromatase gene in cumulus cells. Our previous study showed that epigenetic alterations could change the aromatase gene expression in CCs, which may play a key role in the abnormal folliculogenesis and ovarian function in PCOS.

Study design, size, duration: Cross-sectional study was conducted on 24 patients (12 PCOS patients and 12 women with normal cycling ovaries as control group). Cumulus cells were obtained from women aged 18–36 year old, who underwent ovarian stimulation with antagonist protocol for intracytoplasmic sperm injection between November 2014 to April 2015. Informed consents were obtained from the participants.

Participants/materials, setting, methods: Cumulus oocyte complexes (COC) were obtained from follicles during ovarian puncture. Shortly before ICSI, CCs were stripped from the COC with hyaluronidase. Only cumulus cells with metaphase II oocytes (characterized by the presence of the first polar body) were selected for this study. Gene expression levels of *ERβ* were quantified using Q-PCR. Incorporation levels of ERβ protein to three promoter regions of *CYP19A1* gene (PII, PI.3 and PI.4) were evaluated by Chromatin Immunoprecipitation (ChIP) assay.

Main results and the role of chance: PCOS cumulus cells showed significant up-regulation of *ERβ* gene compared to the control group. Also, ChIP-qPCR analysis of ERβ binding into the three analyzed promoter regions of *CYP19A1* gene showed an increased occupancy of the PII and PI.4 promoters with the ERβ protein in CCs of PCOS patients ($P = 0.01$ and $P = 0.02$, respectively). In contrast, the incorporation of ERβ into promoters PI.3 in these patients is comparable to the control group.

Limitations, reasons for caution: Further studies are necessary to evaluate the interactions of other transcription factors with ERβ and to clarify the detailed mechanisms involved in the *CYP19A1* gene expression in PCOS patient.

Wider implications of the findings: Regarding the importance of the regulatory role of ERβ on the expression of aromatase gene, an augmented ovarian cells reaction in PCOS patients undergoing ovarian stimulation, relevant to aromatase expression, may be described by increased gene expression along with incorporation of the ERβ protein to its promoters.

Trial registration number: not applicable.

P-717 The effect of positive TPO-Ab on pregnancy outcomes of euthyroid IVF women

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Study question: It is still controversial if positive thyroid autoimmunity will affect the pregnancy outcomes of euthyroid women, especially women undergo in vitro fertilization (IVF).

Summary answer: Anti-thyroperoxidase antibody (TPO-Ab) is an independent risk factor of biochemical pregnancy and miscarriage of euthyroid women underwent IVF.

What is known already: Thyroid disease is the second most common endocrine condition in women at childbearing age. Thyroid hormones are involved in control of menstrual cycle and fertility by regulating the actions of follicle-stimulating hormone and luteinizing hormone on steroid biosynthesis by specific triiodothyronine sites on oocytes. Abnormal thyroid function cause adverse effects on maternal and fetal outcomes, for instance, pregnancy loss, gestational hypertension, or pre-eclampsia, pre-term delivery. Thyroid autoimmunity refers to the positive state of antithyroid antibodies, TPO-Ab and/or antithyroglobulin antibody (TG-Ab). Whether positive thyroid autoimmunity will affect the pregnancy outcomes of euthyroid women underwent IVF treatment was not clearly elucidated.

Study design, size, duration: A retrospective cohort study comparing pregnancy outcomes of women underwent IVF with positive or negative TPO-Ab were conducted. This study reviewed 6321 infertility women who received IVF treatment at Reproductive center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University between January 2014 to October 2016.

Participants/materials, setting, methods: Women met the following criteria were included in this study, 1) aged 20-35 years old, 2) with normal thyroid hormone levels, 3) not received treatment against thyroid dysfunction, 4) not complicated with other autoimmunity diseases, 5) did not take any hormones or anti-coagulation medications in recent 3 months. Women with TPO-Ab more than 60IU/L were defined as antibody positive cases. Clinical data and pregnancy outcomes of women with positive and negative TPO-Ab were compared.

Main results and the role of chance: Euthyroid women underwent IVF with positive or negative TPO-Ab showed no differences in age, body mass index, duration of infertility, causes of infertility, basal FSH, LH, AMH, AFC, TSH levels. No significant differences of endometrial thickness, number of oocytes retrieved, number of embryos transferred, implantation rate, clinical pregnancy rate, and ectopic pregnancy rate were detected between euthyroid women with or without TPO-Ab. However, biochemical pregnancy rate and miscarriage rate of euthyroid women with positive TPO-Ab were much higher than those with negative TPO-Ab (biochemical pregnancy rate, 10.5% vs. 5.9%; miscarriage rate, 15.0% vs. 8.7%, $P < 0.05$). Logistic regression analysis showed that TPO-Ab is an independent risk factor of both biochemical pregnancy (adjusted OR 2.53, 95% CI 1.37-2.97, $P < 0.05$) and miscarriage (adjusted OR 3.02, 95% CI 1.62-3.87, $P < 0.01$) of euthyroid women underwent IVF.

Limitations, reasons for caution: This study recruited patients from single center, which might have selection bias, and may not represent other population from China.

Wider implications of the findings: It is necessary to screen thyroid autoimmunity of women undergo IVF, especially those with repeated miscarriage.

Trial registration number: NA.

P-718 Increased proportion of mature oocytes with sustained-releasing growth hormone treatment in poor responders: a randomized controlled study

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Study question: Does sustained-release human growth hormone (GH) supplement in poor responders undergoing in-vitro fertilization using GnRH antagonist protocol improve ovarian response?

Summary answer: Supplementation of sustained-release GH before and during controlled ovarian stimulation would improve ovarian response with more mature oocytes in poor responders.

What is known already: Use of growth hormone in poor responders has been found to show a significant improvement in ovarian response.

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Study design, size, duration: This study was a single center, randomized, open label, parallel study. Infertile women who were diagnosed with decreased ovarian reserve were recruited from March 2014 to September 2015. With power 90% and significant level of 5%, sample size was calculated in reference to result from previous study. To detect significant difference in serum estradiol level of 150 pg/mL on hCG day, 62 samples were required in each group assuming 10% of lost-to follow-up.

Participants/materials, setting, methods: Study eligibility was determined in reference to the Bologna criteria.The eligible participants were randomized into recombinant growth hormone treatment group and control. Women in the treatment group received sustained-release human growth hormone (Eutropin Plus® 20 mg, LG Life Sciences) three times before and during COS (Mid-luteal, late luteal and menstrual cycle day 2). The basal characteristics and IVF outcomes were compared between the two groups.

Main results and the role of chance: A total of 127 patients were included in the final analysis. The mean age of study population was 39.6 years and mean AMH was 0.6. Mean body mass index, medians of age, duration of infertility and previous number of IVF cycles were not different between GH treatment group (N = 62) and control (N = 65). There was no significant difference in the basal characteristics between the two groups. On hCG triggering day, GH group showed higher serum level of IGF-I and number of follicles (> 14 mm) than control (3.1 ± 2.3 vs 2.4 ± 1.6, P = 0.043). Higher proportion of MII oocytes in total retrieved oocytes (67.5 vs 52.3, P = 0.030) were observed in GH group. There were no significant differences in total dose of gonadotropin, endometrial thickness, fertilization rate and number of transferred total/good embryos. The proportion of clinical and ongoing pregnancy and miscarriage were not different between the two groups. In the multivariable linear regression models which controlled for the effect of confounders (i.e. age group, AMH, BMI, infertility duration, previous number of IVF cycles, endometrial thickness, progesterone on hCG and total number of retrieved oocytes), the probability of ongoing pregnancy was not different between the two group.

Limitations, reasons for caution: First, there was no comparison group with daily injection of recombinant GH. Further studies using control group with daily injection of recombinant GH would be needed. Second, we did not measured IGF-1 and IGFBP-3 in follicular fluid. Serum level of those may reflect the local IGF-1 and IGFBP-3 level.

Wider implications of the findings: The sustained-release form of recombinant GH would benefit wide range of poor responders undergoing fresh IVF-ET cycles. Since pregnancy rate in the poor responder group were generally low, larger sample would be needed to detect subsequent improvement in pregnancy rate.

Trial registration number: None.

P-719 GnRH-antagonist and agonist/antagonist conversion based ovarian stimulation protocols did not alter developmental outcomes and the embryonic ploidy

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Study question: Do ovarian stimulation protocols based on GnRH-antagonist and agonist/antagonist conversion affect developmental outcomes and the embryonic ploidy during IVF cycles?

Summary answer: GnRH-antagonist and agonist/antagonist conversion based ovarian stimulation protocols did not alter developmental outcomes and the embryonic ploidy during IVF cycles.

What is known already: Potential adverse effects of gonadotropin analogs employed for ovarian stimulation on embryo development and quality have been a concern during controlled ovarian stimulation treatments. Though controversial, several studies indicated an association between stimulation protocols (gonadotropin type, dosage and duration) and ploidy status of embryos. Proponents of this idea suggest that the ovarian response to the hormonal stimulation affects the ability of recruited follicles to support oocyte growth and maturation, thus altering the competency of resulting oocytes and embryos.

Study design, size, duration: Retrospective cohort study. A total of 170 patients who underwent ovarian stimulation cycles followed by trophectoderm biopsy with array comparative genomic hybridization in a single center in 2014.

Participants/materials, setting, methods: Two different types of controlled ovarian stimulation protocols with gonadotropin-releasing hormone [GnRH] -antagonist, or agonist/antagonist conversion were used to treat 170 patients (antagonist protocol: 114 and conversion protocol: 56). A total of 542 blastocysts were biopsied and screened for aneuploidy using array genomic hybridization. Main outcome measures were frequencies of fertilization, blastocyst conversion and ploidy status of resulting embryos.

Main results and the role of chance: Fertilization and blastocyst conversion rates did not differ between stimulation protocols (% 86 and % 89 fertilization, % 43 and % 48 blastocyst conversion). There was no change in the rate of euploid embryos with different stimulation protocols for antagonist and conversion protocols (%38 and %35 respectively). Although it did not reach a statistical significance, patients who were < 35 yrs old tend to produce more euploid embryos with antagonist protocol than those with conversion protocol (%52 and % 34 respectively). When patients were classified based on their cycle day 3 FSH levels (FSH<8mIU/ml vs FSH>8 mIU/ml), no outcome measure of the study was different between stimulation protocols

Although it is plausible that controlled ovarian stimulation protocols affect the quality of preimplantation stage embryos, our results did not suggest any significant difference between two commonly used current stimulation protocols, in terms of embryo development and ploidy status.

Limitations, reasons for caution: Retrospective analysis with a relatively modest sample size in a single center setting.

Wider implications of the findings: Data presented here adds the growing body of data about possible relationship between ovarian stimulation protocols and embryo competency/characteristics.

Trial registration number: NA.

P-720 Close relationship of hirsutism to hyperandrogenemia in women with polycystic ovary syndrome - with LC-MS/MS measurements

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Study question: There were little overlaps between hirsutism and hyperandrogenemia although they were both representatives of the clinical and biochemical aspects of androgen excess. Is it true or because the unaccurate evaluation?

Summary answer: The incidence of hirsutism assessed by modified Ferriman-Gallwey (mFG) and simpified FG, was 80.2% and 86.6%, in patients deemed hyperandrogenemic by LC-MS/MS (P < 0.001).

What is known already: Hyperandrogenism is critically important for the diagnosis and management of PCOS, and can be determined clinically, usually by hirsutism, or biochemically, through the level and total testosterone (TT) or, better still, free testosterone (FT). However, there were little overlaps between hirsutism and hyperandrogenemia found in published reports. We have published that the improved clinical evaluation of hirsutim had a much closed relationship with TT level, with the measurement of liquid chromatography and tandem mass spectrometry assay (LC-MS/MS). Here we hypothesized that TT measured by LC-MS/MS would predict the extent of unwanted hair growth well in Asian PCOS subjects.

Study design, size, duration: Study designed: A cohort study in an academic hospital for one year. Size and duration: A total of 332 consecutive women with PCOS aged 18 to 44 years were recruited at Sun Yat-sen Memorial Hospital from September 21, 2014 to October 10, 2015.

Participants/materials, setting, methods: All subjects were screened using a standard tool to collect clinical data, undergo a physical examination and blood sampling The relationship between hirsutism and TT and its corresponding FAI

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values measured by LC-MS/MS versus chemiluminescent immunoassay (CLIA). The concordance and the deviation of TT and the mFG and sFG (solely including the scores of the upper lip, lower back, lower abdomen, and thigh) scoring system for diagnosing hyperandrogenism were further analyzed.

Main results and the role of chance: The incidence of hirsutism assessed by mFG and sFG, was 80.2% and 86.6%, in patients deemed hyperandrogenemic by LC-MS/MS ($P < 0.001$), compared to 56.6% and 67.5% of those determined hyperandrogenemic by CLIA, respectively. Rank correlation coefficient between TT level determined by LC-MS/MS and mFG scores was 0.642 (95% CI: 0.571, 0.703, $P < 0.001$); while that between TT by CLIA and mFG scores was 0.045 (95% CI: - 0.067, 0.156, $P = 0.420$). Likewise, TT by LC-MS/MS was positively correlated with sFG scores, with a $R = 0.780$ (95% CI: 0.730, 0.817). FAI estimated from LC-MS/MS TT values was positive related to mFG and sFG scores, with a $R = 0.261$ and 0.204, respectively ($P < 0.01$). There were 80.2% women with increased mFG scores who were also hyperandrogenemic defined by increased TT level measured by LC-MS/MS.Furthermore, the cluster analysis demonstrated that TT level measured by LC-MS/MS clustered with mFG score; these two parameters could explain 84.7% of variance. Logistic regression analysis indicated that the risks of increased TT level measured by LC-MS/MS for those with increased mFG score (mFG score ≥ 5) (OR) was 13.485 ($p < 0.0001$).

Limitations, reasons for caution: The free testosterone level could not be measured by accurate method of LC-MS/MS yet.

Wider implications of the findings: Hirsutism has close relationship with TT measured by LC-MS/MS in Mongoloid Chinese women with PCOS, suggesting that the evaluation of facial and body terminal hair growth should be the first line examination to predict hyperandrogenism.

Trial registration number: ChiCTR-DDT-14005186

P-721 Comparison of fresh versus frozen embryo transfer in women with Polycystic Ovary Syndrome

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Study question: Is frozen embryo transfer in subsequent cycle better than fresh transfer in women with polycystic ovary syndrome (PCOS) when human chorionic gonadotropin (hCG) is used as trigger in antagonist cycles.

Summary answer: Clinical pregnancy rates and live birth rates are better with freezing and transfer in subsequent cycle compared to fresh embryo transfer in women with PCOS

What is known already: Transfer of fresh embryos is a usual practice but in women at risk of ovarian hyper stimulation due to excess follicle development, elective cryopreservation of all embryos and transfer in subsequent cycle is preferred. Fresh cycles have supra physiological steroid levels which may alter the endometrial receptivity and probably affect placentation adversely whereas frozen embryo transfer is done to the uterus after a programmed physiologic cycle of hormone replacement to prepare the endometrium.

Study design, size, duration: A prospective cohort study. Infertile women less than 35 years with the polycystic ovary syndrome diagnosed by Rotterdam criteria who were undergoing their first IVF cycle from 1st Jan 2015 to 28th Feb 2016 were included. Cycles complicated by ovarian hyper-stimulation syndrome (OHSS) were excluded.

Participants/materials, setting, methods: Women (N = 126) with terminal estradiol levels below 2500 pg/ml were triggered with recombinant hCG and based on the number of retrieved oocytes were divided into two groups, Group A- < 15 oocytes retrieved had fresh embryo transfer and Group B where > 15 oocytes were retrieved, all embryos were frozen on day 3 and transferred in subsequent cycle. Primary outcomes were clinical pregnancy rates and live birth rates. Secondary outcome were fertilisation, implantation and miscarriage rates.

Main results and the role of chance: Group A had 73 fresh transfer and Group B had 53 frozen embryo transfer. Both groups were comparable regarding age, body mass index (BMI), basal follicular stimulating hormone (FSH), Antimullerian hormone (AMH) and antral follicle count (AFC). Categorical data was represented as frequency and percentages, differences in these measures between the groups were compared using chi-square tests and quantitative data by student t test. Clinical pregnancy rates (Group A-38.4% versus Group B- 41.5%, $p = 0.88$), Live Birth Rate (Group A: 26.0% versus Group B: 33.9% $p = 0.25$) were slightly higher in Group B though not statistically significant. The misscarriage rate in both the groups were comparable. (Group A 15.1 % and Group B 15.1 %, $p = 0.8$).

Limitations, reasons for caution: Due to the small sample size the study lacks power, which is the main limitation.

Wider implications of the findings: Performing frozen embryo transfer in PCOS women with average estradiol levels yet higher number of recovered oocytes is better than fresh transfer, implying adopting practice of routine elective freezing of embryos and transfer in subsequent cycle for better reproductive outcomes even in hCG triggered cycles in PCOS women.

Trial registration number: MCDH/2014/27.

P-722 Pre-hCG serum progesterone elevation in consecutive 762 IVF cycles: There is a cycle to cycle variation in ovarian response and progesterone levels

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Study question: Should we expect to see similar pre-hCG progesterone (P) levels in two consecutive IVF cycles comparable for gonadotropins, GnRH analog used, and duration of stimulation?

Summary answer: Serum P level at ovulation trigger in the subsequent IVF cycle may change unpredictably and disproportionately with P and ovarian response of a previous cycle.

What is known already: It is well-documented that serum P level at the time of hCG administration is significantly correlated with the magnitude of ovarian response to stimulation (the number of pre-ovulatory follicle≥14 mm, E_2 on hCG day, total and mature oocyte numbers). However, it is largely unknown whether serum P levels and ovarian response change proportionally in two consecutive IVF cycles stimulated with the same protocol and FSH given at a similar dose and duration.

Study design, size, duration: A non-interventional, retrospective cohort data of from single center

Participants/materials, setting, methods: A total of 381 patients (age:31.2 ± 3.2) undergoing two consecutive ovarian stimulation cycles (n = 762) with GnRH agonist long protocol were analyzed. Total FSH dose (2887 ± 1022 vs. 2856 ± 1034 IU respectively, $p = 0.42$) and duration of stimulation (10.3 ± 1.3 vs. 10.4 ± 1.6 days respectively, $p = 0.72$) were comparable between the cycles. The mean interval between the cycles was 6.7 ± 3.5 months. Paired t-test or Wilcoxon matched-pairs signed rank test were used to compare P levels and ovarian response parameters between the cycles.

Main results and the role of chance: Serum P level on hCG day was significantly increased (≥ 50%;≥ +1 SD) in the second IVF cycle in 95 of 381 patients compared to the first cycles values of P (2.06 ± 0.4 vs. 1.03 ± 0.3 ng/mL respectively, $p < 0.01$). Ovarian response (preovulatory follicles≥14 mm, E2 on the day of hCG, and total and mature oocyte numbers) significantly increased along with P in the second IVF cycle in only 45 (47.3%) while it did not change significantly in 11 (11.5%); and significantly decreased in the remaining 39 (41.2%) of the 95 patients compared to their first IVF cycle. In another 41 of these 381 patients serum P level significantly decreased by ≥ 50% (≥ +1 SD) in the second vs. first IVF cycle (0.64 ± 0.3 vs. 2.1 ± 0.5 respectively, $p<0.001$). Despite the decline in P level ovarian response significantly increased in the second cycle in 13 (31.7%); did not change significantly in 7 (17%), and significantly decreased in the remaining 21 patients (51.3%) compared to their first cycle. In the remaining 245 patients (64.3%) serum P level at ovulation trigger, ovarian response and oocyte yield were completely comparable between the first and second IVF cycles.

Limitations, reasons for caution: Retrospective nature of data.

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Wider implications of the findings: These findings indicate that serum P may change in the next IVF cycle unpredictably and disproportionately with P and ovarian response of previous cycle suggesting that growth characteristics and steroidogenic activities of antral cohorts that produce P before ovulation in response to FSH may exhibit considerable cycle to cycle variations.

Trial registration number: None.

P-723 Outcomes of in vitro fertilization in poor responders of ≤ 40 years of age using either daily gonadotropin stimulation or long acting FSH stimulation

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Study question: Evaluation of outcomes of in vitro fertilization in poor responders in two stimulation regimens, daily gonadotropins (HMG-HP) and long acting FSH stimulation protocol.

Summary answer: The primary and secondary outcomes in poor responders showed no significant difference in the various clinical and embryological parameters in the two groups studied.

What is known already: Long acting FSH is a novel recombinant FSH analog which can be administered in the follicular phase and sustains the follicular development for the first week unlike other gonadotropins. Cochrane review concluded its efficacy in normoresponders and was comparable to daily recombinant FSH. Poor responders were excluded from these studies. Due to its two fold longer half life and sustained pharmacological FSH activity, it has a critical role to play in initial spurt of follicular growth in poor responders. Previous studies have been small and retrospective with no difference in results between two regimens.

Study design, size, duration: This study was conducted as prospective observational study from Jan 2016- September 2016 at FakihIVF Fertility center, UAE.

One hundred and thirty three women, ≤40 years of age undergoing in vitro fertilization with fresh cycle transfers were included.

Inclusion criteria – Women with previous poor response (< 4 COCs after maximal stimulation), FSH ≤ 20 IU/L, BMI ≤ 32 kg/sq m.

Exclusion criteria – Women > 40 years.

Participants/materials, setting, methods: Using Antagonist protocol.

Group A – Long acting FSH in a single dose of 150 microgram on day 2 and followed by HMG from day 7 in a dose of 225 units.

Group B – HMG-HP 300 IU daily from day 2 of the cycle.

Primary outcome was ongoing pregnancy rate.

The secondary outcome measures were length of stimulation protocol, number of eggs retrieved, number of embryos transferred, number of cancelled cycles.

Main results and the role of chance: The two groups did not differ in mean age, AMH levels, number of stimulation days, number of eggs retrieved and number of embryos transferred.

The ongoing pregnancy rates per cycle in Group A were 36.7% (11/30) and in Group B were 34.2% (25/73). The difference was not statistically significant (P = 0.815). The pregnancy rate per embryo transferred was 23.9 % (Group A) and 21.4 % (Group B) respectively. The implantation rates were Group A (30.4%) and Group B (23.9%) (p = 0.0.39) respectively. The cancellation rates (no embryos transferred) were lower in Group A (14.3%) than Group B (25.5%) but the difference was not found statistically significant. ROC analysis indicates that AMH is not a significant predictor of cumulative pregnancy status as evidenced by Area Under Curve (AUC), which shows 0.646 for Antagonist group and 0.553 for Long acting FSH group.

Limitations, reasons for caution: Large randomised studies are needed to prove the efficacy of Long Acting FSH in poor responders.

Wider implications of the findings: Long acting FSH may provide a reduction in the daily burden of injections for poor responders with comparable results to conventional regimens. This can improve the drop out rates for in vitro fertilization in poor responders where multiple cycles are needed to achieve success.

Trial registration number: The study was approved by the local ethical committee and Institutional review board.

P-724 The role of pigment epithelium-derived factor (PEDF) in the pathogenesis and treatment of polycystic ovary syndrome (PCOS)

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Study question: Does pigment epithelium-derived factor (PEDF) play a role in the pathogenesis and treatment of polycystic ovary syndrome (PCOS)?

Summary answer: Androgen-induced low PEDF level enables increase of VEGF and IL6/8 expression. Treatment with rPEDF reduces VEGF and IL6/8 levels, alleviating the angiogenic and inflammatory imbalances.

What is known already: PCOS is the most common endocrine disorder in women. Whereas the etiology of PCOS remains elusive, there is compelling evidence that chronic, systemic inflammation is a significant component of this disorder. However, the relationship between inflammation and hyperandrogenism, the hallmark of PCOS, is not well understood. PCOS patients commonly have increased expression of ovarian VEGF and cytokines (such as IL6/8). This overproduction of VEGF and IL6/8 is associated with ovarian hyperstimulation syndrome (OHSS). We showed that PEDF, a potent anti-angiogenic and anti-inflammatory factor, which counteracts VEGF and IL6/8, plays fundamental role in the pathogenesis and treatment of OHSS.

Study design, size, duration: In-vitro studies included cultures of human primary granulosa cells (hpGCs) from follicular fluids aspirated from women undergoing IVF treatments. Primary cells were allowed to reach quiescence after 5 days of culture in hormone-free medium. In-vivo studies included PCOS mice model: pregnant ICR mice were administered once daily with dihydrotestosterone (DHT) or with oil, as control, on days 16–19 of pregnancy. Female offspring were allowed to grow to reproductive age (8 weeks).

Participants/materials, setting, methods: We used a mouse PCOS model and cultured hpGC. Changes in the levels of PEDF, VEGF and IL6/IL8 were measured by qPCR, Western blot and ELISA.

Main results and the role of chance: Stimulation of hpGCs with DHT (10 ng/ml) resulted in significant downregulation of PEDF mRNA expression (~70%, P < 0.001), concomitantly with significant increase of IL6 and IL8 mRNA expression (P < 0.05). Co-stimulation with DHT (10 ng/ml) and rPEDF (5 nM), restrained the increase in the expression of IL6 and IL8 mRNA (P < 0.05). Stimulation of hpGCs with IL8 increased the expression of VEGF and decreased that of PEDF mRNA; whereas co-stimulation with IL8 and rPEDF restrained VEGF upregulation (P < 0.04). Finally, we found in the PCOS mice model an increase in the level of ovarian VEGF concomitant with a decrease in the level of PEDF mRNA (P < 0.05).

Limitations, reasons for caution: The in-vivo experiments were performed in a mouse model.

Wider implications of the findings: Our findings suggest a novel role of PEDF in PCOS pathogenesis and treatment. Hyperandrogenism reduces ovarian PEDF level, enabling high VEGF and IL6/8 levels. This angiogenic and inflammatory imbalance can be restored by rPEDF treatment. Further studies of PEDF role in the ovary could reveal new fertility therapeutic avenues.

Trial registration number: Not relevant.

P-725 The lower serum Anti-Müllerian Hormone (S-AMH) threshold measured by the Elecsys assay that predicts development of three mature follicles for In-Vitro Fertilization (IVF)

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Study question: Can serum AMH predict the patients with low ovarian reserve that reach the classical hCG criteria of three mature follicles after ovarian stimulation?

Summary answer: The S-AMH threshold giving the most accurate prediction was 4.15 pmol/L for reaching the hCG criteria and 4.3 pmol/L for positive pregnancy test.

What is known already: AMH is a marker of the ovarian reserve and the response to ovarian stimulation. The classical criteria for hCG-administration is ≥ 3 follicles ≥ 17 mm after controlled ovarian stimulation. S-AMH has shown to predict the ovarian response to gonadotrophin stimulation. Few studies have investigated the optimal S-AMH threshold predicting who will reach the classical hCG criteria among women with low ovarian reserve. A new fully automated assay is able to detect very low values of AMH.

Study design, size, duration: Prospective cohort study of 80 women with an AMH ≤ 12 pmol/L referred for IVF/ICSI. AMH was measured using the Elecsys assay on cycle day 2-3. Patients were treated in a short GnRH-antagonist protocol with a fixed dose of 300 IE HP-hMG. Follicular development was followed by ultrasound. Triggering ovulation with hCG was given when there was at least 1 follicle ≥ 17 mm. S-hCG ≥5 IE/L two weeks after embryo transfer defined positive hCG.

Participants/materials, setting, methods: Participants were women ≤ 40 years of age with a regular menstrual cycle and a low to limited ovarian reserve defined as AMH levels ≤ 12 pmol/L. Three out of 80 cycles (3.8%) were cancelled due to no follicular development. Embryo transfer was performed in 53/80 women (66.3%). Receiver operator curve (ROC)-analysis was used to identify the optimal AMH threshold for reaching the hCG criteria and for positive hCG. Analyses were performed using SAS 9.3.

Main results and the role of chance: The median age was 36.0 (range 26; 39) years, median cycle length was 26.0 (range 17; 34) days. Median AMH was 5.0 (range 0.41; 12.0) pmol/L. Twenty-one women (26.3%) reached the hCG criteria and 19 women (26.3%) had a positive hCG test. The lowest AMH-value observed in the group with positive hCG was 0.98 pmol/L. ROC-analysis showed that an AMH threshold of 4.15 pmol/L predicted those patients that reached the hCG criteria with 90.5% sensitivity and 44.1% specificity, area under curve (AUC) = 0.69 (95% CI; 0.57-0.81). Using AFC as predictor the AUC was 0.70, (95% CI; 0.57-0.82). Including both predictors in the model AUC was 0.70, (95% CI; 0.58-0.82).

As a predictor of positive hCG, AMH ≥ 4.3 pmol/L had the optimal performance with 78.9% sensitivity and 42.6% specificity, AUC was 0.63, (95% CI; 0.48-0.78). AFC was less accurate, AUC = 0.50, (95% CI; 0.34-0.66). When predicting positive hCG the best performance was achieved with the combined test with an AUC of 0.65, (95% CI; 0.51-0.79). Both regarding the classical hCG criteria and positive hCG test a combination of AMH and AFC had the best screen performance.

Limitations, reasons for caution: The study has a limited sample size and only one cycle per woman was included. The optimal would be to analyse AMH in relation to cumulative birth rates.

Wider implications of the findings: In women with lower ovarian reserve S-AMH is unable as a single marker to predict women that reach the hCG criteria or achieve pregnancy after ovarian stimulation as specificity is low. S-AMH cannot be used to allocate women to either IVF or oocyte donation prior to their first stimulation cycle.

Trial registration number: NA.

P-726 Melatonin treatment is able to restore menstrual cyclicity in PCOS women: a pilot study

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Study question: Does a six month treatment with melatonin improve the clinical, hormonal and metabolic features of normal-weight women affected by polycystic ovary syndrome (PCOS)?

Summary answer: Treatment with melatonin may improve menstrual irregularities and biochemical hyperandrogenism in normal-weight PCOS women.

What is known already: Melatonin seems to regulate steroidogenesis, folliculogenesis and oocyte maturation within the ovary, to protect follicles against oxidative stress and to rescue them from atresia. Melatonin may also play a role in the regulation of gonadotropin release hormone - gonadotropin axis. The reduction of melatonin levels due to pinealectomy induces the development of PCOS in rats. Melatonin supplementation is able to reduce long term body weight gain in animals. In addition, lower levels of this hormone have been detected in the ovarian follicles of PCOS women and this finding has been related to the anovulation that characterize the syndrome.

Study design, size, duration: Prospective cohort study including forty normal-weight PCOS women between January 2016 and September 2016 in an academic research environment.

Participants/materials, setting, methods: Forty normal-weight PCOS women (mean BMI 22.7, 18 – 35 years) were enrolled. Ultrasonographic pelvic exams, hirsutism score evaluation, hormonal profile assays (Antimullerian Hormone, Thyroid stimulating hormone, Follicle Stimulating Hormone, Luteinizing Hormone, Estradiol, Prolactin, Testosterone, Androstenedione, 17hydroxyprogesterone, Sex Hormone Binding Globulin and Dehydroepiandrosterone Sulfate), oral glucose tolerance test (OGTT) with the determination of insulin and glucose levels and lipid profile at baseline and after 6 months of melatonin treatment were performed.

Main results and the role of chance: Almost 95% of subjects experienced an amelioration of menstrual cycles during the treatment with melatonin with a significant increase in the number of cycles/six months ($P < 0.01$). A slight, yet significant, decrease in body weight ($P < 0,01$) and, consequently, in BMI ($P < 0.01$) was observed. A significant decrease in androgens levels occurred (Free Androgen Index: $p<0.05$; Testosterone: $p<0.01$; 17hydroxyProgesterone: $p<0.01$). FSH levels significantly raised ($p<0.01$) and AMH serum levels significantly dropped ($p<0.01$). After therapy, we did not observe a significant modification in the insulin secretion during the OGTT and the treatment did not affect peripheral insulin sensitivity in our group of patients. All patients had a normal lipid profile at baseline; total cholesterol, HDL and triglycerides plasma levels remained unvaried after six months of treatment, whereas LDL-cholesterol plasma concentrations showed a significant reduction.

Limitations, reasons for caution: The lack of a placebo group and the small sample size represent limitations of this pilot study. Future randomized controlled trials on this topic are needed to confirm our findings.

Wider implications of the findings: This is the first study focused on the effects of exogenous melatonin in PCOS patients. Melatonin seems to improve menstrual irregularities and biochemical hyperandrogenism independently of effects on insulin metabolism, Based on our results, melatonin could be considered a potential future therapeutic agent for women affected by PCOS.

Trial registration number: NCT02663570.

P-727 Is the performance of commercial progesterone assays suitable for determining fresh-cycle embryo transfer or freeze policy?

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Study question: Can commercially available progesterone assays be used to guide a policy of fresh embryo transfer versus segmentation if the progesterone concentration is deemed too high?

Summary answer: Commercially available progesterone assays show progressively greater inter-laboratory and inter-assay variability as progesterone concentrations decrease (Variance of >15% at <8.0 nmol/L).

What is known already: An early rise in progesterone concentration during ART stimulation is associated with asynchronicity between endometrial development and the subsequent embryo development, resulting in reduced implantation rates. Previous research states that if the progesterone concentration is > 4.5 nmol/L on day of trigger, it may be expedient to vitrify all suitable embryos (segmentation). This increases the risk of losing viable embryos. There

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are uncertainties about the precision of commercially available progesterone assays at low concentrations. We aimed to assess performance over an extended time-scale across multiple laboratories, to determine suitability for guiding a policy of fresh embryo transfer versus segmentation.

Study design, size, duration: Objective multicentre comparative cohort study. Sixteen successive monthly distributions of 80 serum samples were sent to contributing laboratories running one of five different progesterone assay systems (Abbott Architect®, Beckman Coulter Access 2®, Roche Elecsys®, Siemens Centaur® and Siemens Immulite®) through the NEQAS quality control system. The mean and percentage coefficient of variation (%CV) of the progesterone concentrations (as published by UK NEQAS) were compared.

Participants/materials, setting, methods: The absolute progesterone concentrations and inter-laboratory variance were assessed for sample values from the 80 samples analysed in 16 distributions for five different assay formats. A recovery test at follicular phase concentrations was also effected for one commercial platform (Beckman Coulter Access 2®).

Main results and the role of chance: At a mid-luteal progesterone concentration of 40 nmol/L the mean %CV was 8%. As the progesterone concentration decreased, the %CV increased. At progesterone concentrations between 8 and 10 nmol/L, the %CV was 14%. Variance at concentrations lower than this increased markedly.

The recovery test at approximately 5 nmol/L showed a variance of 20%.

The data are strong evidence questioning the reliability of commercial assays at follicular phase levels.

These statistical analyses undermine the potential role for these tests.

Limitations, reasons for caution: Recovery tests should be performed in all of the methods to confirm that the test weaknesses are universal.

Wider implications of the findings: Commercially available progesterone assays show progressively greater inter-laboratory and inter-assay variability as progesterone concentrations decrease. This increased variability could result in inappropriate decisions when deciding to proceed with a fresh embryo transfer or to employ segmentation and should be taken in to consideration by ART clinics.

Trial registration number: not applicable.

P-728 Change in endocrine, metabolic and body composition profiles of lean women with polycystic ovary syndrome after 1-year treatment with oral contraceptives

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Study question: Do 1-year treatment with oral contraceptives (OC) change endocrine, metabolic and body composition profiles of lean women with polycystic ovary syndrome (PCOS)?

Summary answer: Treatment with OC for 1 year could change not only endocrine and metabolic profiles, but also body composition profiles of lean women with PCOS.

What is known already: Body composition profile is a useful clinical predictive marker for development of PCOS, a common reproductive endocrine and metabolic disease with ethnic variation. Although OC is commonly used to manage the symptoms of menstrual irregularity and a- or oligo-menorrhea with PCOS, most previous studies of its effect on endocrine, metabolic and body composition profiles have been cross-sectional, retrospective or case–control.

Study design, size, duration: One hundred and forty of women with BMI<25 Kg/m^2 and PCOS diagnosed by Rotterdam criteria were recruited to check endocrine, metabolic and body composition profiles with 1-year OC use and annual follow-up in this cohort study.

Participants/materials, setting, methods: Before and after 1-year OC use, endocrine and metabolic profiles were analyzed by serum sample and body composition profiles were checked using multi-frequency bioelectrical impedance analysis. Data were analyzed by Paired t-test and McNemar test where appropriate.

Main results and the role of chance: Among endocrine profiles, SHBG (100.6 ± 95.6 nmol/L vs. 171.5 ± 52.1 nmol/L, P = 0.002) and DHEAs (192.3 ± 88.4 μg/dL vs. 228.8 ± 110.6 μg/dL, P = 0.002) were increased, and testosterone (0.9 ± 0.5 ng/mL vs. 0.6 ± 0.4 ng/mL, P < 0.001), 17-α-OHP (2.3 ± 1.6 ng/mL vs. 1.2 ± 0.7 ng/mL, P < 0.001) and AMH (12.5 ± 10.0 ng/mL vs. 10.8 ± 8.5 ng/mL, P = 0.011) were decreased after 1-year OC use. In metabolic profiles, total cholesterol (195.2 ± 33.5 mg/dL vs. 201.8 ± 32.5 mg/dL, P = 0.007) and 25-OH vitamin D (16.2 ± 9.5 ng/mL vs. 21.6 ± 14.8 ng/mL, P = 0.039) were increased by 1-year OC use. For body composition profiles, protein mass (10.3 ± 1.1 Kg vs. 10.1 ± 1.1 Kg, P = 0.004), mineral mass (2.4 ± 0.2 Kg vs. 2.3 ± 0.2 Kg, P = 0.004), muscle mass (38.5 ± 4.0 Kg vs. 37.7 ± 4.1 Kg, P = 0.004), fat free mass (40.8 ± 4.2 Kg vs. 40.1 ± 4.3 Kg, P = 0.006) and basal metabolic rate (1364.5 ± 102.0 Kcal vs. 1330.9 ± 107.0 Kcal, P < 0.001) were decreased with 1-year OC use. Before and after 1-year OC use, women with abnormal free androgen index (≥5) were 48.6% and 21.4% (P < 0.001), women with abnormal HOMA-IR (> 2) were 52.9% and 57.1% (P = 0.282), and women with abnormal percent body fat (>28%) were 34.3% and 31.4% (P = 0.703).

Limitations, reasons for caution: Further study should be necessary with larger sample size and longer follow-up.

Wider implications of the findings: One-year OC use could change androgen activity, lipid profiles and body composition of muscle and fat in lean women with PCOS.

Trial registration number: N/A.

P-729 Prediction of metabolically unhealthy phenotype by the visceral adiposity index, lipid accumulation product and triglyceride-glucose index in Chinese women with polycystic ovary syndrome

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Study question: Can visceral adiposity index (VAI), lipid accumulation product (LAP) and triglyceride-glucose (TyG) index predict metabolic unhealthiness in Chinese women with polycystic ovary syndrome (PCOS)?

Summary answer: Chinese-VAI (CVAI) and LAP are the best predictors of metabolic unhealthiness encompassing metabolic syndrome (MetS), insulin resistance (IR) and dysglycaemia in Chinese women with PCOS.

What is known already: Women with PCOS, both obese and non-obese, are at increased risks of metabolic derangements compared with the general population. VAI, LAP and TyG are established composite indices derived from some components of the MetS, and have been validated by previous studies as effective markers of metabolic unhealthiness as defined by the presence of MetS, IR and/or dysglycaemia. CVAI is the Chinese version of VAI calculated as -187.32+1.71*Age + 4.23*body mass index + 1.12*waist circumference + 39.76*$\log_{10}$[triglycerides] – 11.66*[HDL-C] (Xia et al, 2016); it has been shown to be more accurate than the original VAI in reflecting metabolic health in Chinese.

Study design, size, duration: This cross-sectional observational study was based on metabolic data obtained from 459 women diagnosed with PCOS according to the Rotterdam criteria. These subjects were prospectively recruited in the Hong Kong Chinese population between 2010 and 2013. A cohort of 253 ovulatory and age-matched healthy women in the same population served as controls.

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Participants/materials, setting, methods: Subjects were recruited from a university hospital or community family planning clinics. Blood was taken after 8-hour fasting for metabolic assessment. MetS was defined according to the joint interim statement (Alberti et al, 2009); IR was defined by the upper quartile of the homeostasis model assessment of insulin resistance (HOMA-IR), and dysglycaemia was defined by the American Diabetes Association criteria. Prediction on metabolic unhealthiness by CVAI, VAI, LAP and TyG was analysed by ROC curve.

Main results and the role of chance: The prevalence of MetS was 71 (15.5%) out of 459 PCOS subjects, compared to 3 (1.2%) out of 253 controls. We defined metabolic unhealthiness by the combined criteria as the presence of either MetS, IR and/or dysglycaemia. The prevalence of metabolic unhealthiness was 229 (49.9%) in PCOS subjects versus 68 (26.9%) in controls. For the prediction of MetS, the areas under the ROC curve (AUROC) were higher for LAP (0.945) and CVAI (0.936) compared to VAI (0.919) and TyG (0.892) ($p<0.05$). For prediction of IR, the AUROC were significantly higher for LAP (0.846) and CVAI (0.841) compared to VAI (0.777) and TyG (0.770) ($p<0.05$). For prediction of dysglycaemia, the AUROC were not significantly different among LAP (0.828), CVAI (0.821), VAI (0.812) and TyG (0.831). For prediction of metabolic unhealthiness by our combined criteria, the AUROC were significantly higher for LAP (0.860; sensitivity 70.3% and specificity 90.0% at best cut-off of 16.8) and CVAI (0.854; sensitivity 70.3% and specificity 88.7% at best cut-off of 29.2) compared to VAI (0.790; sensitivity 81.7% and specificity 62.2% at best cut-off of 0.88) and TyG (0.779; sensitivity 69.9% and specificity 74.4% at best cut-off of 8.03) ($p<0.05$).

Limitations, reasons for caution: The cross-sectional nature of this analysis did not allow the validation of the studied metabolic indices in predicting long term cardio-metabolic morbidity, which would worth further exploration by longitudinal follow-up of the study cohort. This study was targeted at the Chinese population, and generalisability to other ethnicities needs further exploration.

Wider implications of the findings: Using the MetS criteria alone will miss a number of metabolic problems like IR or dyglycaemia in women with PCOS. CVAI and LAP are simple composite metabolic indices which serve as accurate surrogate markers of the metabolically unhealthy phenotypes including MetS, IR and dysglycaemia in women with PCOS.

Trial registration number: Nil.

P-730 Can likelihood of natural pregnancy be predicted from demographics and LH surge characteristics?

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Study question: Predicting likelihood of conceiving naturally could help those considering whether to attempt IVF. This analysis examined whether demographics and LH surge characteristics can predict success?

Summary answer: Predictors of pregnancy were length of time trying to conceive, other well-known demographic factors, lower basal LH levels and steeper LH rise during the surge.

What is known already: There are many factors already known to influence the probability of conceiving naturally, such as age, BMI, smoking and previous obstetric history. Fertility declines during the perimenopause, but onset of the perimenopause is not currently predictable and often not recognisable. In addition, conditions such as PCOS may influence fertility. These conditions can manifest in disturbances in the LH surge profile. Therefore, it is possible that characterisation of the LH surge, along with basic demographic information, could provide important information on the chances of natural conception.

Study design, size, duration: This was a home based observational study. Volunteers seeking to conceive naturally (>18 years old) were recruited via internet advertisements from across the UK. Demographic data was self-reported. Volunteers collected daily urine samples for 1 entire menstrual cycle. Cycles from 185 women who achieved pregnancy and 200 women who failed to become pregnant were included in the analysis.

Participants/materials, setting, methods: Urinary luteinising hormone was measured using AutoDELFIA (Perkin Elmer, Waltham, MA, USA) across the whole cycle. The surges were characterised to examine baseline levels, surge day, peak day, peak concentration and magnitude of surge. The best description of baseline levels was found to be square root of (LH concentration on cycle day 6 - 15)^2, and description of magnitude was (LH on peak day - LH on surge day)/LH on surge day.

Main results and the role of chance: Mean age (SD) of those who achieved pregnancy was 30.55 (5.05) compared to 30.75 (5.14) who did not conceive. Those who failed to conceive had higher BMI (27.91 compared to 26.90) and were more likely to be current smokers. An extremely significant predictor of pregnancy was number of months trying to conceive; mean of 7.72 months for those that became pregnant versus 17.75 for those that did not. Number of previous livebirth was also significantly different (mean of 0.92 for pregnant group versus 0.66 for not pregnant group). Both self-reported endometriosis and PCOS was more prevalent in the group that failed to conceive.

Volunteers who conceived had slightly shorter self-reported menstrual cycle characteristics (average cycle, shortest cycle, longest cycle), but this finding was not significant. In the study cycle, surge day or concentration was not significantly different between groups (day 16.21 Pregnant, 16.61 Not Pregnant; 54.84mIU/ml Pregnant, 58.22mIU/ml Not Pregnant). However, volunteers who conceived had a lower basal LH level, which was significantly different on day 6. The surge profile was also steeper in the group that became pregnant. This indicates that LH cycle profiles may assist in predicting pregnancy success.

Limitations, reasons for caution: The study was for one menstrual cycle, thus intra-individual cycle variation was not considered. Demographic and pre-study cycle length characteristics were self-reported, but as they were reported prior to study conduct, inaccuracies are likely to be equal between the two groups. Male factors were not considered.

Wider implications of the findings: Providing robust information on chances of natural conception can provide realistic expectations on likelihood of success and whether to seek IVF treatment earlier or later. This may be valuable to women who wish to try for natural conception to enable them to make objective decisions on their path to pregnancy.

Trial registration number: NCT01577147.

P-731 The assessment of follicular fluid metabolite profile in poor and high responder patients undergoing intracytoplasmic sperm injection

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Study question: Is the metabolite composition of follicular fluid (FF) associated with the response to controlled ovarian stimulation (COS) for IVF?

Summary answer: The metabolite composition of FF is associated with the response to COS.

What is known already: Bidirectional communication between the oocyte and surrounding cumulus cells is essential for the acquisition of oocyte competence, and due to their close connection, cumulus cells and FF may retain a footprint of the follicular conditions experienced by the oocyte. Therefore, this may be a valuable tool to understand ovarian physiology. Low-responder young patients have reduced oocyte viability, perhaps as a consequence of altered ovarian microenvironment. On the other hand, the overproduction of oocytes, in high-responders, can cause considerable patient discomfort, and important short-term complications including ovarian hyperstimulation syndrome, high incidence of multiple pregnancies and ethical/legal implications of embryo storage.

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Study design, size, duration: For this prospective study, 69 samples of FF were collected from patients undergoing routine ICSI cycles in an University-affiliated assisted reproduction centre, from October/2015 to June/2016. Samples were split into three groups according to the response to COS: Low responder, when ≤ 4 oocytes were retrieved (LR, n = 11, 33.2y-old), normal responder when >4 and < 15 oocytes were retrieved (NR, n = 47, 33.1y-old), and hyper responder patients, when ≥ 15 oocytes were retrieved (HR, n = 11, 32.8y-old).

Participants/materials, setting, methods: Patients received rFSH for COS and GnRH-antagonist for pituitary blockage. Metabolites were extracted using Bligh and Dyer protocol and individually analysed by electrospray ionization mass spectrometry in the positive ionization mode. Data for NR group were compared with other groups. Principal Component and Partial Least Square Discriminant analyses were used to perceive general clustering and to confirm the discriminatory metabolites, respectively. These were based on the VIP metabolites hiperrepresented in each group.

Main results and the role of chance: Dithienyl disulphate was hiperrepresented in the LR group, which was associated with the cellular oxidant detoxification, oxidoreductase and glutathione peroxidase activity pathways. Sulfonic acid was increased in the HR group, which was associated with gamma glutamyltransferase activity and glutamate metabolic process. Those pathways and metabolic processes were obtained through enrichment analysis using the CytoScape 3.4.0.

Limitations, reasons for caution: Although these results may add to the general knowledge of ovarian biology and its response to COS, these must be investigated in plasma, favouring the implementation of individualized COS protocols.

Wider implications of the findings: Our data suggest that identification of metabolites correlated with ovarian response to COS may support the implementation of individualized COS protocols, offering a valuable opportunity for achieving the treatment success with reduced discomfort, emotional and financial costs for the patients.

Trial registration number: N/A.

P-732 Administration of FSH 8 hours post-triggering of final oocyte maturation with hCG does not improve oocyte maturation rate in normal responders undergoing ICSI

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Study question: Does FSH administration 8 hours post-triggering of final oocyte maturation with hCG increase oocyte maturation rate as compared to hCG-only in normal responders undergoing ICSI?

Summary answer: Oocyte maturation rate is similar following FSH administration 8 hours post-triggering final oocyte maturation with hCG as compared to hCG-only in normal responders undergoing ICSI.

What is known already: In IVF programs, the most common form for triggering final oocyte maturation involves administration of hCG that mimics LH surge. However, there is evidence to suggest that, similarly to spontaneous ovulation or GnRH agonist trigger, an FSH surge around the time of triggering final oocyte maturation may have biological importance for the recovery of a fully mature and developmentally competent oocyte. During standard ovarian stimulation protocols, the last dose of FSH may be administered usually 1 day before and rarely on the day of hCG triggering, allowing serum FSH levels to decline prior to the time of final oocyte maturation.

Study design, size, duration: Retrospective cohort study of 229 patients undergoing ovarian stimulation for ICSI. In order to induce final oocyte maturation, patients were administered 250 mcg rec-hCG followed by a single dose of 50 IU of rec-FSH 8 hrs later (FSH/hCG group, n = 57) or rec-hCG only (hCG group, n = 172). The primary outcome measure was oocyte maturation rate, defined as the proportion of MIIs/COCs. The study was conducted from January 2015 until September 2016 at a private IVF Unit.

Participants/materials, setting, methods: Eligible patients were ≤42 years old, were treated with GnRH antagonist protocol and had 5-18 follicles ≥11 mm on the day of triggering. Patients undergoing oocyte donation, PGD, those who had GnRH agonist triggering for final oocyte maturation, and patients with poor (<5 follicles) or excessive ovarian response (>18 follicles) were excluded. Patients were included in the study only once. Continuous variables are expressed as median (interquartile range) and binary variables as proportion and 95% CI.

Main results and the role of chance: Baseline characteristics were similar in the two groups, except for a higher baseline oestradiol in the FSH/hCG compared to the hCG group [46.0 (21.9) vs. 37.5 (19.8), p = 0.020]. There were no significant differences between the FSH/hCG and hCG groups regarding the total gonadotrophin dose [1762.5 (762.5) vs. 1650 (650) IU, p = 0.209], oestradiol [1607 (1517) vs. 1731 (1320.8) pg/ml, p = 0.279] and progesterone levels [0.9 (0.6) vs. 0.8 (0.5) ng/ml, p = 0.316] on the day of triggering, days of stimulation [11 (3) vs. 10 (2), p = 0.407], number of follicles developed [12 (12) vs. 11 (7), p = 0.233], number of oocytes retrieved [10 (10) vs. 10 (7), p = 0.312], number of metaphase-II oocytes [8 (8) vs. 7 (6), p = 0.360], number of 2PN oocytes [5 (6) vs. 5 (5), p = 0.816], number of embryos transferred [2(1) vs. 2(2), p = 0.533] and number of embryos cryopreserved [2.5 (2) vs. 3 (2), p = 0.176], respectively. No differences were also present between the FSH/hCG and the hCG groups regarding clinical pregnancy rate (47.4%; 95% CI:34.0-60.7 vs. 54.1%; 95%CI:46.5-61.6, p = 0.445). Oocyte maturation rate did not differ between FSH/hCG and hCG groups [80% (17.1) vs. 82.4% (22.8), p = 0.710]. No difference was present in maturation rates between the two groups in multivariable analysis controlling for baseline estradiol.

Limitations, reasons for caution: This is a retrospective study and although a multivariable analysis was performed controlling for confounders, some form of unknown bias cannot be excluded. Thus the present findings should be confirmed by relevant RCTs Moreover, the optimal timing and dosage of FSH administration might need to be further explored.

Wider implications of the findings: Although FSH surge accompanies LH surge during spontaneous ovulation, the simulation of this event by administration of 50 IU of FSH 8 h following hCG administration does not appear to be beneficial for enhancing oocyte maturation rate in normal responders undergoing ovarian stimulation for ICSI.

Trial registration number: Not applicable.

P-733 Short-time effect of N-acetyl-L-cysteine (NAC) against natural follicular depletion in pre-pubertal and adult mice – A randomized study

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Study question: Can antioxidant substances, such as N-acetyl-L-cysteine (NAC), be used to reduce the natural steady-state follicle depletion in pre-pubertal or adult mice?

Summary answer: Our preliminary data indicates that intraperitoneal administration of NAC could reduce the natural programmed ovarian follicle depletion in pre-pubertal mice but not in adult mice.

What is known already: Follicular depletion starts early in life, with a massive loss of small follicles before puberty. Thereafter, cyclical follicle recruiting occurs throughout the reproductive lifespan until exhaustion and menopause onset. Oxidative stress by reactive oxygen species (ROS) contributes to somatic aging

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in general, and also has been implicated in reproductive aging. NAC is an antioxidant substance that has been shown to effectively rescue oocytes and embryos from ROS-induced telomere shortening and apoptosis. NAC is also a highly soluble substance that diffuses easily in biological tissues, it is non toxic and can be used for parenteral treatment.

Study design, size, duration: Randomized controlled study. Thirty four C57BL/6 N mice, 16 pre-pubertal (3 weeks) and 18 adults (8-10 weeks), were randomly assigned to intraperitoneal NAC injection or to control (pre-pubertal N = 8; adult N = 9 in each group). The animals were sacrificed 3 or 7 days after the first day of NAC treatment and the ovaries dissected for histological analysis. Follicle density of small size follicles was calculated. The study was approved by the regional ethics committee for animal experiments.

Participants/materials, setting, methods: Intraperitoneal NAC injection (150 mg/Kg) was administered on two consecutive days. Ovarian sections (5 µm) were H&E stained and scanned (3Dhisthech®). Follicles were counted by two independent observers according to Pedersen & Peters (1968). Follicle density was calculated as the total number of small follicles divided by the area of ovarian tissue examined.

Main results and the role of chance: We found 3 and 7 days after the treatment with NAC a higher follicle density in the ovaries of pre-pubertal mice that received NAC (13.0/mm^2 ± 4.7) than in the control group (10.2/mm^2 ± 4.4). However, this was not observed in adult mice that received NAC (7.8/mm^2 ± 3.1) when compared to adult controls (7.2/mm^2 ± 2.7).

The effect of NAC was statistically significantly different between pre-pubertal and adult mice (p = 0.01) with a higher follicle density in pre-pubertal mice (13.0/mm^2 ± 4.7) compared with adult mice treated with NAC (7.8/mm^2 ± 3.1). This difference was not observed in the control groups.

Limitations, reasons for caution: We estimated effects on follicle density after a short-time experimental treatment with NAC. Although we observed a differential effect between pre-pubertal and adult mice, a transitory effect also in the ovary of adult mice that was not persistent at the point of estimates several days later cannot be ruled out.

Wider implications of the findings: Our results suggest that antioxidant substances such as NAC might reduce natural ovarian reserve depletion, according to our findings using pre-pubertal mice. The absence of effect in the ovaries of adult mice might indicate that the mechanisms of programmed ovarian follicle depletion are different between adult and pre-pubertal mice.

Trial registration number: not applicable.

P-734 Ovarian reserve testing- Antral follicle count (AFC) remains the single best predictor in comparison to AMH and FSH

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Study question: The aim of the study was to determine the single best predictor of ovarian reserve and response in patients undergoing an ART program.

Summary answer: In our study, AFC emerged as an independent predictor of response and pregnancy rates.

What is known already: Age is strongly correlated with ovarian response and anti mullerian hormone (AMH) has been emerging as an effective predictor. Comparisons have been made between the value of antral follicle count versus follicle stimulating hormone and AMH with variable conclusions.

Study design, size, duration: A prospective study was conducted from January 2015 - Dec 2016 with the sample size of 369 women undergoing assisted reproduction. The mean age of women is 31.33 years.

Participants/materials, setting, methods: The mean AFC, AMH and FSH values were determined based on the age group < 30 years and > 30 years. The oocyte retrieval rates and pregnancy rates were determined based on the three key fertility predictors as mentioned above. Data's were analyzed using the SPSS version 16.0 for statistical significance.

Main results and the role of chance: The Mean values of AFC, FSH and AMH in women <30 years (A) was 10.49 ± 5.46,7.44 ± 3.49 and 5.59 ± 3.63 respectively. The Mean AFC, FSH and AMH in women > 30 years (B) was 8.46 ± 4.69, 7.78 ± 3.11, and 4.33 ± 3.34. The oocyte retrieval rates were correlated with age and the corresponding AFC, AMH and FSH as mentioned above. The mean numbers of oocytes in women in-group A was 10.36 ± 5.75 and for group B 7.89 ± 5.36. Pregnancy rates for these women in group A was 58% and for group B was 47.7%.

The mean serum levels of AMH as well as the total number of oocytes retrieved and AFC were significantly higher in the women in-group A. However, there was no significant difference in pregnancy rates between groups (A and B). No significant difference was observed between groups (A and B) with respect to FSH levels. The maximum numbers of oocytes was retrieved at high AMH values (>5) with a mean age of 30.33 years. The total number of AFC was positively and significantly correlated (p = 0.01) with pregnancy rates.

Limitations, reasons for caution: Only fresh embryo transfers were considered in this study with exclusion of donor programs. AMH values may correlate with age and response but not conclusively with pregnancy rates in comparison to a more valuable indicator such as AFC

Wider implications of the findings: FSH values do not seem to add significance to response or pregnancy rates as much as the AMH or AFC. In our study, AFC emerged as an independent predictor of response and pregnancy rates.

Trial registration number: not applicable.

P-735 Elevated progesterone levels negatively influence cumulative live birth rates by affecting both endometrial receptivity and embryonic competence

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Study question: Could late follicular phase serum progesterone (P) concentrations be associated with embryonic competence?

Summary answer: Embryo quality and cumulative pregnancy rates in women with high P levels are decreased. High P affects fertilization rates after IVF but not after ICSI.

What is known already: The success of IVF depends intrinsically on two important factors: embryo quality and endometrial receptivity. It is still a matter of much debate whether among ART patients high or low levels of late-follicular-phase P negatively affects the oocytes or the endometrium, since both are simultaneously subjected to the influence of P. Both oocyte donation and embryo cryopreservation models are the best clinical tools to distinguish the effects of high serum P levels on oocytes from those on the endometrium.

Study design, size, duration: A single-centre retrospective cohort analysis of 3350 gonadotropin-releasing hormone (GnRH) antagonist down-regulated cycles with a fresh embryo transfer was performed between 2010 and 2014. The sample was categorized according to the following P levels on the day of ovulation triggering: < 0.5 ng/mL, 0.5-1.5 ng/mL and >1.5 ng/mL. Embryo development and live birth rates (LBR) after the fresh and subsequent frozen embryo transfers were compared among these groups, followed by pairwise group comparisons whenever significant.

Participants/materials, setting, methods: Cycles using pre-implantation genetic diagnosis, in-vitro maturation and donor oocytes were excluded. We performed multivariable generalized estimating equation (GEE) regression analysis accounting for the following potential confounders: female age, cycle rank, total dose of exogenous follicle-stimulating hormone (FSH), the late-follicular-phase estradiol (E2) levels, the number of oocytes/embryos produced and the number, stage and grade of the embryos transferred.The frozen embryo transfer (FET) took place either in a natural or an artificial cycle.

Main results and the role of chance: Comparing the < 0.5 ng/mL, 0.5-1.5 ng/mL and >1.5 ng/mL, as late-follicular-serum P levels increased, female age decreased significantly (median age 34, 33 and 31 years/old, respectively; p = 0.002). Conversely, the total dose of exogenous FSH consumed, late-follicular E2 levels and the number of oocytes retrieved (median 7, 9 and 12, respectively; p < 0.001) increased with increasing trigger P levels. Regarding oocyte and embryo quality, fertilization rates following IVF decreased significantly with increasing P levels (65.8%, 59.5% and 46.5%, respectively; p = 0.0051). Importantly, even after disregarding the oocytes that did not

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fertilize, women in the P > 1.5 ng/mL group had a significantly lower number of embryos of sufficient quality to transfer or cryopreserve at both cleavage and blastocyst stage (49.6%, 48.0% and 43.1% of all fertilized oocytes were useable at blastocyst stage, respectively; p = 0.029). In the multivariable GEE regression models for live birth, fresh LBR varied significantly (29.3%, 34.3% and 25.5%, respectively; p = 0.0029) among the groups. Most importantly, while both women with P < 0.5 and >1.5 ng/mL had similarly low cumulative LBR (38.8%, 43.9% and 38.3%, respectively; p = 0.0211), only the difference between the women with P levels between 0.5-1.5 ng/mL and >1.5 ng/mL remained significant after pairwise comparison.

Limitations, reasons for caution: The main limitation of the study was its retrospective nature. Furthermore, the analysis was restricted to patients under GnRH antagonist pituitary suppression and some potential confounding factors such as female smoking could not be accounted for.

Wider implications of the findings: Our results are in contrast with previous studies suggesting that high P is not associated with reduced embryo quality or cumulative LBR. The knowledge that high P levels may hinder oocyte/embryo competence and, ultimately, diminish cumulative LBR could play an important role in how we perform ovarian stimulation henceforth.

Trial registration number: not applicable.

P-736 Alteration in gastrointestinal health and dysbiosis of gut microbiota in women with Polycystic Ovary Syndrome

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Study question: Do women with Polycystic Ovary Syndrome (PCOS) present a dysbiosis of gut microbiota and an increase in gastrointestinal symptoms?

Summary answer: PCOS women report a high rate of functional gastrointestinal symptoms (abdominal discomfort, bloating, flatulence, constipation), which are presumably associated with a dysbiosis of gut microbiota.

What is known already: Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women in reproductive age. The clinical expression of the syndrome is characterized by the combination of menstrual irregularities, anovulation and hyperandrogenism. It is now well established that PCOS is associated with a chronic state of inflammation and insulin resistance, though the underlying triggers for these two key imbalances still remain unknown. Changes in gut microbiota composition have been associated with different metabolic disorders, including obesity and diabetes; the gut microbiota may play a role in the development of obesity, inflammation and insulin resistance that represent the hallmarks of PCOS.

Study design, size, duration: Here we present the preliminary results of an ongoing observational prospective pilot study comprises 23 women with PCOS and 10 healthy controls from 18 to 35 years, recruited in an academic research environment from January to September 2016.

Participants/materials, setting, methods: 23 PCOS women diagnosed according to the Rotterdam Criteria (19 – 33 years, mean BMI 26) and 10 healthy women (19 – 33 years, mean BMI 21) were enrolled. Gynecological evaluation included anthropometric characteristics, hirsutism score, ultrasound ovarian features, and hormonal parameters. Each participant also underwent an oral glucose tolerance test (OGTT). The evaluation of the gastrointestinal health consisted of Bristol stool charge, Gastrointestinal Symptoms rating Scale (GSRS), psychometric tests, and lactulose/mannitol breath test.

Main results and the role of chance: PCOS participants were affected by oligo-amenorrhea, hirsutism, accompanied by elevated free testosterone and AMH levels. An increased Body mass index (BMI) was also observed in PCOS subjects. The lipid profile and the insulin secretion during the OGTT did not significantly differ between PCOS women and controls. The prevalence of altered Bristol stool scale (constipated or inflammation) was significantly higher in the PCOS group (P < 0,05). On examining individual gastrointestinal symptoms, GSRS was higher compared with control group (P < 0,05). Specifically, abdominal discomfort, borborygmus, bloating, increased flatus and constipation were more frequently observed in PCOS subjects. Severity of the symptoms was not correlated with the androgen levels and the AUC insulin at the Spearman test. Psychometric tests showed no significantly differences in anxiety, depression and vitality; the perception of quality of life was lower in PCOS, in according with literature. PCOS women presented a good resilience evaluated with CD risk and Self Efficacy questionnaires. At lactulose/mannitol breath test PCOS group displayed a significant alteration of intestinal transit and an estimated dysbiosis was present in more PCOS patients than controls. The direct evaluation of gut barrier dysfunction with urine analysis after lactulose/mannitol breath test and of the inflammatory status is now being carried out.

Limitations, reasons for caution: The small sample size represents a limitation. The recruitment is ongoing.

Wider implications of the findings: The finding of a dysbiosis or microbial imbalance in the gut microbiota of PCOS women creates opportunities for potential treatments (probiotics/ prebiotics) and adds a new player in the physiopathologic picture of the syndrome.

Trial registration number: N/A.

P-737 Baseline AMH is associated with ovulation induction dose and ovulatory response and predicts ovulation rate in women with Polycystic Ovary Syndrome

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Study question: To investigate association between baseline AMH levels, ovulatory response to ovulation induction dose among women enrolled in the Polycystic Ovary Syndrome Acupuncture and Clomiphene Trial (PCOSAct).

Summary answer: Higher baseline AMH is negatively associated with lower ovulatory response and requires higher ovulation induction dose to achieve ovulation in women with PCOS.

What is known already: AMH plays an inhibitory role in follicular development and recruitment, contributing to follicular arrest.AMH also reduces aromatase activity and sensitivity of follicles to FSH stimulation. Therefore, elevated serum AMH may have higher threshold response to ovulation induction and affect pregnancy outcome in women with PCOS.

Study design, size, duration: A secondary analysis of the Polycystic Ovary Syndrome Acupuncture and Clomiphene Trial (PCOSAct), a large-sample, multi-center, randomized placebo-controlled clinical trial conducted from 2012 to 2015 in mainland China.1000 PCOS women were randomly assigned in a 1:1:1:1 ratio, to receive true acupuncture or control acupuncture integrated with clomiphene or placebo clomiphene for up to four cycles treatment. Baseline serum AMH concentrations were measured by AnshLabs AL-105-I Ultra-Sensitive AMH/MIS ELISA kits.

Participants/materials, setting, methods: Logistic regression models to test association of demographic, metabolic and biochemical profiles with ovulation, conception, pregnancy and live birth adjusted by the interventions.OR and 95% CI to compare ovulation induction dose and ovulatory response according to the logistic models. ROC to confirm prediction values of AMH for ovulation. Kaplan–Meier curve to compare time to ovulation in the four quartiles.

Main results and the role of chance: Age, BMI(body mass index), WHR (whist to hip ratio),duration of infertility,baseline AMH,progesterone, total testosterone, E2(estradiol), SHBG (sex hormone-binding globulin)and glucose were significantly associated with ovulation, but not conception, pregnancy and live birth. AMH levels were significantly higher among women who did not achieved ovulation than those women who achieved ovulation both overall and within intervention groups (P < .0001) and required higher ovulation induction dose to achieve ovulation. Baseline AMH significantly predicted ovulation

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(AUC0.60, P < .001) and lower baseline AMH has higher chance of ovulation (87.0%vs 71.1%) and shorter duration to ovulate (23 days vs 58 days for 50% ovulation).

Limitations, reasons for caution: The significant association of baseline AMH with ovulation in PCOS requires prospective study to validate.

Wider implications of the findings: This is the first large-scale trial to show the association of baseline AMH with ovulatory response to ovulation induction dose and the prediction values of baseline AMH on ovulation. AMH should be measured prior to infertility treatment in PCOS women.

Trial registration number: The PCOSAct Chinese Clinical Trial Registry: ChiCTR-TRC-12002081.

P-738 Ovarian response to controlled ovarian hyperstimulation: what does serum FSH say?

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Study question: Do serum follicle stimulating hormone (FSH) levels on the day of human chorionic gonadotropin (hCG)-trigger differ between women with a poor, normal or hyperresponse to 150 IU recombinant FSH (rFSH)?

Summary answer: Hyperresponders showed slightly higher serum FSH levels on day of hCG-trigger as compared to poor responders. Poor or hyperresponders did not differ from normal responders.

What is known already: When ovarian response to stimulation for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) is suboptimal, the FSH dose is often adjusted in a subsequent cycle. This is based on the assumption that serum FSH levels were inadequate for optimal stimulation and a suboptimal response will be averted in the new cycle by choosing a different rFSH dose.

Study design, size, duration: Nested cohort study within a randomized controlled trial conducted at the University Medical Centre Utrecht (CETRO trial). Blood was drawn from 124 women on cycle day 2 and on day of hCG triggering. Expecting a difference in serum FSH on day of hCG of 2 IU/L, a sample size of 64 women (16 poor, 32 normal and 16 hyperresponders) would provide 80% power to detect this difference (standard deviation (SD) of 2 and alpha 0.05).

Participants/materials, setting, methods: Women aged ≤ 39 years with a regular cycle and fixed FSH dose of 150 IU. Exclusion criteria: BMI > 32 kg/m^2 and > 2 previous unsuccessful IVF/ICSI cycles. The primary outcome measure was serum FSH level on day of hCG-triggering. Serum FSH levels were determined by the Beckman-Coulter Unicel DXi800 chemiluminescence assay. The interassay coefficient of variation varies from 5.7% to 6.6% for the concentration range of 7-46 IU/L.

Main results and the role of chance: Median [range] age was 32.5 [28-38], 33.0 [22-39] and 28.0 [22-37] for poor (n = 16), normal (n = 94) and hyperresponders (n = 17), respectively. Age differed significantly between poor and normal vs. hyperresponders (p = 0.024 and p = 0.024). Median bodyweight was 70.0 kg [55- 86], 68.0 kg [52-94] and 60.6 kg [51-78] respectively and differed significantly between normal vs. hyperresponders (p = 0.036). Mean (SD) serum FSH levels on day of triggering were 9.5 IU/L (2.4) in poor, 10.4 IU/L (2.3) in normal and 11.5 IU/L (2.2) in hyperresponders. Serum FSH levels in the poor responders differed significantly as compared to those in hyperresponders (p = 0.03). There was a weak, but significant correlation between serum FSH level on day of hCG and the number of obtained oocytes (correlation r = 0.203, p = 0.024, r^2 = 0.041). The multivariable model including age, body weight, basal serum FSH, basal anti-Mullerian hormone (AMH) and serum FSH level on the day of hCG showed a standardized regression coefficient of 0.151 (p = 0.063) for the number of retrieved oocytes.

Limitations, reasons for caution: The number of retrieved oocytes was only slightly determined by serum FSH level on the day of hCG trigger. After correction for age, body weight, basal serum FSH and basal AMH the correlation between serum FSH level on the day of hCG and ovarian response disappeared.

Wider implications of the findings: The current study shows that a poor response is not related to inadequate serum FSH levels. Therefore, increasing the rFSH dose in poor responders is probably meaningless. In hyperresponders however, lowering the dose in a subsequent cycle may lead to lower serum FSH levels thereby moderating ovarian response.

Trial registration number: NCT00866034 (CETRO trial).

P-739 Prediction of IVF outcome based on the follicular output rate

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Study question: Does the follicular output rate (FORT) differ between recombinant FSH (rFSH) and corifollitropin alpha (CFA) during controlled ovarian stimulation in a GnRH antagonist IVF protocol?

Summary answer: FORT was significantly higher in CFA-stimulated cycles and accurately predicted oocyte output and metaphase II oocyte number; however, the relationship to pregnancy outcomes was uncertain.

What is known already: The FORT, defined as the number of pre-ovulatory follicles in response to FSH administration divided by the pre-existing antral follicle count (AFC), may be a better and more immediate measure of the efficacy of ovarian stimulation than mean oocyte numbers retrieved. The responsiveness of antral follicles to FSH may predict their reproductive competence, such that patients with a larger proportion of FSH-responsive antral follicles may be more likely to become pregnant after assisted reproductive technologies.

Study design, size, duration: Retrospective analysis comparing the FORT between women treated with CFA or rFSH from three randomized controlled trials, Pursue (N = 1388), Engage (N = 1476) and Ensure (N = 395). Women underwent controlled ovarian stimulation in a GnRH antagonist protocol followed by hCG trigger prior to IVF.

Participants/materials, setting, methods: Women received 150 µg CFA or 300 IU rFSH in Pursue (ages 35-42, ≥50 kg), 150 µg CFA or 200 IU rFSH in Engage (ages 18-36, >60 kg), and 100 µg CFA or 150 IU rFSH in Ensure (ages 18-36, ≤60 kg). AFC (<11 mm) and pre-ovulatory follicle count (>15 mm) were used for FORT, defined as pre-ovulatory follicles/AFCx100.

Main results and the role of chance: CFA elicited the growth of more pre-ovulatory follicles from the existing AFC versus rFSH. For CFA and rFSH, respectively, the mean FORT values (adjusted for trial and age) were 85.0 versus 76.8 (p<0.001) for the combined cohort, and 74.1 versus 71.2 in Pursue (p = 0.180), 86.0 versus 75.0 in Engage (p<0.001), and 96.2 versus 79.2 in Ensure (p = 0.070). For CFA and rFSH, respectively, the overall mean oocyte output values (oocytes retrieved/AFCx100, adjusted for age) were 100.6 versus 98.1 in Pursue (p = 0.463), 121.9 versus 107.3 in Engage (p = 0.001), and 133.5 versus 102.3 in Ensure (p<0.001). FORT and oocyte output were consistent with the number of metaphase II oocytes for CFA and rFSH: 7.5 versus 7.2 in Pursue (p = 0.37), 10.4 versus 8.8 in Engage (p<0.001), and 10.3 versus 7.6 in Ensure (p<0.001). Significant increases in FORT with CFA compared to rFSH were observed in the younger cohorts of women from Engage and Ensure, but not in the older cohort in Pursue. No differences in pregnancy rates based on FORT were observed in any study. As this analysis only included pregnancy outcomes from fresh embryo transfers, future studies utilizing cumulative

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pregnancy rates are needed to further evaluate the correlation between FORT and IVF outcomes.

Limitations, reasons for caution: This was a retrospective analysis. Measurement of antral follicles by transvaginal ultrasound was not standardized across study centers or observers. A larger study sample may be necessary to discern clinically meaningful differences in pregnancy outcomes based on FORT.

Wider implications of the findings: The association between oocyte output and antral follicles that effectively respond to FSH suggests that FORT may be a qualitative indicator of differences in ovarian response and oocyte competence based on type of gonadotropin used during stimulation. Additional research regarding the correlation between FORT and IVF pregnancy outcomes is warranted.

Trial registration number: NCT01144416, NCT00696800, NCT00702845.

P-740 Vitamin D and live birth rate after IVF

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Study question: How do Vitamin D levels prior to IVF treatment differ between women with unsuccessful IVF-outcome and those with a live birth?

Summary answer: A cut-off of 16,5 ng/ml discriminated best between women with and without a live birth with good sensitivity, but poor specificity.

What is known already: Vitamin D regulates 3% of the human genome and specifically target genes of the Vitamin D Receptor (VDR) involved in cell-proliferation and differentiation, oxidative stress and apoptosis, membrane transport, and cell adhesion. Weather conditions during the month before IVF treatment have been found to have an impact on live birth rate, but in women undergoing euploid embryo transfer, vitamin D status was unrelated to pregnancy outcomes (not including live birth rate). A recent meta-analysis found lower vitamin D Status (< 20 ng/ml vs. >/= 20 ng/ml) was associated with a lower live birth rate (RR 0.76, 95 % CI 0.61-0.93).

Study design, size, duration: This prospective observational cohort pilot-study included 83 patients scheduled for IVF treatment in a university-affiliated IVF-Center. Participants had a serum dermination of their vitamin D and calcium levels prior to downregulation and stimulation. Follow-up included the pregnancy outcome until live birth.

Participants/materials, setting, methods: The relationship between Vitamin D and IVF outcome was modelled by multiple regression analysis. Age, serum-cacium, BMI and Vitamin D were the independent confounders, IVF outcome was the dependant variable. The association was further studied using receiver operating characteristics (ROC) modelling.

In order to find the cut-off, which would best discriminate between patients with a later live birth and those without, Youden's J Index (sensitivity - (1- specificity)) and a tree diagram were performed.

Main results and the role of chance: Average participant age was 37,6 (± 3,37) years, average BMI was 22,95 (± 4,2), serum calcium was 2,4 (± 0,09) and Vitamin D 19,64 (± 9,1). Median Vitamin D values were 21 ng/ml (average 23) in women with ongoing pregnancy, and 17 ng/ml (average 19) im women aborting or not becoming pregnant.

Multiple regression analysis revealed no significant influence of Vitamin D on birth rate. The ROC area under the curve was 0,67, not sufficient to predict IVF success from a vitamin D determination. However, the results hint at some connections which could be relevant, if validated in a larger cohort:

no patient with a vitamin D value below 13,5 ng/ml had a live birth, the birth rate of all women over 13,5 ng/ml was 15%, while women with values over 29,5 ng/ml had a birth rate of 27,3%. A cut-off of 16,5 ng/ml showed a sensitivity of 0,889 and a specificity of 0,446.

Limitations, reasons for caution: This analysis did not take into account all factors related to IVF outcome, such as male factor, prior pregnancy, or therapeutic regime. Also, the small sample size precludes any generalization for clinical practice at this time. Also, there is no proof of a therapeutic value of giving exogenous vitamin D.

Wider implications of the findings: These findings may serve to plan a prospective trial on the value of giving Vitamin D to Vitamin D- insufficient infertilitiy patients.

Trial registration number: not applicable.

P-741 Polycystic ovary syndrome (PCOS): markers of responsiveness to Metformin therapy

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Study question: Is it possible to identify reliable predictors of response and compliance of Metformin therapy in PCOS patients, also studying the proper duration of treatment?

Summary answer: Baseline insulin resistance, BMI, androgenemia and LH/FSH ratio are strictly related to the success of therapy in restoration of ovulatory cycles and reduction of hyper-androgenemia

What is known already: PCOS diagnosis is based on the presence of chronic anovulation, polycystic ovaries and hyperandrogenism. Insulin-resistance is a common even not mandatory feature of the syndrome. The insulin-sensitizing Metformin, currently one of the treatment of choice in PCOS patients, has been shown to improve metabolic abnormalities, decrease androgen levels and improve menstrual pattern and ovulatory function. Nevertheless the extensive use of this medication is contained by the highly variable compliance and response rate

Study design, size, duration: In this prospective not randomized cohort study were analyzed 105 PCOS consecutive patients of reproductive age treated with Metformin 1500 mg/daily by the Gynecological Endocrinology outpatient clinic of the Reproductive Medicine Unit, San Paolo Hospital – University of Milan, Italy. Metformin outcomes were considered: the recovery of regular menstrual cycles, the BMI reduction in overweight patients, the decrease of hyperandrogenemia and hyperandrogenism, the insulin-resistance mitigation, the lipid profile normalization

Participants/materials, setting, methods: All patients met the ESHRE/ASRM 2003 Rotterdam criteria. At inclusion, before treatment, they underwent to a complete clinical evaluation, to an endocrine/metabolic laboratory assessment and to a transvaginal ultrasonography. Later were carried out semestral checks of all parameters. Therapy outcomes were compared to baseline data in order to notice the degree of variation of every single output and to eventually identify reliable markers of compliance and response to the therapy

Main results and the role of chance: The Metformin treatment resulted in a clear improvement of the glucose metabolism in all the treated patients. Nevertheless a great level of baseline insulin resistance do not automatically means improvement of all therapy outcomes. Insulin-resistance, obesity, hyperandrogenemia and also high LH/FSH ratio are singularly markers of treatment efficacy in terms of restoration of regular ovulatory cycles and of reduction of hyperandrogenemia. The predictive value of these markers significatively increase when they are combined. A lower baseline insulin resistance as much as regular androgenemia, irrespectively of the basal BMI, correlates to an easier weight and visceral adiposity containment as a result of treatment.In our population we have not observed severe side effects of Metformin therapy. The drop-out rate was mainly due to mild gastro-intestinal symptoms and to inadequate motivation. Comparing the data of this group of patients (n: 27) with the group who carried out at least six months of therapy (n: 74), the first showed lower endocrine-metabolic impairment. The comparison of the results obtained after 6, 12 and 18 months of treatment leads for optimal duration of therapy of 12 months because of generally achieving in this time the great part of clinical and endocrine-metabolic positive effects

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Limitations, reasons for caution: During this study no specific alimentary regimen has been recommended. Patient's lifestyle and eating habits during the study could potentially interfere with the syndrome expressions.

Wider implications of the findings: Metformin is effective in the treatment of PCOS. The different sensitivity to therapy may lie in the heterogeneity of PCOS population. Greater efforts have to be done to develop a clinically useful predictive model of Metformin treatment response, in order to reduce the poor/null response and the drop out rate

Trial registration number: Not applicable.

P-742 Maternal anti-mullerian hormone (AMH) as a predictor of embryo aneuploidy in young patients undergoing in vitro fertilization with preimplantation genetic diagnosis (PGD)

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Study question: Is the low ovarian reserve determined by AMH serum levels associated to the incidence of blastocyst aneuploidy in young patients submitted to in vitro fertilization (IVF)?

Summary answer: Low AMH levels isn't a predictor of blastocyst aneuploidy in young patients, suggesting the low ovarian reserve in <37yrs-old patients isn't an indication to PGD.

What is known already: The AMH, produced by granulosa cells, is postulated to be associated with both ovarian reserve and function. Serum AMH declines with age and ovarian reserve reducing, and it has the advantage of stability, keeping constant levels throughout the menstrual cycle and is not influenced by other hormones or oral contraceptives. Then, it has been considered a better predictor of ovarian reserve. The ovarian reserve declining by ageing is associated with oocyte quality, which in turns affect the embryo quality. Nevertheless, the relationship between low ovarian reserve and oocyte/embryo qualities in young patients has not been clearly stablished.

Study design, size, duration: This is a retrospective study including 236 IVF cycles with embryo biopsy for PGD between February 2014 and May 2015. Patients had serum AMH measured to evaluate ovarian reserve, and underwent a standard ovarian stimulation and IVF protocols. A total of 562 embryos were biopsied and analyzed by comparative genomic hybridization array (CGHa) in a reference genetic laboratory.

Participants/materials, setting, methods: The embryos were cultured until the fifth day of development, when the trofectoderm biopsies were performed according to standard procedures. Embryos were cryopreserved for future transfers as routine using standard protocols. After CGHa analysis, the embryos were classified as euploid or aneuploid. Also, the patients were categorized by ages subgroups: ≤37 yrs-old (n = 77) and >37 yrs-old (n = 159), and the association of AMH and aneuploidy rates were analyzed for each subgroup.

Main results and the role of chance: The indication for CGHa in patients included in the study were advanced maternal age (67.4%), recurrent miscarriage (8.9%), implantation failure (9.3%), severe male factor (4.2%), genetic factor (2.5%) and screening for patient choice (7.6%). Women ages varied from 30 to 49 yrs-old (39.3 ± 3.4), and had mean AMH levels of 1.9 ± 2.1 ng/ml. In general, the patients had 9.7 ± 6.6 oocytes recovered, 2.4 ± 1.6 blastocysts biopsied and the aneuploidy embryos rate was 68.5%. The comparison between subgroups showed similar mean number of embryos biopsied (≤37yrs-old: 2.4 ± 1.5 and >37yrs-old: 2.4 ± 1.6; p = 0.746). As expected, younger patients had a higher levels of AMH (≤37yrs-old: 2.5 ± 2.7 and >37yrs-old: 1.7 ± 1.8; p = 0.005) and lower aneuploidy embryos rate (≤37yrs-old: 54.7% and >37yrs-old: 75.2%; p<0.001). The levels of AMH were inversely correlated to age in both subgroups of patients (Pearson correlation: ≤37yrs-old: r = -0.256; p = 0.025 and >37yrs-old: r = -0.215; p = 0.007). However, there was not association between AMH measurements and eneuploidy embryos rate for ≤37yrs-old (Pearson correlation: r = 0.034; p = 0.766) or >37yrs-old (Pearson correlation: r=-0.083; p = 0.301) subgroups. Finally, we compared the aneuploidy rates of ≤37yrs-old patients between those presenting AMH < 1.0 ng/ml (55.2%) or AMH≥1.0 ng/ml (54.5%; p = 0.943) and >37yrs-old (AMH < 1.0 ng/ml: 76.1%, AMH≥1.0 ng/ml 74.2%; p = 0.725).

Limitations, reasons for caution: Besides the retrospective design of this study, it is known the AMH measurements methods presents a high variation between laboratories, which can be considered limitations of this study. A higher number of patients should be included in the analysis in order to minimize those biases.

Wider implications of the findings: Our findings confirmed the association between ageing and aneuploidy. However, low ovarian reserve represented by AMH is not associated to aneuploidy rates, regardless of patient age. It suggest that oocytes quality is preserved in young patients presenting low ovarian reserve, and there is no indication for PGD in those cases.

Trial registration number: not applied.

P-743 Telomere content is not altered in girls with Idiopathic Central Precocious Puberty treated with GnRH analogue: a preliminary study

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Study question: What are the implications of idiopathic central precocious puberty (ICPP) on telomere content in girls treated with gonadotropin releasing hormone analogue (GnRHa)?

Summary answer: Telomere Content is not altered in girls with ICPP and metabolic and endocrinology aspects were not correlated with telomere.

What is known already: The ICPP is related to the appearance of physical and hormonal signs of pubertal before eight years old. It is observed an increase in growth velocity, disproportionate advancement of bone age and maturation, metabolical and endocrinological alterations. Polycystic Ovarian Syndrome (PCOS) seems to be common in patients with ICPP treated with gonadotropin releasing hormone analogue (GnRHa). The suppressive therapy with GnRHa is related to suppression of oestrogen and excessive exposure to androgenic hormones, with clinical and/or laboratory hyperandrogenism. Metabolic alterations and hyperandrogenism associated with ICPP is related to changes in telomere biology and maybe an important feature of this disease.

Study design, size, duration: Observational case-control study, in which 45 patients with a history of treatment of ICPP with GnRHa and 40 controls (CO) were included

Participants/materials, setting, methods: Patients with ICPP previously treated with leuprolide acetate (3.75 mg per month) at least six months and healthy control women were included. Height, age, weight, body mass index (BMI) were measured. Insulin, triglycerides and testosterone were evaluated. Insulin resistance was predicted by HOMA (Homeostasis Model Assessment). Telomere content was measured using quantitative real-time PCR (qPCR). Statistical analyses were determined by Wilcoxon test and Spearman correlation was carried out.

Main results and the role of chance: Age mean was 15.96 (±2.73) in ICPP and 16.08 (±3.98) in controls. The variables weight (p=.0001) and BMI (p=.0001) was increased in IPP group. Insulin (p = 0.0048) and HOMA (p = 0.0144) was higher in IPP than in CO. Triglycerides were not different between the groups (p = 0.46). Telomere content was not different between ICPP (1.35 ± 0.30) and control (1.30 ± 0.56) (p = 0.2919). We did not observe any correlation between telomere and testosterone, BMI and weight in the subjects analysed. Only a negative correlation was observed between telomere and age (r2=- -0.39, p = 0.01).

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Limitations, reasons for caution: Limited number of cases of the study group. An increase of sample size is recommended to make the data more robust and confirm the results.

Wider implications of the findings: Telomere content was not different in ICPP and control women and no correlations were observed in metabolic and endocrinology variables analysed.

Trial registration number: N/A.

P-744 Low anti-Müllerian hormone resulted in reduced pregnancy rates within two years of fertility treatment irrespective of female age; a prospective cohort-study of 546 infertile women

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Study question: Can serum-AMH predict long-term success rates in infertile women aged below 40 initiating fertility treatment?

Summary answer: Low AMH was associated with a reduced chance of conceiving within two years after start of fertility treatment in women below 40 years of age.

What is known already: AMH is a well-established predictor of the ovarian response to controlled ovarian stimulation, but a weak predictor of pregnancy and live-birth rates after assisted reproduction.

Study design, size, duration: A prospective cohort study of 546 infertile women aged 20–40 years initiating fertility treatment at a tertiary centre during 2011–2013 with a two-year follow-up on treatment outcome. Oocyte recipients were excluded.

Participants/materials, setting, methods: All women underwent a throughout examination at cycle day 2–5 before initiating their first treatment cycle. Examination included blood sampling (AMH, LH, FSH and thyroid hormones) and ovarian sonography (antral follicle count, pathology). Data on reproductive outcome within two years of the examination was based on the clinic's register.

Main results and the role of chance: Women with a mean age 32.3 ± 4.0 were stratified in low AMH (1st quintile), median (range) pmol/l: 7.8 (< 3;12), intermediate AMH (2nd–4th quintiles, reference): 25 (13;49), and high AMH (5th quintile): 66 (50;417). The proportion of women having achieved a pregnancy within two years of fertility treatment was reduced in women with low AMH: 66.7%, 81.5%, and 76.4%, respectively (p = 0.03). Even after age-adjustment, the chance of having achieved a pregnancy within two years was halved in women with low AMH compared with the reference group (aOR 0.5; 95% CI: 0.3;0.8), whereas no difference in pregnancy rates was observed between the intermediate and high AMH groups (aOR 0.7, 95% CI: 0.4;1.2). The cumulative pregnancy rates were independent of female age; 80.7% in age group 20–29, 78.5% in age group 30–34, and 73.0% in age group 35-39 (p = 0.2). Nine women had an undetectable serum-AMH (<3 pmol/l) at inclusion of whom 3 (33%) achieved a pregnancy during the two-year follow-up period.

Limitations, reasons for caution: The presented data are preliminary. Further analysis including competing risk survival analysis will be conducted. Data were not adjusted for sperm quality.

Wider implications of the findings: The presented results indicate that AMH may be used to predict not only the response to controlled ovarian stimulation but also to advice women on their cumulative chance to achieve a pregnancy.

Trial registration number: Not applicable.

P-745 Thyroid status and prevalence of Thyroperoxidase Antibodies (TPO-Ab) in women referred to a Danish University Fertility Clinic

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Study question: How does thyroid status and Thyroperoxidase Antibodies (TPO-Abs) differ in infertile women compared to fertile women and how do they affect success of in vitro fertilization (IVF)?

Summary answer: Level of thyrotropin and frequency of TPO-Abs did not differ between infertile and fertile women and did not affect success of IVF

What is known already: Thyroid hormones are vital for a woman's fertility. TPO-Abs increase the risk of developing hypothyroidism, also during assisted reproduction and pregnancy. In many studies, infertility is associated with increased risk of thyroid autoimmunity such as TPO-Abs. Recent data from observational studies have questioned the importance of TPO-Abs on results of fertilization treatment. Level of thyrotropin and frequency of TPO-Ab in Danish women seeking fertility treatment is unknown, and a possible effect on success of fertilization treatment has not previously been investigated.

Study design, size, duration:

(1) The cohort consists of women referred for fertility treatment at Aarhus University Hospital, Denmark, January 1st 2012 until March 31th 2014. Here we present preliminary data from 2012 (n = 558). In a cross sectional study, level of thyrotropin and frequency of TPO-Abs were compared between infertile and fertile women. In a prospective cohort study (N = 281) we investigated the effect of these parameters and of grouping on success of IVF.

Participants/materials, setting, methods: Single women treated with donor semen and women with male infertility as primary diagnosis were considered fertile, others infertile (57 %). Biochemistry was obtained as part of infertility work-up. Body mass index(BMI), smoking, details on fertilization treatment, and outcome of each treatment cycle was collected prospectively in a treatment database.

Main results and the role of chance: 559 women were referred for treatment. 41 were excluded from the study due to co-morbidity, mainly cancer. Known or newly diagnosed thyroid diseases were comparable between groups. These women were excluded from analysis. 317 women were infertile (57 %). Thyrotropin level was 1.63 (95%CI 1.52;1.74) among the infertile women and did not differ from the fertile women (1.60 (95%CI 1.48;1.73)) p = 0.76. Frequency of TPO-Abs was 15.18 % among infertile and 14.94% among fertile (chi square test for difference, P = 0.96). Age at referral, BMI, and smoking were comparable between groups.

276 women were treated with IVF. In this sub cohort, infertile women were 1.58 (95%CI 0.53;2.63) years older (p = 0.003) but groups were otherwise comparable. Success of first IVF (positive human chorion gonadotropin (hcg), loss of embryo, and child birth (overall 32.6 %) did not differ between groups. Increasing age significantly reduced chance of biochemical and clinical pregnancy and chance of child birth, whereas level of thyrotropin, TPO-Abs, smoking, and BMI did not. Only 30 women had TPO-Abs. They did not differ from TPO-Ab-negative women and TPO-Abs did not predict chance of IVF.

Limitations, reasons for caution: Level of thyrotropin and TPO-Abs were only available for 448 and 199 respectively, however women without available biochemistry were comparable in terms of demographics. We were not able to look separately at success of ICSI.

Wider implications of the findings: To our knowledge this is the first study describing thyroid status with thyrotropin and TPO-Abs in Danish women seeking fertility treatment. Findings do not differ from similar populations. Our data adds to the ongoing debate regarding the impact of thyroid autoimmunity on fertility treatment. Larger studies are warranted.

Trial registration number: This study was approved by the Danish Dataprotection Agency (1-16-02-487-13) and by the Danish Health and Medicines Authority (3-3013-495).

P-746 Sonographic measures of bone density and markers of bone turnover during pregnancy and lactation

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Study question: Does bone mineral density during pregnancy and lactation measured with ultrasonometry (QUS) correlate to serum markers of bone turnover?

Summary answer: Serial measurements of BMD showed no significant change during pregnancy and lactation and did not correlate to observed changes in markers of bone turnover.

What is known already: A high bone turnover during pregnancy has been shown using biochemical markers of bone resorption and formation and also reflected in a decrease in BMD during pregnancy. To our knowledge, no study has thus far compared these assessment techniques in the same cohort to test for a possible correlation and to evaluate the use of a simple hand ultrasound-based screening method during pregnancy.

Study design, size, duration: This prospective longitudinal 2-year study was designed with 50 female patients followed during pregnancy, in the puerperium, and the lactation period. Measurements of bone mineral density and laboratory markers of bone turnover were repeated at five different time points (once each trimester, 6-8 weeks post-partum, and once during lactation.)

Participants/materials, setting, methods: 51 female pregnant patients were included in this study after obtaining written consent. Quantitative ultrasonometry to assess BMD was performed at the distal metaphyses of the proximal phalanxes II-V in the outpatient department of a university hospital. Biochemical markers of bone remodeling, osteoprotective factors, and serological markers of bone turnover were measured. Statistical analyses evaluated percentage of change in BMD over time and correlated measures of BMD with serum markers for potential use for screening.

Main results and the role of chance: BMD of the proximal phalanxes assessed by AD-SoS and UDPI showed no significant change from baseline during pregnancy ($p = 0.16$ and $p = 0.7$, respectively).

Correlation analyses showed a significant association between AD-SoS and UDPI, $\rho = 0.52$ and 0.63 ($p < 0.01$, respectively).

Markers of bone turnover showed significant changes throughout time (Friedman's test). Biochemical markers of bone turnover increased over the time of pregnancy ($p < 0.001$). Markers of bone resorption (TRAP-5b, ß-Croßlaps) rose and peaked in the puerperium. Markers of bone formation, namely OC, rose significantly ($p < 0.001$) after having decreased in the second trimester and peaked during lactation.

However, no statistically significant correlations between changes in BMD measures and serum parameters of bone turnover and remodeling were found.

Limitations, reasons for caution: No non- pregnant control group was analyzed during the same period. The cohort is too small to analyze subgroups based on parity, BMI, diet or age. The patients in this cohort may not be representative of all pregnant women, especially those with risk factors for bone loss.

Wider implications of the findings: Our results contradict those of previous studies which show a decline in bone density during pregnancy. It is possible that the ultrasonometry used was not sensitive enough to detect subtle changes in BMD. It is possible that the proximal phalanx is not a location of high bone turnover during pregnancy.

Trial registration number: UN 3641277/ 4.3 at study institution.

P-747 A prospective randomised controlled study (RCT) evaluating the effect of early hcg trigger on pregnancy rates in patients undergoing IVF-ICSI cycles using GNRH- antagonists

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Study question: Does an early Administration Of Human Chorionic Gonadotropin (hCG) Improve Pregnancy rates In IVF-ICSI Cycles With GnRH Antagonists?

Summary answer: Yes: Early HCG-triggering of final oocyte maturation result in an improved probability of pregnancy by leading to a less deranged and more receptive endometrium.

What is known already: The criteria used for hCG administration in GnRH - Antagonist cycles (hCG triggering for the final oocyte maturation as soon as ≥ 3 follicles ≥ 17 mm) have not been evidence-based, and their importance for the probability of pregnancy was unknown until recently. Studies have shown that delaying hCG administration is associated with a decreased probability of ongoing pregnancy because of premature closure of the Implantation-Window.

Early HCG-triggering is expected to result in lower progesterone levels on the day of hCG and this intervention might result in an improved pregnancy rates by leading to less deranged and more receptive endometrium.

Study design, size, duration: The purpose of this randomized controlled trial of 416 infertile patients between 2006 to 2016, below 42 yrs of age, was to evaluate whether triggering of final oocyte maturation as soon as ≥ 3 follicles ≥ 16 mm present on ultrasound or one/two day later affects the probability of pregnancy in patients stimulated with GnRH-antagonists in IVF-ICSI-cycles.

PRIMARY OUTCOME: pregnancy rate

SECONDARY OUTCOME: number of MII oocytes, endometrial-thickness, progesterone levels and complications like OHSS

Participants/materials, setting, methods: Patients were randomized by a computer-generated list and after proper consent were divided into two groups of 208 patients each (group one - Early HCG and group two - Late HCG).

Main results and the role of chance: Significant differences were observed between the group One(early-hCG) and group Two (the late-hCG group) regarding -

Pregnancy Rates (33% Vs 21%, respectively) and

Progesterone Levels (0. 7 ± 0. 5 Vs 1. 1 ± 0. 4 ng/mL, respectively) on the day of hCG administration and

Endometrial Thickness (10. 8 +/- 2. 3 mm Vs 7. 3 +/- 2. 5 mm) on day of embryo transfer.

the number of Metaphase II Oocytes (6. 1 ± 4. 1 Vs 9. 1 ± 4. 8, respectively). and

less Complications like OHSS (3 % Vs 7%)

More similar multicentric trials are needed to assess its potential in improving the implantation chances in IVF-ICSI cycles, thereby improving pregnancy rates and reducing the complications of ART.

Limitations, reasons for caution: Why delaying HCG administration leads to an increased incidence of endometrial advancement is not clear?

The adverse effect of Progesterone elevation on the day of hCG is explained by the induction of differences at the histological level and gene expression level between endometrial samples exposed to high concentrations of progesterone.

Wider implications of the findings: Our study suggests that prolongation of follicular phase is associated with a higher incidence of premature secretory changes of the endometrium due to Progesterone elevation and supraphysiological E2 levels in late-hCG group.

Since earlier hcg- triggering results in lower progesterone levels, such an intervention might give better pregnancy rates.

Trial registration number: NONE

POSTER VIEWING SESSION

REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

P-748 Changes in Assisted Reproductive Technology Use After Large Increases in Patient Cost-sharing: Is Something Amiss in Denmark?

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Study question: How did the large increase in patient cost-sharing for Assisted Reproductive Technology (ART) in Denmark impact its use?

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Summary answer: After patient cost-sharing increased to 50% of ART costs in 2011, ART use dropped by 23% when compared to use between 2007-10.

What is known already: Recent advancements in assisted reproductive technology (ART) have improved birth rates and reduced complications; however, ART treatment remains expensive and often requires multiple treatments before a live birth. Many countries increasingly are limiting use of expensive treatments in an effort to reduce spending growth. Several countries have limited ART use by requiring patient cost-sharing, e.g., coinsurance. In general, cost-sharing for medical care has been an effective means for reducing use and spending, but often can have undesirable or unintended clinical consequences. There have been few studies examining the impact of a sudden increase in ART cost-sharing.

Study design, size, duration: We examine the impact of a large cost-sharing increase on ART use within a historical population cohort, i.e., a pre-post longitudinal design focused on within-clinic effects. In Denmark, ART coverage had been generous and use levels amongst the highest in the world. In 2010, Denmark announced plans to require substantial cost-sharing starting in 2011, i.e., a 50% coinsurance (from <10%). The policy was expected to reduce ART treatment use, and thus reduce national expenditures.

Participants/materials, setting, methods: Eligible subjects were Danish women who received publicly-financed ART between 2007-2013. We obtained data from public datasets, e.g., Danish ART and birth registries. To assess changes in the number of ART uses, we used linear models with clinic-level fixed effects. We examined the percent of treatments that had a live birth within six to ten months after ART treatment, using logistic regression and adjusting for maternal age and clinic.

Main results and the role of chance: In 2010, 10,718 women received 24,681 treatments with ART (mean = 2.3 treatments/person-year); and, among women receiving ART in 2010, 52% had a live birth within 6-10 months after treatment. In 2011, after the policy change, both the number of women receiving ART and the number of treatments per woman dropped (8,565 women, 18,986 treatments, mean = 2.2 treatments/person-years); among women receiving ART in 2011, 53% had a live birth within 6-10 months after treatment. Use dropped in each subsequent year from 2011-13; moreover, use initially increased in 2010 (the year of the policy announcement but before implementation), when compared to use in 2007-09. In analyses examining ART use between 2007-13, the 2011 coinsurance introduction was associated with a 23% decrease in ART use (mean of 181 fewer treatments per clinic per year; 95%CI: 93-270 fewer treatments). The odds of a live birth after treatment increased slightly for those treated in 2011, compared with those treated in 2007-2010 (OR=1.13; 95%CI: 1.09-1.18).

Limitations, reasons for caution: The pre-post design does not address any potential secular changes in ART use, though any such alternative explanations would need to coincide with the cost-sharing policy announcement and introduction.

Wider implications of the findings: There are growing financial barriers to ART use; fewer women are seeking treatment, and those that do have fewer treatments. Some women might have initiated ART earlier in anticipation of the policy implementation. Overall, the findings help explain the drop in all live births within Denmark, starting in 2011.

Trial registration number: Not applicable.

P-749 A comparison of cost - effectiveness of freeze - all protocol and fresh embryo transfer in women undergoing IVF - ICSI

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Study question: What is the cost-effectiveness of a freeze-all versus fresh embryo transfer in women undergoing IVF/ICSI?

Summary answer: In women undergoing IVF/ICSI, the use of fresh embryo transfer protocol is cost-effective as compared to a freeze-all protocol.

What is known already: Conventionally, IVF embryos are transferred in the same cycle in which oocytes are collected, while remaining embryos are frozen and stored. Although recent data suggest that pregnancy outcomes could be improved after frozen compared with fresh embryo transfer, the immediate cost of freezing all embryos are higher than a policy in which the first transfer is done in a fresh cycle. The cost-effectiveness of both policies has not been compared.

Study design, size, duration: We performed a cost-effectiveness analysis (CEA) from a patient perspective alongside a RCT comparing freeze-all versus fresh transfer in non-PCOS women undergoing IVF. Cost data were calculated from used resources and prices based on micro–costing per patient. Data were collected from chart review and from patient questionnaires.

Participants/materials, setting, methods: For all patients, we measured direct medical costs relating to treatment (IVF, pregnancy follow-up, delivery), and indirect costs (travel, accommodation, income lost). We evaluated the cost-effectiveness of the Freeze-all and fresh Embryo transfer and then calculated an incremental cost-effectiveness ratio (ICER). One-way sensitivity Analysis (PSA) was used to explore the robustness of our findings.

Main results and the role of chance: Between June 2015 and April 2016, we randomized 782 women. Cost data were based on 704 women who returned questionnaires. The live birth rates after the first cycle were 33.8% for freeze-all versus 31.5% for fresh transfer (P = 0.542). The cost per live birth was 7,456 EUR for a freeze-all transfer, versus 5,699 EUR for the fresh transfer. The incremental cost of one live birth gained by freeze-all compared to fresh embryo transfer was 28,928 EUR. One–way sensitive analysis indicates live birth rate and IVF laboratory cost are factors that have largest effects on ICER.

Limitations, reasons for caution: This was a single IVF center study limited to the first embryo transfer cycle only. To make firm conclusions on the economic impact of freeze-all and fresh embryo transfer on pregnancy outcomes (live birth rate, complications), a cost-effectiveness analysis study based on all cycles is needed.

Wider implications of the findings: In non-PCOS couples undergoing IVF/ICSI, a freeze-all strategy is not cost-effective over a fresh embryo strategy.

Trial registration number: NCT02471573.

P-750 Assisted reproductive technology treatment and risk of breast cancer and gynecological cancers in women

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Study question: Is assisted reproductive technology (ART) treatment associated with risk of breast cancer or gynecological cancers during 16 years of follow-up?

Summary answer: Ovarian cancer risk was increased after initiated ART treatment. Breast cancer risk increased after 10 years. Risk of endometrial and cervical cancer were decreased.

What is known already: Concern has been raised about the potential adverse effects of ovarian stimulation hormones in ART treatment, and previous findings on the impact on breast cancer incidence and incidence of

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gynecological cancers have been conflicting. Parity and type of hormones used have been shown to influence cancer risk, and a long-term elevated risk of breast cancer has been suggested. Risk of ovarian cancer has been shown to be elevated among ART-treated. Endometriosis, which also causes infertility, is associated with increased risk of ovarian cancer.

Study design, size, duration: The Danish National ART-Couple (DANAC) cohort includes all ART-treated Danish women during 1994-2009. After relevant exclusions, the population consisted of 42,510 ART-treated women identified from the Danish IVF Register and 202,595 age-matched controls from the background population with no previous history of ART treatment. The total study population of 245,105 women with no previously diagnosed cancer were followed in population-based registers until ultimo 2010. Cancer diagnoses were retrieved from the Danish Cancer Registry.

Participants/materials, setting, methods: Up to three fresh ART treatment cycles were accessible free of charge to heterosexual couples during 1994-2007 and also for homosexual female couples and single women during 2007-2009. Hazard ratios (HR) and 95 % confidence intervals (95% CI) were estimated in cox proportional hazards regression adjusted for age, calendar year, time since initiation of treatment, educational level, parity and partnership status as time-dependent variables.

Main results and the role of chance: During follow-up, the number of women diagnosed with cancer of the cervix were 589 (80 among ART-treated), endometrium 177 (22 among ART-treated), breast 2,304 (427 among ART-treated) and ovary 192 (56 among ART-treated). For the overall follow-up period there was an increased risk of ovarian cancer among ART-treated women (HR 1.52, 95 % CI 1.36-1.69), and the increased risk was accentuated during the first six years after initiated ART treatment. The highest incidence of ovarian cancer was found 2-4 years after initiated ART treatment (HR 1.81, 95 % CI 1.46-2.24). No overall increased risk of breast cancer was found (HR 1.01, 95 % CI 0.98-1.5), although hazard ratios tended to increase towards the end of follow-up at 10-12 years (HR 1.26, 95 % CI 0.97-1.62) and 12-14 years after initiated ART treatment (HR 1.21, 95 % CI 0.83-1.75). An overall lower risk among ART-treated women was found for cancer of the cervix (HR 0.70, 95 % CI 0.64-0.77) and insignificantly lower for endometrial cancer (HR 0.92, 95 % CI 0.80-1.06). Endometrial cancer risk appeared to be elevated among ART-treated 12-14 years after initiated ART (HR 1.89, 95 % CI 0.90-3.98), although this result was insignificant.

Limitations, reasons for caution: An increased risk of ovarian cancer in the years after initiated ART treatment probably reflects detection bias. A low number of women followed as long as 10-16 years after ART treatment limited the statistical strength of estimates during this period. Endometriosis could explain the elevated ovarian cancer risk among ART-treated.

Wider implications of the findings: Monitoring and longer follow-up in large cohorts could clarify whether long-term breast and endometrial cancer risk is elevated in ART-treated women. The short-term elevated ovarian cancer risk is important to address in studies taking cause of infertility into account, as well as risk of detection bias linked to ART treatment.

Trial registration number: Not relevant.

P-751 Knowledge, perceptions and acceptability of IVF treatment among women in an urban West African city

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Study question: In a society where childbearing is almost mandatory and a third of couples experiencing infertility, what is the knowledge and perceptions on in-vitro fertilisation (IVF)?

Summary answer: Religious beliefs still pose a barrier to the perceptions and acceptability of IVF treatment by religiously-entrenched women, but it is acceptable to more educated women.

What is known already: IVF treatment is a widely accepted option for assisted conception in the developed world. In developing countries, where couples have difficulty getting pregnant, a visit to a fertility clinic may not be a top priority. Rather, many preferentially visit a religious leader (e.g. pastors, imams) or try out some local herbal preparations claimed to offer help to women to conceive. For some. IVF treatment is still perceived as a new phenomenon, mainly due to lack of awareness and general acceptability. The cost implications of medical assisted conception are also prohibitive for many people in developing countries.

Study design, size, duration: This was a prospective study performed between September - December 2016. Open-ended questionnaire was administered via interviews to 150 women in the study area to gain information on their knowledge and acceptability of IVF treatment. Five healthcare professionals were also sampled purposively for their views on IVF practice. Participant responses were reviewed and grouped and when a theme/concept became apparent, codes were assigned. The chi-square test was used to analyze statistical difference in the data.

Participants/materials, setting, methods: One hundred and fifty (150) respondents aged between 18 – 50 years were recruited from the general population. The study area was an urban harbour city (Accra, Ghana) and can be described as a mix of middle to high and low-income families. Interview responses were transcribed, coded and analysed in SPSS software quantitatively and qualitatively.

Main results and the role of chance: When respondents were grouped according to age, only about half of younger respondents (≤25 years) were aware of IVF treatment, whereas all older respondents (≥46 years) had a basic knowledge of the IVF procedure. Those with secondary (high school) and tertiary (university) education had a significantly better knowledge of IVF treatment than their counterparts with basic or no formal education at all ($p = 0.011$). The internet served as the major source of information (72.3%) for those who had knowledge of IVF. On attitude, a good number of respondents accepted IVF treatment as a reliable means to conceive, but to them this will only be an option when 'all else has failed". Many stated cost was the main reason why IVF treatment would not be on their top priority when they are faced with infertility and that such treatment was 'for the rich". Others also viewed IVF treatment 'as sinful and against their religious belief". Such opinions came from Christians and Muslims alike. Others also perceived IVF as 'unnatural and had the propensity to make people lose faith in God". Some respondents had the perception that children born as a result of IVF were 'not normal and unhealthy".

Limitations, reasons for caution: The low number of study respondents should be considered when drawing conclusions based on this study.

Wider implications of the findings: With increasing education and public awareness through social media and other internet based approaches, knowledge of IVF treatment should become more widespread, and make the technique more acceptable in religiously-entrenched societies in developing countries

Trial registration number: N/A.

P-752 Increased rate of inferior neonatal outcomes among twins following assisted reproductive technology

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Study question: Do twins born following assisted reproductive technology (ART) have higher rates of inferior neonatal outcomes compared to non-ART twins?

Summary answer: ART twins have higher rates of preterm birth, low birthweight, need for resuscitation, admission to Neonatal Intensive Care Unit (NICU), and prolonged hospital stay.

What is known already: Twin pregnancies have a higher risk of maternal and neonatal complications than singleton pregnancies. The higher incidence of twin pregnancy following ART results in higher rates of adverse maternal and perinatal outcomes compared to non-ART pregnancies. It remains unclear whether ART twins have similar perinatal outcomes compared to non-ART twins.

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Study design, size, duration: A retrospective population study using the Australian National Perinatal Data Collections (NPDC). The study included 19,662 twins of ≥ 20 weeks gestational age or ≥ 400 grams birthweight born in Australia between 2007 and 2011. The analysis included 2290 (23.3%) ART twin sets and 7541 (76.7%) non-ART twin sets.

Participants/materials, setting, methods: The rates of stillbirth, preterm birth, low birthweight, low Apgar score, need for resuscitation, admission to NICU, prolong hospital stay, and neonatal death were compared between ART and non-ART twins. Generalized Estimating Equations was used to assess the likelihood of any neonatal outcomes following ART, with adjusted odds ratio (AOR) and 95% confidence intervals (CI) presented. Weinberg's differential rule was used to estimate the rate of monozygotic twins.

Main results and the role of chance: ART mothers were 3.3 years older than non-ART mothers. Compared to non-ART mothers, higher proportions of ART mothers were primiparous, non-smoking, with normal body mass index and with private health insurance. The rates of pregnancy-induced hypertension and gestational diabetes were significantly higher for ART mothers than non-ART mothers (12.2% vs. 8.4%, $p < 0.01$) and (9.7% vs. 7.5%, $p<0.01$) respectively. The incidence of monozygotic twins was 2.0% for ART twins and 1.1% for non-ART twins. Compared with non-ART twins, ART twins had higher rates of preterm birth (AOR: 1.13, 95% CI: 1.05-1.22), low birth weight (AOR: 1.13, 95% CI: 1.05-1.22), and need for resuscitation (AOR: 1.26, 95% CI: 1.17-1.36). In contrast, ART twins had significantly higher live birth rate than non-ART twins (98.6% vs. 97.7%; $P < 0.01$). Liveborn ART twins had 28% (AOR 1.28, 95% CI 1.09-1.50) increased odds of having any adverse neonatal outcome compared to liveborn non-ART twins, especially for opposite-sex ART twins (AOR 1.42 95% CI 1.11-1.82).

Limitations, reasons for caution: Since there is no information on ART procedures in the NPDC, we were unable to investigate the impact of single versus multiple embryo transfer on outcomes. The large proportion of mothers with missing data on body mass index and smoking during pregnancy may impact the validity of the comparison.

Wider implications of the findings: As ART twins had higher rates of adverse outcome, special antenatal care is recommended for them. Couples access ART should be fully informed the risk of adverse outcome of twin pregnancies. Single embryo transfer should be continued encouraged to reduce the rate of ART twin pregnancies.

Trial registration number: N/A.

P-753 Summary of 3 years of Polish state-founded in-vitro fertilization infertility treatment program for years 2013-2016. Results after 3 years

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Study question: What are the results of Polish state-founded *in-vitro* fertilization infertility treatment program for years 2013-2016 in June 2016 when program was terminated?

Summary answer: Results are comparable with data collected by ESHRE from European registers despite limitation of fertilized eggs to six.

What is known already: This was the first time Polish government conducted *in vitro* fertilization (IVF) programme financed from public funds. Due to infertility treatment regulations being developed by the Polish government at the time programme started, some limits in number of fertilized eggs (only six) and transferred embryos (only one) for women under 35 years of age were introduced.

Study design, size, duration: 3 IVF procedures were available for women aged <40 with FSH<15 mU/mL on beginning of a cycle or AMH>0.7 ng/mL. For women aged <35 number of fertilized eggs was limited to six and single embryo transfer (SET) was obligatory. For women >35 years of age there were no limitations in number of fertilized eggs and double embryo transfer (DET) was approved. Presented results contain data from 3 years of programme.

Participants/materials, setting, methods: Participants: In 17990 patients 27650 controlled ovarian stimulation in various protocols were initialized. Settings: 34 clinics realizing the IVF programme were obligated to report every procedure within 2 weeks. Methods: retrospective analysis of treatment outcomes including clinical and embryological data.

Main results and the role of chance: Cumulative pregnancy rate per cycle was 44.93%. Clinical pregnancy rate per cycle was 30% and 33.7% per transfer. The pregnancy rate by thawing in frozen embryo transfer (FET) was 29.73% and per SET and DET was 28.03% and 37.59% respectively. In the group of women under 35 years clinical pregnancy rate per fresh embryo transfer was 33.31% and multiple pregnancy rate was 4.61%. Clinical pregnancy rate per fresh embryo transfer in group of women between 35 and 40 years was 34.11% and multiple pregnancy rate was 14.12%. Miscarriage rate was 10.11% and single pregnancy was 92.94%. Ovarian hyperstymulation syndrome (OHSS) rate was 2.37%. High numbers of analyzed cycles limit the impact of chance in the presented data.

Limitations, reasons for caution: This presentation contains data form June 2013 to June 2016, while procedures were financed from public funds. When all live-birth data will be submitted and analyzed the result will be complete and final.

Wider implications of the findings: Despite limiting factors, pregnancy rates of the programme were relatively high. This may have impact on future public funded IVF programs.

Trial registration number: N/A.

P-754 Prevalence and impact of elective gender selection in oocyte donor recipient cycles in the United States 2005-2013

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Study question: What is the prevalence and impact of elective gender selection (eGS) in oocyte donor recipient cycles?

Summary answer: Elective gender selection reduced the odds of live birth in oocyte donor recipient cycles.

What is known already: Elective gender selection has been criticized for possible 'gender bias, social harm, and the diversion of medical resources from genuine medical need". Cleavage stage as well as trophectoderm biopsy have previously been associated with decreased embryo viability.

Study design, size, duration: Utilizing data between 2005-2013 from the national Assisted Reproductive Technology Database of the Society for Assisted Reproductive Technology (SART), we report on the prevalence of eGS and its effect on birth outcomes in first fresh oocyte donor-recipient cycles (ODRCs) and their subsequent frozen-thawed embryo cycles. Statistical models adjusted for patient and donor ages, number of embryos transferred, race, and cycle year were created to compare outcomes in eGS cycles relative to and non-eGS cycles.

Participants/materials, setting, methods: 33,756 patients initiated a first ODRC, among which 570 (1.68%) underwent eGS for assessment.

Main results and the role of chance: Live birth rates were significantly lower for eGS than non-eGS cycles (48.1 vs. 55.7%, $P = 0.04$). Adjusted for patient and donor ages, oocytes retrieved, embryos transferred, race and reporting year, the odds of live birth in cycles with eGS were reduced by 30% (OR 0. 70 95% CI 0. 59 to 0.84; $P < 0.001$) in comparison to non-eGS cycles. While Asian

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women represented only 7.2% of the overall ODRC population they were 19.1% of the population choosing to undergo eGS.

Limitations, reasons for caution: Techniques for embryo biopsy have changed over the study period so findings in this analysis may not be generalizable to current practice.

Wider implications of the findings: Like other forms of preimplantation diagnosis, elective gender selection can have a significant negative impact of cycles outcomes in oocyte donor recipient cycles. Patietns should be appropriately counseled before choosing to have such elective procedures.

Trial registration number: n/a.

P-755 Assisted reproductive technology treatment and risk of death in women

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Study question: Is assisted reproductive technology (ART) treatment associated with risk of death during a 16 year follow-up period?

Summary answer: ART-treated women had a lower risk of death short-term, although the risk was similar to untreated women from 10 years after ART treatment initiation.

What is known already: Previous studies have shown decreased risk of death among ART-treated women compared to women in the background population. Because reasonably good health is a prerequisite for initiating ART treatment, the association may reflect selection of healthy women into ART treatment. This process is known as the 'healthy patient effect'. To the best of our knowledge, this 'healthy patient effect' has not been directly addressed in prior investigations on this subject. ART implies delayed or obstructed parenthood for women in treatment, and factors such as childlessness, older age at first childbirth and non-marital status are associated with increased risk of death.

Study design, size, duration: The Danish National ART-Couple (DANAC) cohort includes all ART-treated Danish women during the years 1994-2009. After relevant exclusions, the population consisted of 42,897 ART-treated women identified from the Danish IVF Register and 204,514 age-matched comparison women from the background population with no previous history of ART treatment. The total study population was made up of 247,411 women, who were followed in population-based registers until ultimo 2010.

Participants/materials, setting, methods: Up to three ART treatment cycles were accessible free of charge for heterosexual couples during 1994-2007 and also for homosexual female couples and single women during 2007-2009. Associations between ART treatment and death were estimated using Cox proportional hazards regression adjusted for age, educational level, parity, time since ART treatment initiation and partnership status as time-dependent variables and prior comorbidity as a fixed variable. Hazard ratios (HR) with 95% confidence intervals (95% CI) were estimated.

Main results and the role of chance: In total 2,041 women died, 235 among ART treated (0.6 %) and 1,806 among the untreated women (0.9 %). An initial lower risk of death among ART treated women persisted with adjustment for socio-demographic factors and comorbidity prior to treatment (1-2 year HR 0.84, 95 % CI 0.75-0.94), but within 10 years after treatment initiation the risk of death among ART-treated and untreated was similar (10-11 year HR 1.01, 95 % CI 0.71-1.45). When incorporating reproductive status before and during the follow-up period, differences between ART-treated and untreated women were minor and/or insignificant. Risk of death was highest among childless women regardless of ART treatment status, particularly death caused by cancer, psychiatric factors and suicide. Among women with children, having children during observation was associated with lower risk of death compared to those who only had children prior to observation, regardless of ART treatment status.

Limitations, reasons for caution: Number of deaths was insufficient to investigate separate causes of death. Measurement of comorbidity was based on hospital in- and outpatient diagnoses, other minor conditions could be relevant.

Wider implications of the findings: ART-treated women have a transient decreased risk of death, which diminishes with increased time since ART treatment initiation. ART-treated women are not necessarily healthier than an untreated comparison group, but the initial lower risk of death may also be caused by pregnancy planning, pregnancy and shorter duration since achieved parenthood.

Trial registration number: Not relevant.

P-756 Anxiety and ART: should we be anxious about it?

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Study question: Can patients at risk of anxiety after embryo transfer (i.e, during the waiting period) be identified and reassured about the impact of anxiety on outcome?

Summary answer: Pre-existing anxiety is the primary determinant of anxiety during the waiting period, but pre-treatment and waiting period anxiety do not significantly impact clinical outcome.

What is known already: Dealing with subfertility and its treatment is reported as being a stressful life experience for couples. Stress and anxiety are known to affect the experience of ART, and have been suggested to influence pregnancy rates. It has been proposed that emotional response to treatment results, and possibly compliance to treatment, is conditioned by demographic and clinical factors, as well as level of pre-existing anxiety. However, it has not yet been established to what extent these factors are responsible for stress during the waiting period of treatment. Coping interventions have been suggested to reduce stress in the time after embryo transfer.

Study design, size, duration: Archival data from the routine care control arm of a randomized controlled trial, examining the effects of a coping intervention, were used in the present study (n = 124) (Ockhuisjen et al. 2014). Participants completed the hospital anxiety and depression scale (HADS) just prior to the first scan during stimulation (T1), on Day 10 of the 14-day waiting period (T2), and 6 weeks after the start of the waiting period (T3, about 4 weeks post treatment results).

Participants/materials, setting, methods: Respondents were recruited over two years from a Dutch University Hospital fertility clinic and composed of Dutch-speaking women undergoing ART. Self-reporting and medical records yielded demographic (e.g., age, education) and clinical data (e.g., number of previous cycles, fresh or frozen cycle), as well as outcome of treatment. Multiple regression analyses were used to examine whether demographic and clinical factors were associated with anxiety at T2 and T3, whilst controlling for anxiety at T1.

Main results and the role of chance: Most of the respondents (mean age 34.8 years), had received higher education (n = 77, 63%), were nulliparous (n = 85, 69%), had been infertile for three years or more (M = 3.13, SD=2.27), and were undergoing a fresh stimulated IVF/ICSI cycle (n = 96, 77.4%). A total of 28 (24.8%) participants achieved a positive pregnancy test and 18 (14.2%) achieved a live birth with treatment. Levels of anxiety were significantly higher at T2, compared to T1 and T3 (p < .001). The mean difference in anxiety between T1 and T2 was 2.02 (95% CI 7.94 - 5.92). Greater age was associated with significantly less anxiety at T2 (P < .01) but none of the other demographic or clinical variables were associated with anxiety at T2 and T3. Pre-existing anxiety levels at T1 were significantly and positively associated with anxiety at T2 and T3 (P < .001), as was anxiety at T2 with anxiety at T3 (P < .001). There

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was no evidence to support a relationship between overall anxiety and success of treatment.

Limitations, reasons for caution: Sample size was small and there was possible bias of relying on self-report measures of anxiety in an RCT. Moreover, the majority of the sample had higher educational attainment, meaning that the results may not necessarily apply to other educational groups, and from which socio-economic status cannot be reliably inferred.

Wider implications of the findings: Pre-treatment assessment of anxiety may identify those that could benefit from earlier intervention before embryo transfer. While younger women may be seen as 'having time', they may be at greater risk of increased anxiety. Interventions to reduce anxiety may include reassurance about the impact of anxiety on outcome.

Trial registration number: Not applicable.

P-757 Twelve years of the first "Embryo adoption" program worldwide

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Study question: Is Embyo Adoption (EA) an alterantive to international child adoption? Is it the best choice for thousands of embryos that accumulate on IVF laboratories worldwide?

Summary answer: Almost 1.000 newborns, since we created the concept of « Embryo Adoption » in 2004, to give a chance to our IVF laboratory abandoned embryos.

What is known already: Abandonment of frozen embryos in treatment centres is a common phenomenon. Adopting these embryos offers them a chance of life, and the recipient the opportunity to carry a child.

Embryos available for recipients come from donation (where progenitors specify they would like to give their embryos to other couples) or adoption (where progenitors fail to respond to letters from the treatment centre for over 4 years, and, by spanish law, the centre takes over their legal custody). Thus the difference between EA and donation is simply a legal one. No official adoption papers are necessary.

Study design, size, duration: Retrospective descriptive study including 1.728 patients that underwent EA between 2004 and 2016. Approximately 50% of patients from 124 different countries chose EA for reasons common throughout reproductive medicine (ovarian factor or severe male infertility, previous failed IVF alternative treatments), but the remainder chose EA primarily for social reasons: women without a male partner who wanted to have a child and couples or single women who were on the waiting list for standard adoption.

Participants/materials, setting, methods: The embryos offered for adoption all came from young healthy parents: women under 35 years for whom we had a complete medical and family history, and also information regarding children born.

To avoid the possibility of future consanguinity, sibling embryos were distributed to recipients living in different regions or countries. Racial matching was respected, but there was no matching of specific physical characteristics or blood groups.

Recipient patients underwent hormonal replacement treatment for cryotransfer

Main results and the role of chance: In our study only 3% of the patients donated their frozen embryos to other couples; almost 55% of the patients didn't answer to the requirement on how they wished to destine their frozen embryos, although spanish law gives them all options (donation to other couples, to science or destruction).

Between January 2004 and December 2016, 2.656 embryo transfers took place, with an average number of 2,2 embryos transferred, that has decreased to 1,3 during the last 3 years. We observed a pregnancy rate/transfer of 43%, with a miscarriage rate of 12%. To date 972 children have been born in our EA program following this treatment.

Limitations, reasons for caution: The main reason for caution in our study is the heterogenicity of the patients that came for EA treatment: half of them were healthy and didn't correspond to patients with previous fertility problems.

Wider implications of the findings: Embryo adoption is an effective IVF treatment, and offers the possibility of life to frozen embryos. Worried about our responsibility towards these cyopreserved embryos, that accumulate, in 2004 we inaugurated the first Embryo Adoption program worldwide; After 12 years, almost 1.000 babies have been born.

Trial registration number: Not applicable.

P-758 National Italian ART register, eleven years (2005-2015) of data collection with 3 different legislation changes

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Study question: Analyze and compare the trends of eleven years of ART application in Italy (from 2005 to 2015) through four radical changes in the law governing these procedures

Summary answer: The law changes reflected in the application of ART treatments, have positively affected the results and the achievement of the best practices in infertility treatments

What is known already: In Italy a severe law has regulated the application of ART. Banning embryo cryopreservation, genetic preimplantation screening and diagnosis, gametes donation, obliging to the transfer of all the embryos created for a maximum of three. For five years Italy has had one of the highest triplet pregnancy and delivery rate (3.3% and 2.7 % respectively) and a quite low pregnancy and delivery rate in all cycles. The Constitutional Court, has radically changed the law

Study design, size, duration: Retrospective evaluation of eleven years of ART application through the analysis of the data collected by the Italian National ART Register (IARTR) since 2005. 611,941 ART Cycles were collected altogether, including 532,347 homologous fresh (FRESH), 49,766 frozen embryo (FER) and 27,342 frozen/thawed oocytes (FO), plus 2,496 donation cycles

Participants/materials, setting, methods: About 179 clinics per year participated to the study (range 169 to 185). Among those clinics about 53% were private settings 37% were public and 10% were private payed by NHS. The records were kept in the archive of IARTR. The analysis has been performed utilizing SPSS 22.0

Main results and the role of chance: Number of total cycles reported was 609,445 including 532,347 homologous fresh (FRESH), 27,342 frozen/thawed oocytes(FO) and 49,766 frozen embryo (FER) plus 2,287 donation cycles. Pregnancy rates per transfer change overtime with respect to FRESH, FO and FER treatments from 24.5% to 26.5%, from 11.4% to 20.8% and from 16.3% to 28,5% respectively. Delivery rate per cycle with at least one live birth change from 8.0% to 11.7%, from 4.9% to 11.1% and from 7.8% to 18.6% respectively. Twin and 'triplet or more" birth rate per delivery for fresh cycles changed from 21.6% to 17.3% and from 2.7% to 0.8%. During the study period 93,414 ART infants were born, corresponding to the 0.7% of all the national births in the beginning and to the 2.2% in 2015

Limitations, reasons for caution: Since the national ART Register collect only summary data more deep analysis could not be performed. The donation cycles, has been permitted in Italy only at the end of 2014, so the implementation of this procedure was not yet complete in 2015

Wider implications of the findings: The analysis of the Italian situation during these years, has demonstrated how Countries specific legislations could affect the results and outcomes of these procedures, and how the application of a restrictive law in the field of human reproduction has contributed to offer to the patients a suboptimal kind of treatment

Trial registration number: not applicable.

P-759 A randomised controlled trial comparing the Cost-effectiveness of blastocyst (Day 5/6) versus cleavage stage (Day 3) embryo transfers in IVF-ICSI Cycles in developing countries

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Study question: To determine whether blastocyst stage (day 5 / 6) embryo transfer is cost-effectiveness in improving the pregnancy rate, and other outcomes, compared with cleavage stage (day 3) embryo transfer?

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Summary answer: Cleavage stage-day 3 transfers with top quality embryos, are more cost-effective and do not compromise the pregnancy outcome, compared with day 5/6 Transfers

What is known already: There are only a few studies comparing the cost-effectiveness of Day 2/3 Vs 5/6 embryo transfer outcomes.

Embryos from IVF-ICSI are commonly transferred into a woman's uterus at either the cleavage stage (day 2 / 3) or blastocyst stage (day 5 / 6).

Until recently, most ART cycles transferred embryos at cleavage stage, however there has been a trend to transferring embryos at the blastocyst stage as this timing is about the same as natural cycle embryos moving into the uterus.

Advances in cell culture media have led to a shift in IVF-ICSI from cleavage stage to the costly blastocyst transfer.

Study design, size, duration: The rationale for blastocyst transfer is to improve uterine and embryonic synchronicity.

As we practice in a developing country, it has become necessary to perform more economical Day 3 transfer in order to reduce the cost of extended Day 5/6-blastocyst culture.

Day 3 transfers were assessed to determine if the outcome was comparable with that obtained following Day 5/6 blastocyst transfer.

This RCT is conducted at our centre between 2007 and 2016 for patients below 40 years.

Participants/materials, setting, methods: If more than 06 oocytes were retrieved and three top quality embryos (6-8 blastomeres & less than 20% fragmentation without multinucleation) were observed at day 2,couples were included in the study.

Total 438 patients were assigned to Day-3 or Day-5/6 transfer by computer generated randomised list after proper consent.

Primary outcomes: pregnancy-rates and cost of cycle

Secondary outcome: multiple pregnancy, miscarriage rates, cumulative pregnancy rates, failure to transfer embryos, and embryo freezing

Main results and the role of chance: Pregnancy rates were 31% for Day- 3 Cleavage stage transfers and 34% for Day- 5/6 Blastocyst transfers, the difference being not statistically significant.

Failure to transfer any embryos was higher in the Day-5/6 blastocyst transfer group and more embryos were available for freezing in the Cleavage stage transfer group.

Although more Abortions and Multiple pregnancies were seen in the Cleavage stage Day-3 transfer as compared to the Blastocyst Day-5/6 transfer, the difference is not statistically significant.

Our data show clearly that for IVF-ICSI for women under 40 years at day -2 with three top quality embryos, the pregnancy outcome are similar after embryo transfer at cleavage stage (Day-3) or blastocyst stage (Day 5/6).

These preliminary results have shown that Day -3 Cleavage stage transfers with top quality embryos having the highest implantation potential, do not compromise pregnancy outcome as compared to the costlier Day -5/6 blastocyst transfers.

Limitations, reasons for caution: Thus, although there is no benefit favouring blastocyst transfer it remains unclear whether the day of transfer impacts on cumulative pregnancy rates.

Future more studies should report cumulative live birth rates to enable couples to make decisions on the best cost-effectiveness treatment option available in poor & developing countries.

Wider implications of the findings: Our results have shown that day 3 transfers do not compromise pregnancy outcome compared to day 5/6.

Day 3 transfer has also provided a unique opportunity to study day 3 morphology critically and avoid costly extended blastocyst culture, therefore be a feasible low-cost alternative to expensive day 5/6 blastocyst culture.

Trial registration number: NONE.

P-760 A prospective randomised contolled study comparing a low-cost antagonist-protocol using oral ovulation inducing agents in ivf-icsi-cycles with a standard agonist long protocol in developing country

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Study question: To compare the cost-effectiveness of a low cost antagonist protocol using oral ovulation induction agents (Clomiphine Citrate or Aromatase Inhibitors) with a standard long GnRH-Agonist-Protocol in IVF-ICSI -Cycles in developing-countries?

Summary answer: GnRH-Antagonist in combination with oral-ovulation-inducing-drugs offers the advantage of an economical method of stimulation in IVF-ICSI-Cycles, as compared to the agonist-protocol, with a similar pregnancy-rate.

What is known already: Infertility affects 10 - 20% of couples trying to conceive in whom IVF-ICSI has improved chances of achieving pregnancy. In standard IVF method with controlled-ovarian-hyperstimulation (COH), development of multiple follicles is stimulated by using gonadotrophins, combined with a gonadotrophin-releasing hormone (GnRH) - Agonist or Antagonist. It is expensive with many adverse effects.

The objective of this prospective randomised controlled study is to compare the cost-effectiveness of a low-cost antagonist protocol using oral ovulation induction agents (Clomiphine Citrate or Aromatase Inhibitors) with a standard long GnRH agonist protocol in IVF-ICSI cycles in developing countries, where IVF is self financed.

Study design, size, duration: This prospective randomised controlled study is to compare the cost-effectiveness of a low cost antagonist protocol using oral ovulation induction agents (Clomiphine Citrate) with a standard long GnRH agonist protocol in IVF-ICSI cycles.

The patients in the study underwent a long GnRH-Agonist or an Antagonist protocol with randomisation using a computer generated list, after proper consent.

A total of 378 IVF-ICSI cycles were prospectivly studied in patients below 40 years of age.

Participants/materials, setting, methods: A total of 378 IVF-ICSI patients below 40 years of age (2010-2016) were randomised into two groups using a computer generated list.

The agonist-group underwent the standard-long-GnRH-analogue-protocol.

The antagonist-group received oral-ovulation-inducing drugs (Clomiphene Citrate / Aromatase-Inhibitor) for the first five days of cycle followed by gonadotropins and 0.25 mg antagonist (Cetrorelix) injection daily till the day of hCG, using a flexible antagonist start approach.

Primary-outcome: Pregnancy-Rate and Cycle-Cost

Secondary-outcome: Gonadotropins-used, number of Mature-Oocytes and Embryos

Main results and the role of chance: There was a significant difference in the gonadotropin usage between the two groups.

As a result, the cost of the oral ovulation inducing drugs (Clomiphene Citrate / Aromatase- Inhibitor) - gonadotropin - antagonist cycle was significantly lower than the standard long GnRH agonist - gonadotropin protocol. (600 - 800 USD Vs 1000 - 1500 USD)

Though the mean number of Mature -Oocytes retrieved was higher in the long protocol group (M2: 6. 1 Vs 11. 6),

yet the clinical pregnancy rate per transfer was similar in both groups (31 % Vs 34 %)

The oral-ovulogen-antagonist cycles were more cost-effective and more patient friendly.

The group of patients who were treated with oral-ovulogen-gonadotropins-antagonist, had lesser injections, fewer visits, and significantly reduced cycle cost with comparable pregnancy rates.

Therefore, it is advisable to use this less expensive option of oral ovulation inducing agents with flexible antagonist protocol for IVF-ICSI in developing countries.

Limitations, reasons for caution: In ART, different stimulation protocols are used, including natural cycle IVF, modified natural cycle IVF, and standard IVF with controlled ovarian hyperstimulation(COH).

Our study is to assess the cost-effectiveness of the oral-ovulation-inducing-drugs(Clomiphene-Citrate / Aromatase- Inhibitor) -Antagonist-cycle compared with standard -long-GnRH-Agonist-protocol and similar multicentric RCTs are required

Wider implications of the findings: Usage of GnRH antagonist in combination with oral-ovulation-inducing-drugs offers the advantage of an economical

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method of stimulation in IVF-ICSI-cycles, as compared to the standard long-agonist protocol, with a similar pregnancy rate in poor and developing countries where ART is self-financed and hence cost is a major factor to be considered.

Trial registration number: NONE.

P-761 The hurdles to parenthood: Exploring Chinese lesbians' opinions towards the use of assisted reproductive technologies

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Study question: What are the prospect of childbearing and perceived difficulties in achieving parenthood through the use of assisted reproductive technologies (ART) among Chinese lesbians?

Summary answer: Participants expressed hesitation in childbearing due to concerns over the lack of genetic connection of the child with both mothers, inter-generational discrimination, and financial burden.

What is known already: A growing body of literature has emerged in the past two decades concerning the process of decision-making and transitioning to parenthood among lesbian couples in the West. Despite the fact that parenthood is generally considered an essential life goal and fulfilment of filial piety in Chinese societies, Chinese lesbians' aspirations for reproduction and family formation have rarely been paid attention in a predominantly heterosexist environment where access to ART is restricted to heterosexual couples and same-sex marriage is not legally recognized.

Study design, size, duration: Semi-structured interviews were conducted with 12 lesbian-identified Chinese women who aged between 18 and 35 and resided in Hong Kong in 2015. Participants were recruited through local lesbian, gay, bisexual, and transgender (LGBT) organizations, a university in Hong Kong, and personal networks.

Participants/materials, setting, methods: A list of open-ended questions was used to guide the discussion and allow respondents to articulate their attitudes towards the use of ART, childbearing, and family formation. Each interview lasted around 90 minutes and was audio-recorded and transcribed. Data was analyzed with the assistance of NVivo which is a qualitative data analysis software. Based on Grounded theory, coding (namely open coding, axial coding and selective coding) and categorizing were the major techniques employed.

Main results and the role of chance: All lesbian participants in this study expressed hesitation in childbearing. Some participants claimed that the use of ART was not worth the effort due to the lack of genetic connection of the child with both mothers. The internalized stigma also urged some of them to worry that their children born as a result of ART would face discrimination due to their non-normative family formation and structure. All participants expressed the need to accumulate financial resources so that they might have the capital not to worry about the negative costs of coming out or/and to emigrate to Western countries where same-sex marriage was legally recognized or/and ART was provided. Traditional Chinese belief about family plays a key role in our participants' attitudes towards ART and fertility plans. Most of them felt reluctant to disclose their sexual orientation particularly to their parents since being homosexual would be considered a shame to the family of origin. Lesbian parenthood would mean that they had to come out to family, relatives, and even the public.

Limitations, reasons for caution: With a small sample size, this qualitative research cannot be considered representative of the Chinese lesbian population. Meanwhile, there has been neither census data identifying lesbian individuals nor research based on a random sample of this subgroup. Further quantitative and qualitative studies are needed to understand Chinese lesbians' reproductive health.

Wider implications of the findings: The nature of childlessness among Chinese lesbians is not only a medical issue but also a psychosocial issue which is linked with gender and sexual norms. This research sensitizes healthcare professionals to the alternative needs, wants, and psychosocial burden of Chinese lesbians in relation to fertility plan.

Trial registration number: N/A

POSTER VIEWING SESSION
REPRODUCTIVE SURGERY

P-762 Pain relief during oocyte retrieval by transcutaneous electrical acupoint stimulation: a single blind randomized controlled multi-centered trial

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Study question: To study the effect of transcutaneous electrical acupoint stimulation (TEAS) of pain-relieving in women undergoing transvaginal ultrasound-guided oocyte aspiration.

Summary answer: Oocyte retrieval causes pain and discomfort. TEAS suppressed the pain and also alleviated nausea and vomiting associated with the operation.

What is known already: Transvaginal ultrasound-guided oocyte aspiration is a standard procedure in the process of in vitro fertilization and embryo transfer (IVF-ET). In many clinics, conventional medical analgesia (CMA) during OPU includes sedative pre-medication with benzodiazepine, local analgesics administered as a paracervical block (PCB) and intravenous administration of fast-acting opiates during OPU. But CMA is known to produce a number of side effects such as nausea, tiredness and confusion.Acupuncture has demonstrated its pain-relieving effect both intra-operatively and post-operatively. Transcutaneous electric acupoint stimulation (TEAS), increase the reproducibility of this acupuncture-like technique and to reduce invasiveness, an user-friendly technique, instead of using manual needling or electro-acupuncture.

Study design, size, duration: Participants were20-45ages individually randomly assigned (in a 1:1 ratio) to receive either TEAS or Mock TEAS. Specifically, randomization numbers were produced by SAS 9.13 software and were put in sealed random envelopes.Patients were divided into two groups according to the order in which the subjects were enrolled in the group. Mock TEAS (group I, n = 194) and TEAS (group II, n = 196). The study was completed between May 2013 and May 2015.

Participants/materials, setting, methods: A total of 390 women undergoing oocyte retrieval,was completed at four reproductive centers: Peking University People's Hospital; Peking University Third Hospital; Beijing Obstetrics and Gynecology Hospital; Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine.The levels of pain, nausea and vomiting associated with oocyte retrieval were evaluated immediately and one hour after operation using visual analog scale (VAS)Serum concentrations of β-endorphin were measured by commercially available original auxiliary reagent for humanβendorphin ELISA Kit.

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Main results and the role of chance: In 390 subjects, pain levels evaluated with VAS instantly (18.63 versus 24.39 P < 0.01) and 1 hour after oocyte aspiration (4.64 versus 6.77, P < 0.05) were both lower in TEAS group than mock TEAS group. Nausea assessment revealed a significant decrease instantly (2.9 ± 0.66 versus 1.24 ± 0.40, P = 0.033) but not 1 hour (0.10 ± 0.1 versus 0.21 ± 0.16, P = 0.59) after oocyte aspiration. Furthermore, serum β-endorphin level was higher in TEAS group after the oocyte retrieving procedure than mock TEAS group (11.41 versus 9. 14, P < 0.001).The present study substantiated the efficacy of 2/100 Hz TEAS in ameliorating the pain induced by ovum pick up(OPU), without paying the intention of comparing the efficacy between fixed frequency and mixed frequency.Several underlying mechanisms have been proposed for the EA/TEAS-induced reduction of postoperative nausea and vomiting.TEAS seems to be a safe and effective method to reduce pain, nausea and vomiting associated with OPU.

Limitations, reasons for caution: Considering the characters of TEAS procedure, the operator of TEAS stimulation is not blinded, only the patients and doctors to evaluate the degree of pain, nausea and vomiting were blinded.

Wider implications of the findings: TEAS seems to be a safe and effective method to reduce pain, nausea and vomiting associated with OPU.we hope It can be widely applied to around the world.

Trial registration number: The project was registered at Chinese Clinical Trial Registry, a World Health Organization International Clinical Trial Registration Platform (http://www.chictr.org.cn; Registration No. ChiCTR-TRC-13003952).

P-763 Final report of clinical efficacy of modified adenomyomectomy in infertile women with adenomyosis

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Study question: Does reduction surgery of adenomyosis have clinical efficacy in infertile women?

Summary answer: Modified adenomyomectomy as a uterus-sparing surgery could be an effective method for increasing pregnancy rate and conservation of fertility potential in infertile women with adenomyosis.

What is known already: The relation between infertility and uterine adenomyosis is controversial, but it appears to affect endometrial receptivity and increase abortion rate. In infertility where uterine conservation is paramount, the treatment of adenomyosis is often complicated, that is to say, medical treatment is often transient and hysterectomy for eradication could not preserve their fertility. At this stage, there is no agreement on the most appropriate therapeutic methods on fertility outcome in infertility patients with adenomyosis. Regarding surgical removal of adenomyosis, including laparoscopic reduction, uterus-sparing surgery appears to be satisfactory and reduced the need for hysterectomy, but needs well designed prospective study.

Study design, size, duration: Prospective clinical trial was conducted. The subjects consisted of 50 infertile patients with adenomyosis and were enrolled after the failure of In Vitro Fertilization (IVF) for pregnancy from December 2007 to September 2016.

Participants/materials, setting, methods: All cases were classified as having unexplained infertility, adenomyosis with severe periodic dysmenorrhea and occasional menorrhagia. This newly designed operative procedure included pediatric foley insertion into the uterine cavity, injection of diluted vasopressin along the uterine incision, T- or transverse H-incision on the adenomyotic wall, careful excision of adenomyosis tissue using argon laser under intra-operative ultrasonography. After debulking surgery, patients underwent follow up examination for symptom relief, reduction of adenomyosis by MRI and pregnancy rate.

Main results and the role of chance: The mean age and the duration of infertility were 35.60 ± 3.37 years and 55.48 ± 48.24 months, respectively. The mean volume of excised specimens of adenomyosis was 94.15 ± 56.63 g. The relief of dysmenorrhea was observed clearly in all patients at 6 months after operation (NRS; 7.28 ± 2.29 vs. 1.56 ± 1.29, $p < 0.001$). The amount of menstrual blood was also significantly decreased (140.44 ± 91.68 vs. 66.33 ± 65.85, $p = 0.009$). The CA 125 level was significantly decreased at the time of 6 months after operation (187.75 ± 229.52 vs. 20.36 ± 19.19, $p = 0.026$). Postoperational complication occurred in four patients (subfascial hematoma, ureter fistula, shrinkage of uterus and premature ovarian insufficiency). Five patients were lost in the follow-up. Of 33 patients who attempted pregnancy, 18 patients conceived by natural or IVF or thawing ET after the operation (18 of 33; 54.5%). However, miscarriage occurred in five patients, ectopic pregnancy in three patient, preterm delivery in two patient and eight patients (8 of 33; 24.2%) delivered by cesarean section at term. The rest of the patients have been trying to conceive by IVF-ET or natural course.

Limitations, reasons for caution: The sample size is small, so further study with larger number of patients will be helpful to investigate the possibility of this result.

Wider implications of the findings: This modified adenomyomectomy was related to symptom relief of dysmenorrhea, menorrhagia and increasing pregnancy rate, implying that reduction surgery could be considered as a successful method for infertile women with adenomyosis who need fertility preservation. This is the first report on the clinical pregnancy outcome of uterus-sparing surgery in adenomyosis.

Trial registration number: N/A.

P-764 Combination treatment of preoperative embryo cryopreservation and endoscopic surgery (surgery- assisted reproductive technology hybrid therapy) in infertile women of late reproductive age with uterine tumour

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Study question: What are the factors of successful combination treatment of preoperative embryo cryopreservation and endoscopic surgery in infertile women of late reproductive age with uterine tumours?

Summary answer: Sufficient preoperative frozen embryos are needed for a successful 'surgery-assisted reproductive technology (ART) hybrid therapy'.

What is known already: Endoscopic surgery is gold standard for infertile women with uterine tumours, but a waiting time for preoperative GnRH agonist administration and postoperative contraception period is required. Ageing is the most important factor in infertility treatment due to a decrease in the quality and number of eggs and an elevated miscarriage rate. It is debatable whether infertility treatment or surgery should be the first choice for women of late reproductive age with uterine tumours. In 2009, we first reported successful cases of a combination of ART for embryo cryopreservation and surgery (surgery-ART hybrid therapy) in infertile advance-aged women with multiple myomas.

Study design, size, duration: This study was approved by the Local Ethics Committee. Women of late reproductive age with uterine myomas underwent ART for embryo cryopreservation preoperatively and reproductive surgery from 2014 to 2015. One warmed embryo was transferred into the uterus after the postoperative contraceptive interval.

Participants/materials, setting, methods: A total of 18 women underwent surgery-ART hybrid therapy in Juntendo University Hospital. One woman acquired no embryo and gave up conceiving. Out of 17 women, 4 were in postoperative contraceptive period and 13 underwent embryo transfer. Three women delivered babies and 3 women had ongoing pregnancy. Seven women had implantation failure or miscarriage. A comparison was made between 6 women who conceived babies (success group) and 7 women of hybrid therapy failure (failure group).

Main results and the role of chance: The women in the success and failure groups were 40.0 ± 1.4 and 42.0 ± 1.1 years (p = 0.026) of age and 3.3 ± 4.5 and 5.3 ± 5.9 years of the duration of infertility (NS), and had 2.2 ± 1.9 ng/ml

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and 1.6 ± 1.7 ng/ml of serum AMH levels (NS), respectively. The numbers of preoperative frozen embryos were 4.8 ± 1.3 in the success group and 2.0 ± 0.9 in the failure group (p<0.001). The success and failure groups had 9.5 ± 8.5 and 9.7 ± 12.4 uterine myomas (NS) with a diameter of 5.8 ± 3.3 and 7.2 ± 2.9 cm (NS), respectively. The 6 women who had a successful surgery-ART hybrid therapy were significantly younger and had a larger number of cryopreserved embryos than the 7 women who had hybrid therapy failure.

Limitations, reasons for caution: The limitation of this study is that it is a retrospective study.

Wider implications of the findings: Surgery-ART hybrid therapy should be considered as one of the infertility treatments for women of late reproductive age or diminished ovarian reserve with uterine tumours as well as ovarian endometrioma.

Trial registration number: None.

P-765 Laparascopic strassman metroplasty for bicornuate uterus

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Study question: is laparascopic metroplasty feasible for unification of bicornuate uterous

Summary answer: laparascopic strassman metroplasty is a good alternative for bicournuate uterous

What is known already: Laparoscopic strassman metroplasty is feasible, it depends on sugeons skill of suturing, second look laparoscopy hysteroscopy is mandatory to evaluate strength of suture line

Study design, size, duration: Laparoscopic Strassman metroplasty were done in five patients with bicornuate uterus with history of at least two second trimester abortion with triple puncture technique, after transverse fundal hysteratomy incision including fundal cleft, unification of uterus was done with intracorporeal sutures in two layers, after three months of sequential hormone therapy HSG repeated,

Three patients got pregnant, delivered at 37 – 3 8 wks of gestation, there was no defect or dehesence in incision line

Participants/materials, setting, methods: Case report, five cases were operated within two years duration, three patients conceived, two patients lost their follow up,they received hormone replacement therapy for three months post operatively,then second look laparoscopy and hysteroscopy done,then they were allowed for conception,three patient got pregnant and cesarian section done on thirty eight weeks of gestation.there was no adhesion at suture line

Main results and the role of chance: normal full term pregnancy outcome with no definite adhesion formation as seen in conventional laparatomy method of strassman metroplasty

Limitations, reasons for caution: normal full term pregnancy outcome with no definite adhesion formation as seen in conventional laparatomy method of strassman metroplasty

Wider implications of the findings: This issue is not related to my presentation and cases

There in not enough literiture, this surgery has not been reported laparoscopicaly from USA or Canada yet, there is one report from India by Sinha

in video clip this technique for llaparascopic strassman metroplasty will be displayed

Trial registration number: 1967.

P-766 Differentiating tissues and organs in endoscopic images using a convolutional neural network

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Study question: Is it possible to identify different tissues and organs during endoscopy supported by a software.

Summary answer: During the learning curve of laparoscopy trainees can get help in differentiating tissues by an automated digital image processing based decision support system

What is known already: Endoscopy surgery is the part of everyday work of surgeon. In an experienced hand provides less postoperative morbidity, complications and the time periods of hospital stay and returning to normal activity, so it has a lot of advantages compared to the laparotomy.Opposing to open-surgery, the recognition of organs is rather difficult in laparoscopy because of the lack of the tactile information. Knowing detailed anatomy, having experience letting us to recognize structures in the abdominal cavity. Because of the anatomical variations, tissue identification is not always sure relying on the visual information.

Study design, size, duration: Our dataset has been collected retrospectively during 35 different gynecological endoscopic operations at the Department of Obstetrics and Gynecology of the University of Debrecen. The videos have been recorded by a high definition 1-MOS endoscopic camera at 30 frames/sec rate and resolution of 1920×1080 pixels.

Participants/materials, setting, methods: Data of patients scheduled for gynecological endoscopic operations are analyzed. The medical expert or an assistant should manually mark the region of interest. Then, the maximum number of sub-images of size 224×224 pixels are cut off along the axis from the video frame. Finally, the classification problem is solved automatically using a convolutional neural network with the resulted labels are pinned on the corresponding organs in the video frame.

Main results and the role of chance: We have presented an approach to develop an application, which helps medical experts with performing endoscopic surgeries. Our effort primarily addressed the drawback of losing tactile information during key-hole surgery in the recognition of different organs. To address this problem, we have developed a semi-automatic tool, which requires a manual annotation regarding the axis of the interested organs first. Then, several sub-images covering the selected organs are extracted and classified by a fine-tuned GoogLeNet convolutional neural network.The classification performance of the fine-tuned GoogLeNet model on our test dataset considering the top-1 error rate is 0.193 at sub-image level. That is, 403 out of the 500 test images have been classified correctly. However, notice that these sub-images are only small, non-overlapping segments of the interested organs. That is, it is reasonable to fuse these label information for recognizing the corresponding organ. To do so, we have applied the simple majority-voting rule on the 4-5 labels supplied by the sub-images for the same organ. In this way, our proposed approach has reached 94.2% final accuracy regarding this binary classification task.

Limitations, reasons for caution: Our collected dataset is relatively small with containing insufficient number of images to train a complex neural network, so we should extend the size of our dataset.

Wider implications of the findings: Using the software made by the results of the study, accuracy of the tissue/organ recognition could be increased during training laparoscopic technique, or for the experts as well.

Trial registration number:

P-767 Neck scarf of ureter and bulldog of the uterine vessel in Da Vinci Robotic deep infiltrative endometriosis excision

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Study question: How to improve the safety of the ureters during Deep infiltrative endometriosis which is one of the most challenging gynecologic operation?

Summary answer: We could always check the ureters safety during difficult dissection with the help of

neck scarf on the ureters

What is known already: Deep infiltrative endometriosis is one of the most challenging gynecologic operation due to the distorted anatomy by the fibrosis of endometriosis gland.

The major surgical procedure including the following

(1) The bilateral ovarian chocolate cyst decompression and then suspension with a suture to abdominal wall as external traction.

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(2) The ureterolysis by trace the ureter course from pelvic brim to crossing with uterine vessels
(3) Dissection of the origin of uterine vessels from the origin of the internal iliac artery and block it with bulldog temporally
(4) 4.Develop the pararectal space, and expose the Cul-de-sac by using the vaginal and rectal probe

Study design, size, duration: Here we present our technique by using the yellow rubber band to collar the ureter as neck- scarf, when using Da Vinci Si robot system with four arms (included robotic laparoscopy), the stable third arm as an assistant of the surgeon to traction the neck scarf to prevent ureter injury when dissection.

Participants/materials, setting, methods: 20 consecutive patients with deep infiltrative endometriosis were safely operated without fistula, ureter injury, laceration.

Main results and the role of chance: We could always check the ureter safety during difficult dissection, especially during the long surgery the surgeon was fatigue and had less concentration while using the energy source.

Limitations, reasons for caution: technical limitation for those who are not very familiar with the anatomy of the ureters course and the uterine vessels crossing with ureters

Wider implications of the findings: it is also feasible to use the technique in laparoscopic hysterectomy for uterine myoma or adenomyosis

Trial registration number: no.

P-768 Outcomes of laparoscopic peritoneal vaginoplasty operation in patients with mullerian agenesis

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Study question: To assess the postoperative outcomes and complications of peritoneal vaginoplasty operation.

Summary answer: Laparoscopic peritoneal vaginoplasty is a good alternative beyond the other techniques.

What is known already: RKMH is a very rare syndrome with mullerian agenesis. Its incidence is 1 in 5.000-10.000 women. The major complaints of these patients are infertility and sexual inability. There are numerous surgical and non-surgical approaches for vaginal reconstruction for better satisfactory results. But there is no consensus whether on which approach is the best.

Study design, size, duration: Retrospective cohort study, consisting 25 women with Rokitansky-Kustner-Hauser-Mayer (RKMH) syndrome between the years 2010-2016

Participants/materials, setting, methods: Women with a surgical history of RKMH syndrome were detected when the operation database of the years 2010-2016 were screened. Patients' age, diagnose time, operation type, peri-operative and postoperative complications, postoperative vaginal depth, and sexual comfort of patients were analyzed.

Main results and the role of chance: Total of 25 patients with RKMH were detected. Mean age at diagnose was 27,6 years (+/- 5.1). All the patients successfully underwent laparoscopic peritoneal vaginoplasty by the three surgeons (GU, KO, BA) There were two complications.

The first one was bladder injury during peritoneal dissection and it was repaired laparoscopically. The second one was postoperative complication. It was rectovaginal fistula. The patient admitted with vaginal defecation after three months of surgery and general surgeons operated her. After one year of surgery she successfully recovered. One patient did not apply appropriate mold exercises and than vagina collapsed. All the patients scored their sexual comfort from 0 to 10 points. The mean score result was 8.17 (+/- 1.9).

Limitations, reasons for caution: Retrospective study desgin, Small sample size.

Wider implications of the findings: Laparoscopic peritoneal vaginoplasty is a good alternative beyond the other techniques. This technique has satisfactory functional outcomes and lower complication rates and could be safely applied to patients with mullerian agenesis.

Trial registration number: not applicable.

P-769 An acute presentation of a Müllerian duct abnormality

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Study question: Case report describing acute on chronic pelvic pain due to a Müllerian duct abnormality.

Summary answer: A rudimentary right uterine horn and absent right kidney and ureter were noted on MRI and removed laparoscopically, resolving the patients abdominal pain.

What is known already: Congenital malformations of the female reproductive tract are common miscellaneous deviations from normal anatomy with a range of reproductive outcomes. Gonadal differentiation occurs between the 6th and 8th week of gestation, a lack of SRY protein results in the pluripotenial gonads developing into ovaries. This results in Wolffaian duct degeneration and persistence of the Müllerian ducts. Following organogenesis the Müllerian ducts fuse to form the fallopian tubes, uterus, cervix and upper two thirds of the vagina. Should the two ducts fail to fuse correctly a range of uterine abnormalities can occur. One such abnormality is a rudimentary uterine horn.

Study design, size, duration: Patient was admitted under the general surgeons with abdominal pain and at laparoscopy referred to Gynecology after observing a dilated right fallopian tube.

Participants/materials, setting, methods: Case report – patient consent obtained.

Main results and the role of chance: 23 year-old nulliparous female presented with menorrhagia and pelvic pain localized to the right iliac fossa for 3 months duration. She was admitted under the surgeons with raised inflammatory markers, appendicitis, urinary tract infection and ovarian cyst rupture or tortiontorsion was suspected. Pelvic and abdominal ultrasound identified an inflammatory, possibly bowel related, mass surrounding the right ovary. At diagnostic laparoscopy hemorrhagic fluid was seen in the pelvis and paracolic gutter; a normal appendix; right hydrosalpinx and a small uterus were identified. A Gynecologicical opinion was sought and a diagnosis of pelvic inflammatory disease and right hydrosalpinx was made. At gynecology follow-up, a repeat transvaginal ultrasound demonstrated a mass (25 × 25 mm) superior to the right ovary adjacent to the right iliac vein and artery. It was oval in shape with increased echogenicity. A uterine abnormality was suspected and an MRI pelvis requested. MRI confirmed a rudimentary right uterine horn which haematometra, right hydrosalpinx and absent right kidney. The rudimentary horn was removed and right salpingectomy undertaken laparoscopically which resulted in complete resolution of her pelvic pain. At laparoscopy minimal endometriosis was noted which was ablated. Series have indicated around 3% of women with congenital uterine abnormalities have endometriosis.

Limitations, reasons for caution: Not applicable.

Wider implications of the findings: Using ESHRE/ESGE consensus on classification of female genital tract abnormalities this case is classified as Class IV, subsection A abnormality – rudimentary horn with cavity.

- Anatomy is the foundation for systematic classification of uterine anomalies.
- Variations deriving from the same embryological origin form the basis of the classes.

Trial registration number: Not applicable.

POSTER VIEWING SESSION
SAFETY AND QUALITY OF ART THERAPIES

P-770 Risk evaluation for pronuclear transfer using for preventing the transmisson of mitochondrial disease: a mouse model

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Study question: Is it safe to prevent transmission of mitochondrial disease using pronuclear transfer as a mitochondrial replacement technology?

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Summary answer: There is a potential risk associated with metabolism in pronuclear transfer offspring

What is known already: Pathogenic mitochondria DNA (mtDNA) mutation is closely linked with many mitochondria diseases that usually lead to fatal results such as premature death. MtDNA is transmitted maternally within oocyte cytoplasm. Management of mitochondrial disease is limited due to its characteristic inheritance. Pronuclear transfer is considered to be effective in preventing the transmission of pathogenic mtDNA from mother to offspring.

Study design, size, duration: Pronuclear transfer technology were performed between zygotes of CD-1 mice to obtain constructed zygotes and offspring. We preformed 263 zygotes to assess embyoes development and 40 PNT offsprings to evaluate risk of PNT procedure.

Participants/materials, setting, methods: The constructed zygotes produced by PNT was fused by electrofusion. By testing mitochondria DNA copy number, the ratio of donor mitochondria was calculated. The cell number of balstocyst was counted and gene expression profiling was identified by NGS technique. Then PNT blastocyst were transferd to pesodu-pregnant mice. For PNT offspring, the body weight, ratio of fat/weight and energy expenditure were measured. The reproductive function was also assessed both in female and male PNT offspring.

Main results and the role of chance: The development efficiency was significantly decreased in PNT embryos compared with normal control embryos. Compared with normal fertilized embryos, total cell numbers of the blastocysts from PNT technology were decreased significantly, and a large amount of genes showed the abnormal expression levels. But there were no significant differences between neonatal body weight and plancenta weight. the ratio of donor mitochondria was no more than five percent. However, the quality of PNT blastocysts was declined. Compared with control blastocysts, implantation rate and birthrate in PNT blastocysts were all impaired. For PNT offspring, compared to the controls, the body weight, ratio of fat/weight, energy expenditure, blood pressure and glucose tolerance showed no difference both in female and male pronuclear transfer mice. Estrous cycle, histogical analysis of ovary and fertility were normal in female pronuclear transfer mice, so were the testicular morphology, sperm concentration and fertility in male pronuclear transfer mice. However, a considerable of genes were differentially expressed between pronuclear mice and control group.In tissues where mebabolism were more vigorous like brain, heart, liver and muscle, an abundant of genes showed the abnormal expression levels. Differentially expressed genes were highly related in metabolism, which suggest a potential risk of pronuclear transfer offspring.

Limitations, reasons for caution: The ratio of donor mitochondria was not assessed in all tissues of PNT offspring during the whole life cycle. In addition, the F2 mice of PNT mice were not evaluated throughout the whole life.

Wider implications of the findings: The result not only emphasize the potential risk associated with metabolism of PNT procedure using to prevent transmission of mitochondrial disease but also facilitate a better understand of the effects of PNT procedure on development of offspring and enhancing the awareness of improving the outcome of PNT procedure.

Trial registration number: not clinical trial.

P-771 Monozygotic twinning following assisted reproduction: a six-year experience base on a large cohort of pregnancy

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Study question: To characterize the incidence and risk factors for monozygotic twinning (MZT) after assisted reproductive technologies

Summary answer: There is a pretty higher prevalence rate of monochorionic-diamniotic(MC-DA) MZT after ART. Both maternal age and blastocyst transfer are risk factors of monozygotic pregnancy independently.

What is known already: MZT pregnancies are associated with an increased risk of both fetal and maternal complications. Although an elevated rate of MZT after ART has been previously reported, but the risk factors of the high incidence in the ART procedures are full of much controversy.

Study design, size, duration: All clinical pregnancies after embryo transfer carried out in our center between 2011 and 2016 (n = 8860) were retrospectively analyzed for the incidence of MZT. The effect of different clinical data and laboratory procedures on the incidence of MZT was evaluated.

Participants/materials, setting, methods: The following ART risk factors were assessed: maternal age, type of ET (fresh versus frozen), ICSI, embryo stage at time of ET (cleavage or blastocyst)

Main results and the role of chance: The overall MZT rate was 2.55% (226/8860). Eighty one MZTs occurred in the fresh cycles and 145 MZTs followed in the frozen cycles (2.67% vs. 2.49%). MZT incidence did not differ significantly whether or not ICSI was performed (2.79% vs. 2.44%). The MZT rate that resulted from single embryo transfer (SET) cycles (1.99%) was slightly lower than multiple embryo transfer cycles (2.61%),but with non-significance. However, women <35 years displayed a higher rate (2.81%) than women ≥35 years old (1.16%). Blastocyst transfer was associated with a significantly increase in MZT incidence than cleavage-stage embryos transfer (2.79% VS 2.02%, P = 0.008). In the logistic regression analysis of subgroups, blastocyst transfer is a major risk factor of MZT in the fresh cycles (P = 0.044), while maternal age plays a more important role in the frozen cycles (P = 0.004).

Limitations, reasons for caution: This study is limited by its retrospective nature. Many parameters could not be assessed as the relatively uncommon occurrence of MZT during ART procedures.

Wider implications of the findings: We should pay more attention to the potential risk of maternal age and blastocyst transfer to increase MZT. More studies are needed to reveal the exact mechanism.

Trial registration number: The study was approved by the Ethical Committee of Anhui Medical University.(ECAMU No 2008035).

P-772 Congenital malformations in offspring of women after IVF treatment with or without a previous maternal malignancy

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Study question: Is there an increased risk for congenital malformations after a maternal history of malignancy and use of IVF?

Summary answer: No increased risk was seen after only a history of maternal malignancy. An increased risk was seen after use of IVF and IVF+ maternal malignancy.

What is known already: Survival after malignancy has increased and the question of risks, including risk for congenital malformations for the offspring of these women has become important. Data on congenital malformations in such offspring are limited.

Study design, size, duration: We compared congenital malformations in offspring, born in Sweden 1994-2011 to women with a history of malignancy (at least one year before delivery) with all other offspring. The study comprises 1 773 926 live born infants, among them 71 954 were identified with malformation diagnoses (4.1%), among which 47 081 were relatively severe (2.7% of total material).

Participants/materials, setting, methods: Data were obtained by linkage between five Swedish national health registers. Adjustment for confounders was mainly made by Mantel-Haenszel methodology.

Main results and the role of chance: We identified 71 954 (4.1%) infants with congenital malformation, of which 47 081 (2.7%) were relatively severe (roughly corresponding to major malformation). Among 7284 infants to women with a history of malignancy 204 relatively severe malformations were found (2.8%, OR = 1.04, 95% CI 0.91-1.20). After IVF (In vitro Fertilization), the risk of a relatively severe malformation was significantly increased in women without a history of malignancy (OR =1.31, 95% CI 1.24-1.38) and still more in women with such a history (RR = 1.85, 95% CI 1.08-2.97). However,

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there were no significant differences neither, for any malformations (OR 1.04, 95% CI 0.92-1.16) nor for relatively severe malformations (OR 1.04, 95% CI 0.91-1.20), when comparing offspring only after maternal history of malignancy.

Limitations, reasons for caution: One limitation is that fetuses with prenatally identified malformations that were selectively aborted are not included. Such events are reported to the Swedish Birth Defect Register but (in accordance with Swedish law) without maternal identification which makes linkage with the cancer register impossible.

Wider implications of the findings: No general increase in malformation rate was found in infants born to women with a history of malignancy. A previously known increased risk after IVF was verified and it is possible that this risk is further augmented among infants born of women with a history of malignancy.

Trial registration number: Not applicable.

P-773 Is a double-embryo transfer detrimental to a patient's chances of pregnancy?

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Study question: How do pregnancy and birth outcomes differ between single (SET)- and double-embryo transfers (DET) when considering blastocyst quality?

Summary answer: A high-quality embryo in a single transfer has better outcomes than in a double-embryo transfer with another embryo of any quality.

What is known already: The transfer of multiple embryos in in-vitro fertilisation (IVF) was originally utilized to maximise rates of implantation and pregnancy; however, its use has subsequently led to a high risk of multiple pregnancy and its associated complications. It is also accepted that SET results in healthier babies. Although SET is typically preferred, a variety of factors may influence the decision to concurrently transfer a second embryo. The quality of embryos is one such factor, and it remains unclear how this may affect pregnancy outcomes.

Study design, size, duration: This was a retrospective cohort study of 4275 single-embryo transfers and 881 double-embryo transfers in the blastocyst stage that occurred between 2005 and 2015. All SETs and DETs during this period were eligible for inclusion in the study (n = 72,867). Exclusion criteria included cases with missing data points (n = 10,564), duplicate transfers (n = 41,721), and transfers not utilising day-5 embryos (n = 8,715). Cases were also excluded where the quality of embryos was not adequate for our analysis (n = 6711)

Participants/materials, setting, methods: Data was extracted from private IVF clinic database. To keep data independent, embryo transfers included in the study were limited to one per woman. An internal embryo quality grading system was used – Grade A (very good) to D (poor). Grade A SETs were compared with DETs with at least one Grade A embryo. Chi-square test used and crude odds ratios quoted. Logistic regression was performed with adjusted odds ratios, controlling for number of factors.

Main results and the role of chance: We found that implantation, clinical pregnancy and live birth rates were higher with single Grade A embryo transfers than DET involving at least one Grade A embryo. The absolute event rate (AER) for implantation was 0.49 for SET and 0.29 for DET with at least one high quality embryo, with a relative risk reduction (RRR) of 41% ($p<0.001$). For clinical pregnancy, the AER was 0.52 for SET and 0.45 for DET, with a RRR of 14% ($p<0.001$). For clinical pregnancy loss, the AER was 0.13 for SET and 0.15 for DET, with a relative risk increase (RRI) of 15% ($p = 0.29$). For live birth, the AER was 0.45 for SET and 0.38 for DET, with a RRR of 16% ($p<0.001$). For multiple pregnancy, the AER was 0.01 for SET and 0.15 for DET, with a RRI of 977% ($p<0.001$).

Limitations, reasons for caution: Although adjustment for all available confounders using statistical analysis was attempted, there will be factors not available or included in analysis that may have biased our results.

Wider implications of the findings: Our results contradict the historical precedent that transferring multiple embryos may compensate for low implantation and pregnancy rates; and suggest that to do so may cause harm.

Trial registration number: The local trial registration number is 15172 M.

P-774 Systematic review of the clinical efficacy of vaginal progesterone for luteal phase support in assisted reproductive technology cycles

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[3]*Besins Healthcare UK Ltd., TBC, London, United Kingdom*

Study question: What is the comparative efficacy and safety of vaginal progesterone (VP) preparations licensed for use in the UK for luteal phase support (LPS) in assisted reproductive technology (ART) cycles?

Summary answer: No studies reported statistically significant differences in efficacy or safety between Utrogestan Vaginal (UV) and Crinone, Crinone and Lutigest or Lutigest and Cyclogest.

What is known already: A variety of progesterone preparations are available for LPS including intramuscular injection, oral, rectal and vaginal administration. The comparative efficacy and safety of these preparations have been well reported in the literature, with oral preparations having lower progesterone concentrations in the endometrium and intramuscular routes complicated with injection-site reactions. VP has similar efficacy to other routes with the advantage of rapid absorption and so is the most common form used for LPS in Europe. Despite this wide use of VP, limited data are available on the comparative efficacy and safety of the different VP products available.

Study design, size, duration: A systematic literature review (SLR) was conducted using the MEDLINE, EMBASE and Cochrane Library Databases on 30th June 2016 with no date limit.

Participants/materials, setting, methods: Randomised controlled trials (RCTs) investigating the comparative efficacy or safety of at least two VP preparations (UV, Crinone, Cyclogest or Lutigest) for LPS in ART cycles were eligible. Bibliographies of identified SLRs and major conference proceedings were also hand-searched for further publications. Abstracts and full-texts were screened for eligibility by two independent reviewers. Extraction of data from relevant studies and quality assessment was performed by one individual with verification by another.

Main results and the role of chance: 1,914 records were screened for relevance with 17 studies included in the SLR. Seven studies compared UV with Crinone, one compared Lutigest with Crinone, one compared Lutigest with Cyclogest and another compared Cyclogest with Crinone. Variations in timing or dosage of UV were investigated in seven studies. The most common primary outcome measure was ongoing pregnancy rate (OPR; seven studies), followed by clinical pregnancy rate (CPR; six studies), patient-reported outcomes (PRO; two studies) and delivery (DR) and expected live birth rates (ELBR; one study each). Adverse events (AEs) were reported in five studies. No significant difference in CPR or ELBR was identified in any comparison of UV with Crinone whilst UV and Lutigest were found to be non-inferior to Crinone in OPR comparisons. Differences in PROs for Crinone and Lutigest were found not to be significantly different to Cyclogest.

Limitations, reasons for caution: Due to the nature of comparisons between different formulations of VP eg. gel versus tablet, true double-blinding of the studies was not possible; however, one study implemented assessor blinding. Furthermore, the time points and definitions of outcome measures differed between studies making comparisons across studies inappropriate.

Wider implications of the findings: The results of this SLR suggest that UV, Crinone, Cyclogest and Lutigest each represent valid, equally safe and effective choices of VP preparation for LPS in ART cycles.

Trial registration number: N/A.

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P-775 Cardiovascular health of 9-year-old IVF offspring is not associated with IVF but with parental subfertility

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Study question: Does the *in vitro* procedure, ovarian hyperstimulation or subfertility affect blood pressure (BP) of 9-year-old IVF children born to subfertile couples?

Summary answer: Our study demonstrates that the higher BP in 9-year-old children born to subfertile couples is not due to IVF-procedures but is associated with couples' subfertility.

What is known already: Possible long-term effects of IVF on child health and development have been studied relatively little. This is surprising, as it is known that environmental conditions may influence embryonic and fetal development which may result in health related problems in later life. Some studies suggested that IVF adversely affects BP at school age. Yet, it is unclear which component of IVF attributes to this potentially less favourable BP.

Study design, size, duration: A prospective assessor blinded study of two groups of children followed from birth onwards: the Groningen Assisted Reproductive Technology (ART) cohort-study and the Long-Chain PolyUnsaturated Fatty Acids (LCPUFA) study. In total 449 children were assessed at the age of 9.

Participants/materials, setting, methods: We evaluated cardiovascular health, focusing on BP (in mmHg and percentiles), and heartrate of 57 children born following controlled ovarian hyperstimulation-IVF (COH-IVF); 47 children born after modified natural cycle-IVF (MNC-IVF); and 65 children who were conceived naturally to subfertile couples. Cardiovascular parameters were measured multiple times on one day. Similar data of a reference group (n = 279) of children born to fertile couples of the LCPUFA-study were available.

Main results and the role of chance: BP was similar in the COH-IVF, MNC-IVF and Sub-NC groups. This allowed us to pool the groups to form a subfertile group (n = 169). The subfertile group had a higher systolic blood pressure percentile (SBP, mean [σ]: 60.7 [19.2]), diastolic blood pressure percentile (DBP, 62.5 [18.8]) and heart rate (81.5 [9.9]) than the fertile reference group: SBP (56.3 [24.5]); DBP (55.8 [23.3]) and heart rate (77.0 [9.7]). In the adjusted analyses the subfertile group still had a higher SBP percentile (adjusted B [95%CI]: 7.86 [2.16-13.55]); a higher DBP percentile (7.64 [2.20-13.08]); and a higher heart rate (7.40 [4.96-9.85]).

Limitations, reasons for caution: Larger study groups are necessary to draw firm conclusions regarding ovarian hyperstimulation and the *in vitro* procedure. Another limitation is that all blood pressure measurements were performed on one day.

Wider implications of the findings: Our findings are in line with other studies describing less favourable cardiovascular outcomes in IVF offspring. Our study suggests that the higher BP and heart rate is not due to procedures of IVF but due to parental subfertility. The findings may have important implications for the counselling of subfertile couples.

Trial registration number: -.

P-776 The effects of repeated hormonal treatments on tubo-ovarian gene expression, on ovulated oocytes and on the sex-ratio of the offspring obtained

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Study question: To understand if gonadotropins could modulate tubo-ovarian gene expression. To evaluate the number and quality of oocytes ovulated, and the sex-ratio of the offspring obtained.

Summary answer: Cyclin D1 and p53 increased after 4 cycles of stimulation in FT of treated mice. Number and quality of oocytes decreased. Sex-ratio was not subverted.

What is known already: Epidemiological data evidenced an increased risk for borderline ovarian tumors in infertile women treated with IVF and about two thirds of serous borderline ovarian tumors are characterized by kras mutations that determines a significant increase of cyclin D1 expression. Ovarian cancer would arise from the fallopian tube (FT), not from the ovarian cortex. Quality and number of oocytes decrease after repeated ovarian hyperstimulations. Sex-ratio is influenced by hormonal levels and stress factors.

Study design, size, duration: Ovaries and FT of naturally-ovulating mice and of mice undergoing 0-4-8 rounds of gonadotropin stimulations were analyzed to detect localization and expression levels of Oct-3/4, Sox-2, p53, β-catenin, cyclin D1, pChk1 and pH2AX. Ovulated oocytes were analyzed to detect meiotic spindles and chromosome alignment. Sex-ratio of the offspring obtained were analyzed and compared with control mice, evaluating two consecutive generations of offspring.

Participants/materials, setting, methods: Four (4 T) to eight (8 T) rounds of stimulations were performed with intervals of 1 week between each. Repetitive cycles of ovarian stimulation were performer according to the protocol of Van Blerkom and Davis. For each experiment, control (Ctr; N = 5) and hyperstimulated (N = 20/round, 10 4 T+10 8 T) mice were sacrificed. The experiment was replicated four times.

Main results and the role of chance: After 4 and 8 cycles of ovarian hyperstimulation with gonadotropins, ovaries and FT of control and treatment groups showed no differences in Oct-3/4, Sox-2, β-catenin intracellular localization nor in Oct-3/4, Sox-2, p53, β-catenin, pChk1 and pH2AX contents. By contrast, cyclin D1 and p53 levels increased significantly in the FT, but not in the ovarian cortex of treated mice. After 4 and 8 cycles of ovarian stimulation, the increase of cyclin D1 remained below 46%, which is the rate recorded in ovarian carcinomas. Number and quality of oocytes decreased meanwhile frequency of abnormal meiotic spindles increased with treatments. After 8 consecutive stimulations, no oocyte was retrieved. Sex-ratio was unchanged in both consecutive generations.

Limitations, reasons for caution: The significant increase of cyclin D1 and p53 levels detected in the FT needs to be further investigated.

Wider implications of the findings: We cannot reject the possibility that in a small percentage of susceptible women also a relatively low increase of cyclin D1, as that recorded in mice, could sensitize epithelial cells towards malignant transformation. It remains ethically proper to inform women at risk, about the potential consequences of infertility treatments.

Trial registration number: Not needed.

P-777 Comprehensive protocol of traceability during in vitro fertilization: the result of a multicentre failure mode and effect analysis (FMEA)

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Study question: Can traceability of gametes and embryos be ensured during in vitro fertilization (IVF)?

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Summary answer: A simple and comprehensive traceability system that can be applied in different settings is proposed and its capacity to minimize the risk of miss-matches shown.

What is known already: Miss-matches in IVF are very rare but unfortunately possible with dramatic consequences for both patients and health care professionals. Traceability is thus a fundamental aspect of the treatment. A clear process of patient and cell identification involving witnessing protocols has to be in place in every unit. To identify potential failures in the traceability process and to develop strategies to mitigate the risk of miss-matches, failure mode and effects analysis (FMEA) has been previously effectively used. To reduce subjectivity and to obtain a widespread comprehensive protocol of traceability, a multicentre centrally coordinated FMEA was performed.

Study design, size, duration: Seven representative Italian centres were selected. The study had duration of 21 months and was centrally coordinated by a team of experts. After mapping the traceability process, each center identified the possible causes of mistakes in their protocol. The results of the FMEA analyses were investigated by the experts and consistent corrective measures suggested. A new FMEA analyses was performed after the recommended implementations.

Participants/materials, setting, methods: This study involved in each centre a team composed by: the laboratory director, the Quality Control & Quality Assurance responsible, Embryologist(s), Gynaecologist(s), Nurse(s) and Administrative. The FMEA analyses were performed according to the Joint Commission International (2002, 2010). For each failure a Risk Priority Number (RPN) score was calculated by multiplying the probability of occurrence (O), severity of impact on the process (S) and chance of detection (D) (RPN=OxSxD).

Main results and the role of chance: The FMEA teams identified 7 main process phases: oocyte collection, sperm collection, gametes processing, insemination, embryo culture, embryo transfer and cryopreservation. A mean of 19,29 (SD+5.8) associated process steps and 41,86 (SD+12,4) possible failure modes were recognized per centre. A RPN > 15 was calculated in a mean of 6,43 steps (range 2-12, SD+3,60). A total of 293 failure modes were centrally analysed 45 of which were considered at medium/high risk. The implementation of consistent corrective measures allowed a significant reduction in the RPNs in all centres (RPN < 15 for all steps). A simple and comprehensive traceability system was designed as the result of the seven FMEA analyses.

Limitations, reasons for caution: Specific situations such as sperm/oocyte donation, import/export and pre-implantation genetic testing were not taken into consideration. Moreover, this study is only limited to the analysis of failure modes that may lead to miss-matches, other possible procedural mistakes are not accounted.

Wider implications of the findings: The results of this study can support IVF groups in better recognizing critical steps in their treaceability protocols, understanding identification and witnessing process, and in turn enhancing safety by introducing validated corrective measures.

Trial registration number: N/A.

P-778 Intracytoplasmic morphologically selected sperm injection (IMSI) procedure affects secondary human sex ratio (SSR)

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Study question: Does the use of the intracytoplasmic morphologically selected sperm injection (IMSI) affect the sex ratio at birth?

Summary answer: The data imply that sex selection emerges after the IMSI procedure with the sex ratio skewed towards females.

What is known already: Among the ART-associated consequences, a skewed sex ratio is an issue of concern. The secondary sex ratio (SSR), which may be defined as the proportion of live-born males out of all live births, is commonly recognized as male biased among naturally born offspring. However, according to published studies, ART methods/procedures(IVF/ICSI, blastocyst transfer, and culture media) or gamete quality may potentially affect the SSR by increasing, reducing or even reversing the ratio. With regard to IMSI, which improves the quality of sperm selected for ICSI using high magnification and MSOME criteria, virtually no information is available about its influence on SSR.

Study design, size, duration: The cohort study included children (n = 938) from pregnancies achieved through IMSI procedures that were conducted in a single fertility clinic. The children were born alive between January 2008 and September 2016. Children from fresh and frozen cycles were included, but no PGD or PGS cases were considered.

Participants/materials, setting, methods: Sperm was selected for IMSI at 15,000x magnification. The most recently available data (2008-2014) from a national demographic database were used as a control. SSR was calculated by dividing the number of live-born male babies by the number of all live-born babies. Chi-squared analysis was used to compare gender ratios. P < 0.05 was considered statistically significant.

Main results and the role of chance: Among the children born from IMSI the SSR was 47.2%. In contrast, the nationwide SSR was 51.2%, which is consistent with what is expected. There was a significant difference ($P = 0.01$) in the SSR between the IMSI population and the general population(Table 1). The reduction in the SSR remains independent of maternal and paternal age, aetiology, semen origin, type of cycle (fresh or frozen), culture medium, and embryo transfer stage and independent of whether it was a single or multiple pregnancy (Table 2).

Table 1. The sex distribution at live birth.

	Gender	
Population	Male	Female
IMSI	443	495
General	10.438.698	9.938.419

$P = 0.01$

Table 2. IMSI cycles and SSR distribution.

	SSR	P
Female age (years)		
-≤35	48.9%(309/632)	0.22
-36-39	42.2%(101/238)	
-≥40	48.5%(33/68)	
Male age (years)		
-≤35	47.7%(193/405)	0.24
-36-39	43.1%(110/255)	
-≥40	50.4%(140/278)	
Aetiology		
-Male	46.3%(167/361)	0.88
-Female	49.2%(147/299)	
-Male+Female	45.9%(17/37)	
-Idiopathic	46.5%(112/241)	
Semen collection		
-Masturbation	47.2%(421/892)	0.93
-Surgical retrieval	47.8%(22/46)	
Culture medium		
-Sydney IVF Cleavage Medium K-SICM-20(Cook®)	41.4%(12/29)	0.78
-Total-LGGT(Life Global Group®)	51.1%(184/360)	

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Continued

	SSR	P
-G-1 Plus/G-2 Plus(Vitolife®)	47.5%(19/40)	
-P-1 Medium SSS(Irvine Scientific®)	43.78%(98/224)	
-IVF-Cleavage +Total-LGGT	40%(2/5)	
-IVF-Cleavage +G-1/G-2	48.0%(12/25)	
-Total-LGGT+G-1/G-2	36.4%(4/11)	
-Total-LGGT+P-1	46.2%(109/236)	
-G-1/G-2+P-1	37.5%(3/8)	
Cycle		
-Fresh	47.6%(352/739)	0.69
-Frozen	45.7%(91/199)	
Embryo stage/transfer		
-Cleavage	47.6%(383/804)	0.60
-Blastocyst	44.8%(60/134)	
Pregnancy		
-Singleton	46.3%(259/560)	
-Twins	49.7%(176/354)	0.22
-Triplets	33.3%(8/24)	

Limitations, reasons for caution: Allocation for IMSI cycles over the four media was not random. The small sample size in some subgroup populations impairs the analysis

Wider implications of the findings: It may be hypothesized that alterations in the Y chromosome may lead to morphological changes in the sperm that prevent its selection under high magnification. However, other possible causes should be considered and discussed. Additional studies with larger sample sizes to examine sex ratios after IMSI are warranted.

Trial registration number: Not applicable. The local ethics committee authorised this study.

P-779 Long-term neurological morbidity in children following in-vitro fertilization and ovulation induction pregnancies

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Study question: To determine the risk for long-term neurological morbidity among children born after in-vitro fertilization (IVF) and ovulation induction (OI) as compared to spontaneous pregnancies.

Summary answer: Singletons conceived following IVF but not OI have an increased risk of long-term neurological morbidity

What is known already: Controversy exists regarding the association between fertility treatments and adverse neurological outcome of the children. While previous studies of children up to school-age, found no significant difference in neurodevelopmental outcome, large cohort studies that followed the participants for a longer period, up to 14 years, reported an increased risk of neurological outcome in children born following infertility treatments. Yet, another study that followed children up to 17 years indicates that children born following fertility treatments perform above average on standardized testing and their cognitive development is normal

Study design, size, duration: A population based cohort analysis was performed, including computerized database of all singleton infants born between the years 1991-2014 in Soroka University Medical Center (SUMC). This database was linked with the computerized dataset of all pediatric hospitalizations in the same medical center. SUMC is the largest birth center in Israel, and is the only tertiary medical center in Israel's southern region, including the single IVF unit in the region.

Participants/materials, setting, methods: This study included all singleton deliveries occurring between 1991-2014 at a single tertiary medical center. Fetuses with congenital malformations were excluded. A comparison was performed between children delivered following IVF, OI and spontaneous pregnancies. Hospitalization rates up to the age of 18 years involving neurological morbidity were evaluated. A Kaplan-Meier survival curve was used to compare cumulative morbidity incidence. A Cox regression model was used to control for confounders.

Main results and the role of chance: During the study period 242,187 singleton deliveries met the inclusion criteria; 1.1% were following IVF (n = 2603), and 0.7% occurred following OI (n = 1721). Hospitalizations up to the age of 18 years involving neurological morbidity were significantly more common in children delivered following IVF (3.7%) and OI (4.1%) as compared with those following spontaneous pregnancies (3.1%; p = 0.017 using the chi-square test for trends). Attention deficit disorders (ADHD), sleep disorders, movement disorders and headaches were more common in the IVF and OI groups as compared to the spontaneous pregnancies groups. Autism, cerebral palsy, eating disorders among other evaluated complications were comparable between the study groups. The Kaplan-Meier survival curve demonstrated a significantly higher cumulative incidence of total neurological morbidity following IVF and OI (log rank p < 0.001). Using the cox regression model, controlling for multiple confounders such as maternal age, preterm delivery, maternal diabetes and hypertensive disorders in pregnancy, IVF (adjusted HR = 1.37, CI 1.12-1.67, p = 0.03), but not OI (adjusted HR = 1.15, CI 0.91-1.46, p = 0.242), was noted as an independent risk factor for long-term pediatric neurological morbidity.

Limitations, reasons for caution: This study was limited by its retrospective nature. Fertility etiology is not applicable as well as the treatment protocol used.

Wider implications of the findings: The increased rate of long term neurological pediatric complications in IVF but not OI conceived pregnancies should improve the consultation given to the patients prior to treatment. Further studies are required in order to address the underlying pathophysiology leading to these complications in IVF pregnancies in order to reduce it.

Trial registration number: N/A.

P-780 IVF is an independent risk factor for third stage of labor complications

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Study question: To determine if third stage of labor complications are more prevalent in term singleton vaginal deliveries following IVF gestations than in matched spontaneous control gestations.

Summary answer: Post-partum hemorrhage, manual lysis and blood transfusion requirement were significantly more prevalent following vaginal deliveries of IVF gestations than in the matched spontaneous pregnancy controls.

What is known already: IVF pregnancies are associated with a higher rate of prenatal and intra-partum complications than spontaneous gestations, even

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after correction of confounding factors such as maternal age, parity and number of fetuses. Little is known about the prevalence of third stage of labor complications in IVF pregnancies, excluding a higher prevalence of placenta accreta reported in a few small studies.

Study design, size, duration: Cohort study reviewing the delivery files of all our IVF patients who vaginally delivered singletons in our hospital between 8/2011-3/2014. Two matched spontaneous pregnancy controls from our obstetrical database in the same time range were randomly selected by software based on age, gravidity, parity and week of delivery. The impact of pregnancy type on the length and complications of the third stage of labor was examined. P values were calculated using the Fisher exact test.

Participants/materials, setting, methods: The study group consisted of 242 IVF gestations, and 484 matched spontaneous pregnancy controls, who delivered vaginally. The average maternal age was 32.74 ± 5.22 years for IVF cases and 32.61 ± 5.14 years for controls. The average gravidity and parity were 2.1 ± 1.24 and 0.63 ± 0.73 for IVF, and 2.07 ± 1.13 and 0.64 ± 0.74 for controls. The delivery week was 38.69 ± 1.75 for IVF and 38.61 ± 2.46 for controls. Post-partum hemorrhage was defined when the estimated blood loss following delivery was higher than 500 mL.

Main results and the role of chance: The prevalence of diabetes, hypertensive complications and placental abruption did not differ between deliveries following IVF and controls. There was also no difference in the rate of labor inductions (26.03% following IVF and 31.2% in controls, NS) and instrumental deliveries (17.77% following IVF and 15.5% in the controls, NS). The average birthweight was 3153 ± 645 gr for IVF gestations and 3179 ± 479 for controls (NS). The 1 minute and 5 minutes Apgar scores following IVF gestations were 8.75 ± 1.13, 9.9 ± 0.62, and for controls 8.89 ± 0.67, 9.95 ± 0.48 (NS).

The average length of the third stage was similar in deliveries following IVF and controls (14.23 ± 8.89 and 13.69 ± 9.19 minutes respectively, NS). The rated of post-partum hemorrhage was 5.79% in deliveries following IVF and 1.45% in controls ($p = 0.001$). Manual lysis of the placenta was performed in 11.98% of the deliveries following IVF and in 7.02% of the controls ($p = 0.025$). Blood transfusion was required in 2.07% following IVF deliveries and 0.41% of the controls ($p = 0.032$). Abnormally adherent placenta was diagnosed in 2 deliveries following IVF (0.83%) and in none of the controls ($p = 0.045$).

Limitations, reasons for caution: Retrospective study.

Wider implications of the findings: Our results indicate that IVF is a risk factor for post-partum hemorrhage, requirement for manual lysis and blood transfusions, even after correction for confounding factors. Therefor complication anticipating management of the third stage is warranted in women delivering vaginally following IVF pregnancies, even in the absence of other risk factors.

Trial registration number: NA, the acquisition of data from the medical records for this study was approved by our IRB.

P-781 Infertility treatment affects endothelial health in early pregnancy

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Study question: Does the unphysiologic maternal milieu after assisted reproduction affects maternal endothelial function already in early pregnancy?

Summary answer: Maternal endothelial function early in pregnancy in women using assisted reproductive technologies (ART) is reduced compared to spontaneously conceived pregnancies in infertile women.

What is known already: ART is associated with an increased risk for vascular disease in pregnancy, e.g. gestational hypertension and preeclampsia. The reasons are still debated, ranging from the maternal susceptibility, hormonal milieu to embryo culture conditions. An impaired peripheral arterial tone, measured as reactive hyperemia index (RHI) is associated with an increased risk of vascular events.

Study design, size, duration: Recruitment for this ongoing prospective, observational study started in June 2014. The overall goal is a number of 45 participants finishing the study visits.

Participants/materials, setting, methods: Infertile women at 7-10 weeks gestation with a viable intrauterine pregnancy after spontaneous or ART conceptions were recruited. Cases with known disorder that affect vascular health or a recent cold were excluded. Endothelial function was evaluated using the Endo-PAT2000 system and measured as reactive hyperemia index (RHI, arbitrary units) at 11-14 weeks. Multiple comparison analysis was performed and endothelial function compared between spontaneously conceived and ART pregnancies. Data are shown as median and standard error.

Main results and the role of chance: RHI was lower in women who received infertility treatment (1.93 ± 0.06, n = 30, $p = 0.009$) compared to spontaneous conceptions (2.36 ± 0.26, n = 6) in infertile women. If broken down to mode of conception a significant effect on RHI was observed after stimulated intrauterine insemination and timed intercourse (1.69 ± 0.15, $p = 0.005$, n = 8) or fresh in vitro fertilization (IVF), (1.87 ± 0.16, $p = 0.05$, n = 8). Although a lower RHI has been observed in medicated frozen-thaw embryo transfer cycles (1.85 ± 0.11, $p = 0.07$, n = 5) this wasn't statistically significant. Interestingly, frozen-thaw embryo transfers in a natural cycle (2.0 ± 0.08, $p = 0.13$, n = 9) had the highest RHI in all ART groups. There were no differences in BMI, systolic or diastolic blood pressure or lipid concentrations.

Limitations, reasons for caution: Due to low numbers of participants there is a risk of chance. At this point of the study it is unclear if the observed differences in endothelial function persist throughout pregnancy and even thereafter and if they are associated with a higher risk for hypertensive disease, e.g. preeclampsia.

Wider implications of the findings: If the observed trend of impaired vascular function after ART (e.g. fresh IVF) holds up a less aggressive ovarian stimulation due to concerns about short-term effects and longer-term issues of fetal and maternal outcomes would have a high probability of driving a change in practice guidelines and standard of care.

Trial registration number: Does not apply.

P-782 Associations between embryo grading and congenital malformations in IVF/ICSI pregnancies

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Study question: Does the quality of transferred embryos in a population of infants conceived by IVF and ICSI have an impact on the rate of congenital anomalies?

Summary answer: A baby conceived from a poor quality embryo may be at higher risk of congenital malformations than one from a good quality embryo.

What is known already: Multiple studies have shown that there is an association between congenital malformations and IVF/ICSI pregnancies. Other studies have investigated the role of ICSI vs. IVF, transferring fresh vs. frozen embryos, ovarian stimulation regimens, assisted hatching and transferring blastocysts vs. cleavage-stage embryos in congenital malformations. The relationship between embryo quality in implantation, clinical pregnancy and livebirth rates is well known. However, there is limited knowledge on the role of embryo quality in congenital malformations. It is thought that the malformation risk is higher in poor quality embryos, but rates of individual anomalies in good and poor embryos have not been compared.

Study design, size, duration: This study was a retrospective cohort study of 10,309 clinical pregnancies in women who underwent IVF or ICSI at a private multisite IVF clinic between January 2005 and December 2015 inclusive. Data on pregnancies were recorded in a standardised database. There were no losses to follow up due to local legislative reporting requirements.

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Participants/materials, setting, methods: 10,309 single embryo transfers graded on Day 5 which resulted in clinical pregnancies were identified. One clinical pregnancy per woman was randomly selected. The type and number of congenital malformations were determined. Clinical pregnancies were further limited to those that resulted in deliveries (n = 7292). Embryos were classified as good quality (grade A or B, n = 6099) or poor quality (grade C or D, n = 1193). Malformation rates between good and poor quality embryos were compared.

Main results and the role of chance: Deliveries resulting from the transfer of poor quality embryos were more likely to have talipes (adjusted odds ratio, aOR 0.39(95% Confidence Interval 0.18-0.87), p = 0.003), birth marks (aOR 0.1(0.11-0.86), p = 0.010) and sacral dimples (aOR 0.24(0.07-0.78), p = 0.018) than births from good quality embryos. There was no significant difference in rates of major malformations between good and poor embryos.

All clinical pregnancies were also analysed to ensure that terminations and clinical pregnancy losses were captured. Comparison of demographic data were unchanged from that of deliveries. The statistically significant results in deliveries were upheld. Additionally, diaphragmatic hernia occurred more commonly in poor quality embryos (aOR 0.08 (0.007-0.95), p = 0.026), as did genetic anomalies (aOR 0.22 (0.05-0.87), p = 0.016) and hypospadias (aOR 0.32 (0.10-0.99), p = 0.11). As a result, 'all anomalies" also became statistically significant (aOR 0.75 (0.58-0.97), p = 0.008).

When all clinical pregnancies were analysed, there were less spontaneous clinical pregnancy losses in good quality embryos compared to poor quality embryos on univariate analysis, but there was no significant difference after adjusting for confounders (crude OR 0.75 (0.68-0.84), aOR 0.79 (0.61-1.03), p<0.001). There was no significant difference between the rate of terminations in good quality and poor quality embryos (aOR 0.65 (0.17-2.46), p = 0.92).

Limitations, reasons for caution: Information about anomalies was obtained from medical notes rather than a dedicated birth defects register. Subfertility and intrinsic parental factors may contribute to our results. As there are very small rate differences between good and poor embryos, studies with larger sample sizes are required to confirm or deny our findings.

Wider implications of the findings: This is the first study comparing the rates of individual congenital malformations for good and poor embryos. It assists clinicians in counselling patients about the risks of transferring poor quality embryos if there are no good embryos available. Poor grade day 5 embryos are associated with minor malformations and talipes.

Trial registration number: Not applicable.

P-783 Preliminary results on cognitive and motor development of 2-year-old IVM children

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Study question: Is there a difference in cognitive and motor development between children born after In vitro maturation (IVM), Intra cytoplasmatic sperm injection (ICSI) and spontaneous conceived (SC) pregnancy?

Summary answer: First results show a significant difference between IVM and SC controls for cognitive development (IVM< SC) and between IVM and ICSI for motor development (IVM<ICSI).

What is known already: Several ART follow-up studies show no significant difference in cognitive and motor development between children born after IVF and ICSI compared with SC controls. Studies that looked at children born after IVM treatment focused on medical factors. There are no more significant premature births or major birth problems after IVM compared with other modes of conception (IVF, ICSI, SC) (Buckett et al., 2007, Cha et al., 2005, Shu-Chi et al., 2006, Söderström-Antilla et al., 2006) Data of birth length- and weight of IVM-children compared with other modes of conception are inconclusive.

Study design, size, duration: A case-control study design were three groups (IVM, ICSI and SC children) were matched according to age, sex, language, ethnicity, mothers educational level, maternal age and birth order. All children are singletons, live in Belgium and have Dutch or French as mother tongue. Recruitment ran from 2014 until 2016. Sixty-nine were invited, 53 participated.

Participants/materials, setting, methods: Seventeen IVM, 18 ICSI were invited to the UZ Brussel. Eighteen SC children were recruited in a nursery. The IVM mean age of assessment was 25 months (SD=3.47), for ICSI and SC 27 months (SD=4.22/ SD=4.63). Cognitive and motor development was investigated using the Bayley Scales of Infant Development II, Dutch version. The scales were administered yielding a mental index score and motor index score with a mean of 100 and a SD of 15.

Main results and the role of chance: The results on the B S I D- II-NL show significant differences between the three groups for cognitive and motor development. There is a significant difference on the mental index score between the IVM and de SC control group (F= 4.68, p= .014). The SC controls (M= 108.78, SD= 4.41) score higher than the IVM group (M= 101.47, SD= 9.37). There is also a significant difference on the motor index score between the IVM and the ICSI group (F= 4.26, p= .019). The ICSI group (M= 111.50, SD= 8.34) score higher than the IVM group (M= 103.89, SD= 9.92). None of the children show a delayed cognitive and motor development.

Limitations, reasons for caution: This study used multiple evaluators, giving a potential evaluators bias. Although significant differences were found, scores in all three conception groups are within a normal range. The small sample size undermines the generalizability of the results to the population. The results presented in this abstract are of a preliminary nature.

Wider implications of the findings: The IVM and ICSI treatment does not have a negative impact on the cognitive and motor development of children compared with SC controls. The small sample size and limited IVM studies require further investigation.

Trial registration number: Non-randomised study design.

P-784 Neonatal outcomes and congenital malformations in children born after treatment with progesterone primed ovarian stimulation in in vitro fertilization and vitrified embryo transfer cycles

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Study question: Does neonatal outcomes including congenital malformations in children born after progesterone primed ovarian stimulation (PPOS) differ from that of children born after conventional stimulations?

Summary answer: Children born after PPOS treatment do not increase the prevalence of congenital malformation and compromise the neonatal outcomes compared with conventional ovarian stimulations.

What is known already: Our previous studies demonstrated that progesterone agents such as medroxyprogesterone acetate (MPA) could prevent premature LH surges during COH in combination with a freeze-all policy, however, the safety of the children born after this new regimen remains unknown.

Study design, size, duration: A retrospective, single-center study was conducted, in which children born after PPOS treatment using exogenous gonadotropins concurrent with MPA, gonadotropin releasing hormone agonist (GnRH-a) short protocol, or mild ovarian stimulation during January 2014 to June 2016 were included.

Participants/materials, setting, methods: A total of 4596 babies were born, of which 1931 babies born after PPOS, 1658 babies born after short protocol and 1007 babies born after mild ovarian stimulation. Neonatal outcomes were anal yzed for singletons and twins separately. Multivariable logistic regression was used to evaluate the possible relationship between various ovarian stimulation methods and congenital malformations.

Main results and the role of chance: Neonatal outcomes both for singletons and twins such as mean birthweight and birthlength, gestational age, the frequency of preterm birth were comparable between groups. Rate of stillbirth, perinatal death were also similar. No differences were found in the overall incidence of congenital malformations among groups. Multivariable logistic regression indicated that children born after PPOS regimen didn't increase the prevalence of congenital malformations compared with those born after short protocol as well as mild ovarian stimulation, with adjusted odds ratio (AOR) of

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1.16 (95% confidence interval (CI) 0.54–2.01) and 1.28 (95% CI 0.51–2.42), respectively, after adjusting for maternal age, BMI, parity, insemination method, cycles with Day 3 or blastocyst transfers.

Limitations, reasons for caution: This study was not a randomized controlled trial and the allocation of different ovarian stimulation treatment was not at random. Furthermore, the neonatal data were obtained by questionnaires from parents rather than access to medical records.

Wider implications of the findings: In terms of neonatal outcomes and congenital malformations, treatment with PPOS during COH is as safe as conventional ovarian stimulations.

Trial registration number: not applicable.

P-785 Improved ovarian hyperstimulation syndrome (OHSS) risk management by individualised dosing of follitropin delta based on serum anti-Müllerian hormone (AMH) and body weight

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Study question: To evaluate the clinical benefits of individualised follitropin delta dosage with regard to OHSS risk management.

Summary answer: Individualised follitropin delta compared with conventional follitropin alfa reduces the need for preventive interventions for OHSS and decreases the risk of OHSS following preventive interventions.

What is known already: The risk of early OHSS increases with increasing ovarian response to gonadotropin stimulation. An individualised dosing regimen of follitropin delta (FE 999049) based on AMH (a preferred predictor of ovarian response to gonadotropins) and body weight (a determinant of systemic exposure to follitropin delta), targeting an adequate number of oocytes retrieved, significantly reduced the incidences of preventive interventions for early OHSS and preventive interventions and/or early OHSS compared to conventional follitropin alfa treatment (Nyboe Andersen and Nelson et al, Fertil Steril, 2016).

Study design, size, duration: Randomised, assessor-blind, controlled trial including 1326 patients undergoing their first IVF/ICSI cycle. Patients were randomised 1:1 to individualised follitropin delta dosing (665 patients) or conventional follitropin alfa dosing (661 patients). AMH was measured by Elecsys® AMH (Roche Diagnostics). The follitropin delta dose was fixed throughout stimulation, while the follitropin alfa dose (starting at 150 IU/day) could be adjusted upwards or downwards from day 6 of stimulation based on the individual response.

Participants/materials, setting, methods: Preventive interventions for early OHSS included cycle cancellation due to excessive ovarian response (≥25 follicles of ≥12 mm in diameter), triggering of final follicular maturation with GnRH agonist (triggering criterion 25-35 follicles ≥12 mm) and/or administration of dopamine agonist (if ≥20 follicles ≥12 mm). Early and late OHSS were defined as OHSS with onset ≤9 days and > 9 days, respectively, after triggering of final follicular maturation. OHSS was assessed using Golan's classification (1989).

Main results and the role of chance: In total, 17 and 20 cases of early OHSS were observed in the follitropin delta and follitropin alfa groups, respectively. Statistically significantly fewer patients in the follitropin delta group required preventive interventions for early OHSS compared to the follitropin alfa group (15 vs 30, p = 0.005). The type of preventive interventions employed were GnRH agonist triggering in 10 women in the follitropin delta group and 23 women in the follitropin alfa group (p = 0.019) and administration of dopamine agonist in 5 vs 10 women, respectively. Three women in the follitropin alfa group received both types of intervention. In spite of these preventive interventions, 10 patients developed early OHSS; 1 in the follitropin delta group and 9 in the follitropin alfa group. Early OHSS, 3 mild and 4 moderate cases, occurred despite triggering with GnRH agonist in 7 out of 23 women in the follitropin alfa group. The improved OHSS risk management observed with individualised follitropin delta in relation to early OHSS was also observed for late OHSS, where the number of OHSS cases were reduced to half in the follitropin delta group compared to follitropin alfa (6 vs 12 cases).

Limitations, reasons for caution: The present trial confirms that GnRH agonist triggering can reduce, but not eliminate the incidence of early OHSS. The risk of early OHSS following GnRH agonist triggering in relation to ovarian response needs to be further evaluated in prospective trials.

Wider implications of the findings: Personalised ovarian stimulation based on AMH and body weight provides clinical benefits in OHSS risk management. The lower incidence of OHSS despite preventive interventions suggests a less profound hyperstimulation associated with an individualised follitropin delta dosing regimen compared to a conventional dosing approach.

Trial registration number: NCT01956110.

P-786 Trends in utilization of cryopreserved embryos in the United States from 2004-2013: an analysis of 411,811 cycles

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Study question: What is the quantity and utilization of embryos cryopreserved at assisted reproductive technology (ART) clinics in the United States from 2004 through 2013?

Summary answer: Embryo cryopreservation without fresh transfer has increased from 7.9% (2004) to 40.7% (2013), with 1,954,548 embryos frozen and 1,237,203 unaccounted for by embryo transfer.

What is known already: Embryo cryopreservation is inherently linked with potential ethical, legal and healthcare policy challenges. Choices regarding disposition of supernumerary embryos which survive cryopreservation involve: a potential future embryo transfer, donation for research, thaw and discard, donation to another couple, or indefinite storage. Evidence suggests that most embryos remain in storage with no specific plan for utilization. In 2003 it was estimated that at least 400,000 embryos were in storage in the United States. The trends in utilization of stored embryos in the U.S. over the past 10 years are unknown.

Study design, size, duration: Historical cohort of U.S. ART cycles reported to the Society for Assisted Reproductive Technologies Clinical Outcomes Reporting System (SART CORS) between 2004 and 2013 in which an oocyte retrieval and embryo cryopreservation took place.

Participants/materials, setting, methods: Over the 10-year period, there were a total of 411,811 autologous fresh ART retrievals from women in whom at least one embryo was cryopreserved. Cycles were categorized by year and by whether they resulted in one of the following: embryo transfer and no pregnancy, embryo transfer and clinical pregnancy, embryo transfer and live birth, or cryopreservation of all embryos with no embryo transfer.

Main results and the role of chance: Of 411,811 IVF cycles in which at least one embryo was cryopreserved, 31.5% resulted in no pregnancy, 48.6% resulted in a clinical intrauterine pregnancy, 41.5% resulted in a live birth and 19.2% had all embryos cryopreserved. The percentage of fresh cycles in which all embryos were frozen increased significantly each year after 2010 with the following percentages of freeze-all cycles: 15.6% (2010), 19.9% (2011), 30.7% (2012) and 40.7% (2013); the trend was significant with P < 0.0001. The mean number of embryos cryopreserved per cycle was 4.75. The number of embryos cryopreserved per year steadily increased from 158,383 in 2004 to 303,203 in 2013 (trend analysis significant, P < 0.0019). During the 10-year period, 1,954,548 embryos were cryopreserved and 717,345 embryos were transferred. In total, 1,237,203 embryos were cryopreserved and are potentially still

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in storage, although we cannot account for embryos that were discarded, donated or did not survive the thaw.

Limitations, reasons for caution: This was a retrospective study, therefore only the available parameters could be included. SART CORS cannot account for embryos that were discarded, donated to research or donated to other couples.

Wider implications of the findings: There has been a sharp increase in the U.S. in the number of cycles in which all embryos are frozen and this may result in more embryos in storage and a subsequent increase in disposition decisions required by patients and clinics.

Trial registration number: This study was supported by the Clinical Research Scientist Training Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development (R25 HD 075737).

P-787 Vitrification of cleavage stage embryos result in lower fetal birthweight than slow-freezing method: a large scale retrospective study in two centers

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Study question: Does the embryo freezing method have an impact on the birthweight of newborns from thawed embryo transfer (TET) cycles?

Summary answer: The mean adjusted birthweight was lower in the vitrified group than the slow-freezing embryo transfer group.

What is known already: Vitrification has been successfully used for embryo cryopreservation and is superior to slow-freezing technique regarding the embryo survival rate and live birth rate. However, whether the vitrification method would affect fetal growth and thus the neonatal birthweight is still controversial. Most studies addressed this issue by comparing the neonatal outcome after transferring vitrified embryos with fresh embryo transfer. So far, comparative data of two cryopreservation methods with respect to the singletons' birth outcome is limited.

Study design, size, duration: The retrospective, two-center study was performed for 1797 singleton neonates born after Day 3 embryo transfer of slow frozen or vitrified embryos from January 2012 to December 2016.Each patient contributed only one cycle per group. Outcome measures was neonatal outcome including birthweight, gestational age and gender.

Participants/materials, setting, methods: All Cycles were performed in the First Affiliated Hospital of Sun Yat-sen University and Jiangmen Maternity and Child Health Care Hospital, which were two large reproductive centers in South China. Blastocyst transfer, PGD and IUI cycles were excluded. Only data from singletons born alive after the 28th week of gestation were included in the data analysis.

Main results and the role of chance: One thousand four hundred and seven singleton babies were born from vitrified group and 390 were from slow-freezing embryo transfer. The absolute birthweight was comparable between two groups (3155.5 ± 503.7 g vs. 3200.9 ± 506.4 g, $P<0.05$), but the adjusted birthweight controlled for gestational age and gender in vitrified group is lower than the slow-freezing group (z-score:0.01 ± 1.01 vs. 0.18 ± 1.08, $P<0.05$). The gestational ages were 38.6 ± 1.8 and 38.4 ± 1.7 weeks in the vitrified and slow-freezing group respectively and preterm birth occurred in 8.6% and 9.2% of these two groups without significant difference. The sex ratio (Odds ratio (OR)1.09, 95% condfidence interval (CI):0.97-1.24), rate of small for gestational age (OR:1.22, 95%,95% CI:0.87-1.71) and large for gestational age (OR: 0.82,95% CI:0.55-1.22), as well as the rate of low birthweight (OR:1.02,95% CI:0.67-1.55) and macosomia (OR:0.65,95% CI:0.40-1.06) were all comparable between the two cryopreservation method. After adjusting for numerous confounding factors, multiple linear regression analysis demonstrated that maternal weight, gestational age, embryo freezing method and infant gender were significantly related to neonatal birthweight

Limitations, reasons for caution: Although the data was from two large centers with big sample size which can represent ordinary experience of reproductive centers in South China, it was a retrospective study. Moreover, researches for the long-term effects of vitrification method on the health of children are essential in the near future

Wider implications of the findings: Vitrified embryo transfer seemed to give rise to a decrease in neonatal birthweight when compared with the slow-freezing technique. Further studies are required to elucidate this issue with larger number of patients. The potential epigenetic changes that might cause the decrease of birthweight should be carefully concerned

Trial registration number: non-clinical trials

P-788 Influence of natural and artificial cycle on obstetric and neonatal outcomes of cryo-thawed embryo transfer: a retrospective study

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Study question: Are pregnancy complications and neonatal outcomes after artificial cycle frozen-thawed embryo transfer(AC-FET) non-inferior to pregnancy complications and neonatal outcomes after natural cycle frozen-thawed embryo transfer (NC-FET)?

Summary answer: AC-FET is associated with a higher incidence of gestational hypertension, neonatal morbidity, preterm and low birth weight rate compared with NC-FET, especially in twins.

What is known already: Pooling prior retrospective studies of AC-FET and NC-FET results in obstetric and neonatal outcomes. Most studies were forced on live birth rates and clinical/ongoing pregnancy rates.

Study design, size, duration: This retrospective study included data from women and their neonates born after FET since January 2014 to January 2015. Patients after AC-FET and NC-FET both were 872 singletons and 206 twins. Hormone levels, the endometrial thickness before the day of embryo transfer, the obstetric and neonatal outcomes was recorded.

Participants/materials, setting, methods: The study design was a single-center, retrospective study. Pregnancies from donated gametes, surgically retrieved sperm, early miscarriage, stillbirth data and the mother who had pre-pregnancy hypertension, diabetes, intrauterine adhesions, uterine malformations, PCOS/PCO, were excluded in our study. HCG-induced/clomiphene citrate induced ovulation cycles were excluded in the study. Patients from NC-FET were randomized based on a 1:1 allocation to AC-FET for matching the BMI, age and basal endocrine level. All embryos were cryopreserved by vitrification.

Main results and the role of chance: The endometrial thickness was thinner in AC-FET than in NC-FET, [10.60 mm(9.70-11.60) vs. 11.40 mm(10.40-12.40); $P < .001$ in singletons and 10.55 mm(9.80-11.90) vs. 11.20 mm(10.30-12.30); $P < .001$ in twins]. Serum Estradiol/Progesterone/Prolactin on the day of before embryo transfer was higher in AC-FET than in NC-FET both on singletons and twins[In singletons: Estradiol: 1066miu/ml(181.7-2213.50) vs. 102.45miu/ml(74.70-142.42); $P < .001$ and Progesterone: 10.92miu/ml(8.30-14.68) vs. 9.64miu/ml(5.97-13.98); $P < .001$ and Prolactin: 28.02miu/ml (18.65-42.48) vs. 17.89miu/ml(13.63-23.16); $P < .001$; In twins: Estradiol: 493.3miu/ml(168.6-1907.5) vs. 93.75miu/ml(71.69-139.93); $P < .001$ and Progesterone: 10.30miu/ml(8.39-13.28) vs. 8.56miu/ml(5.53-13.25); $P < .001$ and Prolactin: 23.30miu/ml(17.09-37.06) vs. 17.24miu/ml(12.83-25.45); $P < .001$]. Pregnancy hypertension was higher in AC-FET than in NC-FET both in singletons(5.29% vs. 3.66%; P=.011) and twins(12.62% vs. 4.85%; P=.003). The incidence of previa was higher in AC-FET than in NC-FET both in the singletons(2.06% vs. 0.57%; P=.010) and in twins(2.43% vs. 0%; P=.061). Neonatal morbidity was higher in AC-FET compared with in NC-FET(5.50% vs. 3.67%; $P = 0.067$ in singletons and 6.3% vs. 2.4%; $P = 0.006$ in twins). For twins, pregnancy duration was longer in NC-FET compared with that in AC-FET [37.14 weeks(36.36-37.86) in NC-FET and 36.57weeks(35.14-37.43), $P < .001$], and the birth weight was higher in NC-FET than in AC-ET (2.65 kg

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(2.40 kg-2.90 kg) in NC-FET and 2.55 kg(2.20 kg-2.85 kg) in AC-FET, P < .001). No other difference in the two groups.

Limitations, reasons for caution: The results of this study should be regarded with caution because of its limitations, mainly the retrospective design, confounding factors, and small sample size.

Wider implications of the findings: AC-FET is associated with a higher incidence of gestational hypertension and worse neonatal outcomes compared with AC-FET, especially in twins. The thinner endometrial thickness and the higher estrogen level may be the main cause for higher risk of gestational diseases in AC-FET.

Trial registration number: not applicable.

P-789 Introduction of a novel device for drug administration for ovarian stimulation

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Study question: What is the experience with an injection port for drug administration during ovarian stimulation instead of standard injections?

Summary answer: i-Port® is a well tolerated and effective alternative in terms of oocytes retrieved and gonadotropin consumption that minimizes skin punctures needed to follow the treatment.

What is known already: Ovarian stimulation involves the administration of exogenous hormones for around 9-14 days. Complaints about the number of injections required are frequents. In fact, it is estimated that at least 10% of adults have a fear of needles, which even leads in the most severe cases to avoid any medical treatment. Furthermore, the risk of misadministration is a serious matter of concern. Diabetic patients reported better tolerance to insulin administration with an injection port (i-Port®, Medtronic). We evaluate the applicability of this device in an attempt to reduce the negative impact of multiple needle sticks and increase patient satisfaction.

Study design, size, duration: Proof of concept study including 12 voluntary and healthy oocyte donors to explore the validity for ovarian stimulation with the i-Port® device, that comprises a subcutaneous soft cannula for medication delivery that is virtually painless applied with the aid of an inserter. The device must be replaced with a new one after 3 days. The study was conducted between November and December 2016.

Participants/materials, setting, methods: GnRH antagonist protocol started with an initial dose of 150-300 UI/day of urinary FSH. When lead follicle reached 13-14 mm, the antagonist was administered daily, and final ovulation was triggered with a GnRH agonist. All drugs were administered exclusively through the i-Port®. The main objective was tolerance evaluation assessed with a questionnaire focused on the comfort and the occurrence of adverse reactions. Retrieved eggs and gonadotropin consumption were also evaluated.

Main results and the role of chance: The mean age was 26.0 ± 4.1 years, being in seven cases the first time they underwent ovarian stimulation. No adverse events were reported, only in one case it was not possible to administer medication due probably to the obstruction of the cannula, which made it necessary to replace the device with a new one. The device was very well accepted taking into account that all patients stated that they would use it again and would strongly recommend it to other patients. The length of stimulation was 9.3 ± 1.2 days, with a mean gonadotropin consumption of 2131.3 ± 623.8 IU and 14.4 ± 8.1 retrieved eggs, similar to clinical efficacy that we observe in our donation program during the same period (2450.0 ± 763.1.8 IU consumed and 15.5 ± 7.1 oocytes, non significant). Considering all drugs administered, 12.5 ± 3.2 shots would have needed, that were replaced by the application of 3.3 ± 1.2 devices. No differences were observed in gonadotropin consumption or retrieved eggs in donors with previous cycles, suggesting that there is no interference in the absorption of the medication.

Limitations, reasons for caution: This was a proof of concept study with a limited number of patients oriented to validate the usability of the device. We are conducting a prospective and randomized comparative study with a higher number of cases to contrast the hypothesis of better tolerance compared with conventional injections.

Wider implications of the findings: Any patient, not only donors, could benefit from using the iPort®. Self-administration usually increases anxiety of patients not only because of needle sticks but also because a poor administration when injected may lead to suboptimal response. iPort® supports drug administration to make easier the follow up treatment.

Trial registration number: Not applicable.

P-790 The ARTHIQS joint action – EU wide institutional practice and inspection guidelines in ART

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Study question: Can heterogeneous levels of regulatory practice and the transposition of the EU Tissue and Cells Directive (EUTCD) be harmonised to levels of established good practice?

Summary answer: Working widely with the European Union (EU) ART community, ARTHIQS has analysed current practice, developed and disseminated institutional and inspection guidelines and established networks.

What is known already: The specificities of reproductive medicine are not perfectly reflected in the EUTCD. Member State legislation is also varied. This complexifies the missions of inexperienced CA and inspectors and could give rise to concerns about quality, traceability, vigilance, safety and ethics in ART cross border care.

Beyond the challenges of developing generally applicable guidelines, the major objective of the joint action is to have them adopted by the CA and modify their practices.

Study design, size, duration: An initial survey completed by 3 interactive workshops led to the development of two guidelines on institutional good practice and inspection of ART establishments. These guidelines form the basis of training and dissemination.

ARTHIQS is a 3 year EU funded Joint Action project - http://www.arthiqs.eu/

Participants/materials, setting, methods: Fifteen EU MS and nine collaborative partners elaborated surveys and guidelines through interactive authoring and technical workshops.

Training and dissemination is on-going. The EC/CHAFEA and the joint action interact with the MS CA committee to build the stakeholder network.

ESHRE provided invaluable feedback and the Council of Europe and WHO are informed of the joint action's progress and results.

A specific work,package and an External Advisory Board continuously evaluate the joint action's processes and products.

Main results and the role of chance:

- **An EU wide Survey** of the organisational and institutional status and needs of ART Competent Authorities/Delegated Bodies was carried out and analysed.
- **Institutional guidelines** were elaborated to help MS develop appropriate ART governance.
- **Inspection guidance** was produced to help guide and standardise ART establishment inspection and training. This has been completed by a Curriculum for the selection and education of inspectors.
- **Dissemination** of these guidelines is on-going through the CA-ART network and through training of CA staff and inspectors. The role of national, EU wide and international organisations in their critique and uptake is essential.
- **A Network** of ART contact points within the CAs has been established conjointly by the project and the EC. It will continue to exist after the joint action. It is expected to facilitate the implementation of the outcomes of this EU Joint Action in the MS and provide the EC with ART experts, helping to improve legislation.

Limitations, reasons for caution: Developing and disseminating EU-wide guidelines perceived as pertinent by all stakeholders is delicate. The guidelines aim to improve quality, traceability, vigilance and safety of all participants and especially mothers and children. Cross border heterogeneity and weak communications continue to make this task difficult. Dissemination is a key activity.

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Wider implications of the findings: ART governance is evolving in the EU, however much remains to be done. The joint action aims at harmonising practices in CA and inspectorates. ARTHIQS feeds into on-going projects and provides the EC/CHAFEA with EU-wide resources. The joint action lays foundations for future CA cooperation.

Trial registration number: not applicable.

P-791 Risk of juvenile idiopathic arthritis among children born to women with fertility problems: a nationwide register-based cohort study

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Study question: To examine whether children born to women with fertility problems have an increased risk of juvenile idiopathic arthritis (JIA) compared with children born to fertile women.

Summary answer: There was an increased JIA risk in children born to women with fertility problems; the risk in children born after fertility treatment was not increased.

What is known already: Potential long-term adverse health consequences after fertility treatment has received increasing attention. The existing studies primarily concern childhood cancer, asthma and mental disorders but it has also been suggested that mechanical manipulations of the gametes and embryos as well as the hormonal stimulation used in fertility treatment may affect the development of autoimmune diseases (e.g. diabetes mellitus type 1, hypothyroidism and JIA). However, only few studies have investigated the association between fertility treatment and risk of autoimmune diseases and only one very small study with few exposed cases and short follow-up has examined the risk of JIA after fertility treatment.

Study design, size, duration: This retrospective register-based cohort study included all 1,084,184 live-born children in Denmark between 1 January 1996 and 31 December 2012. All children were followed from date of birth until first diagnosis of JIA as diagnosed in the Danish National Patient Register, date of 16th birthday, date of emigration, date of death or date at the end of follow-up (31 December 2014), whichever occurred first.

Participants/materials, setting, methods: In total, 911,173 children were born to fertile women. 173,011 children were born to women who had ever experienced fertility problems; of these, 37,120 children were conceived after ART procedures and 52,808 children were conceived after use of fertility drugs only. Information on maternal fertility status and fertility treatment was obtained by linkage to The Danish Infertility Cohort. Cox regression models were applied to estimate hazard ratios (HRs) for JIA with adjustment for potential confounders.

Main results and the role of chance: A total of 2,237 children received a diagnosis of JIA during the follow-up period. Children born to women who had ever experienced fertility problems had a modest, but statistically significantly, increased risk of JIA (HR 1.15; 95% confidence interval (CI) 1.03-1.29) compared to children born to fertile women. However, children conceived after use of any ART (HR 1.03; 95% CI 0.81-1.30), in vitro fertilization (IVF) (HR 0.98; 95% CI 0.72-1.35), intracytoplasmic sperm injection (ICSI) (HR 0.96; 95% CI 0.63-1.46) or fertility drugs only (HR 1.13; 95% CI 0.94-1.37) were not found to be at higher risk for JIA compared with children born to fertile women. We also analysed the risk of JIA among children conceived after respectively any ART, IVF, ICSI or fertility drugs only, compared to a reference group of children born to all other women with fertility problems, but without the type of treatment analysed, in order to investigate whether there was an additional effect of fertility treatment besides the underlying infertility; however, the conclusions remained unchanged.

Limitations, reasons for caution: Even though the present study was based on data from high-quality Danish health registers, we cannot completely rule out that the exposure variables and the outcome variable might have been slightly misclassified, which would have led to a small bias of the results.

Wider implications of the findings: The results are based on national data and our findings can therefore be applied to other similar populations. However, at this point, further studies are needed to confirm our results and elucidate the explanatory factors of a potentially increased risk of JIA in children born to women with fertility problems.

Trial registration number: Not applicable.

P-792 Procoagulant properties of circulating microparticles, and subsequent intravascular coagulation, in women with ovarian hyper stimulation for in vitro fertilization

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Study question: Can endothelial, erythrocyte, leukocyte or platelet cell microvesiculation be responsible for the procoagulant state observed after ovarian hyper stimulation for *in vitro* fertilization (IVF)?

Summary answer: Although no vesicular marker of cell activation quantitatively changed throughout IVF cycle, microparticles' procoagulant qualitative properties increased at ovulation triggering, followed by subsequent intravascular coagulation.

What is known already: IVF and more Ovarian Hyperstimulation Syndrome (OHSS) histories are risk factors for intravascular thrombosis during the first trimester of pregnancy. Several modifications of coagulation factors have been invoked to explain the procoagulant state following IVF; however, the involved mechanisms remain poorly understood. Recently, microparticles (MPs) have emerged as new cell-derived effectors resulting from cell activation in the vascular compartment with a key role in regulating haemostasis. We hypothesized that MPs are associated with the coagulation disturbance observed after IVF.

Study design, size, duration: Prospective cohort study, performed between the 25th of April 2012 and the 2nd of January 2015 in the Department of Reproductive Medicine of the Poissy's University Hospital.

Fifty one not smoking infertile volunteers, aged 18 to 35 years, with neither cardiovascular disease nor Lupus erythematosus related disease, and assessed from their day 3 basal hormonal assessment, at least to the ovarian triggering of their IVF cycle were included. Samples were frozen stored before being processed.

Participants/materials, setting, methods: Included women were scheduled for blood sampling at 6 different times of the IVF cycle, including day 3 basal hormonal assessments, first day of FSH stimulation, ovarian triggering day, day 2 embryo transfer, mid-luteal phase, and pregnancy test. For each sample, MP subsets from endothelial, erythrocyte, leukocyte, and platelet origins, MP functional properties (Tissue Factor-Dependent Procoagulant Activity (MP-TF), Plasmin Generation Capacity (MP-PGC)), and markers of intravascular clot formation (Fibrin monomer, D-dimer) were measured.

Main results and the role of chance: Women were 33.1 years old (95%CI 31.8-34.3). Among the 51 included women, the stimulation protocol was antagonist in 33 (64.7%), long in 10 (19.6%), and ultra-long in 8 (15.7%). Sixteen women (31.3%) were assessed at all times of their IVF cycle. Of the 6 sampling initially scheduled, women had a mean of 3.9 assessments (95%CI 3.3-4.3). The minimum number of available samples was 25 at mid-luteal phase. Endothelial-, erythrocyte-, leukocyte- and platelet-MPs showed no quantitative variation throughout IVF cycle. MP-TF showed a dramatic burst in activity, from 13.2 fM [25th-75th

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percentile, 5.9-20.3 fM] to 32.5 fM [17.3-56.7 fM] (+145%), limited to the time of embryo transfer (p = 0.01). A wave of MP-PGC subsequently occurred during the luteal phase, with maximum activity of 2.8 mDO/min [1.6-4.6 mDO/min], compared to basal activity of 1.7 mDO/min [1.2-2.7 mDO/min] (+65%), shifted at mid-luteal phase (p = 0.028). This thrombolytic reaction was significantly associated with the peak of MP-TF activity (spearman r^2 = 0.63, p = 0.001). Finally, subclinical intravascular clot formation was proved by Fibrin monomer formation, from 2.4 µg/ml [1.8-3.5 µg/ml] to 3.6 µg/ml [3.3-4.8 µg/ml] (+50%, p = 0.001) and D-dimer formation from 0.28 µg/ml [0.23-0.48 µg/ml] to 1.44 µg/ml [1.0-2.1 µg/ml] (+415%, p = 0.001), both shifted at mid to late-luteal phase.

Limitations, reasons for caution: Because of the small size of our cohort and of the heterogeneity of our stimulation protocols, our results should be interpreted with caution.

Wider implications of the findings: We highlighted for the first time, a procoagulant process, occurring throughout IVF cycle, for which microparticles are mediators or markers. MP-TF could thus potentially provide a promising marker of thrombotic risk following IVF.

Trial registration number: Not applicable.

P-793 Effects of FSH vs LH stimulation on bone metabolism

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Study question: Does ovarian stimulation therapy affect bone metabolism – and is there any difference between rFSH and HMG stimulation?

Summary answer: Fertility treatment seems to have a protective influence on bone in short term. There was no significant difference between rFSH and HMG.

What is known already: Osteoporosis is one of the most common illnesses worldwide which causes high morbidity. Since 1947 we know about the positive effects of estrogen on bone [Reifenstein EC et al. Clin Orthop Rel Res 2011]. Since in 2006 Sun et al showed that ovarectomised rats do not lose as much bone mass after hypophysectomy than without hypophysectomy, a direct influence of FSH on osteoclasts has been discussed [Sun L et al. Cell 2006]. As FSH as well as LH/FSH combinations are used for ovarian hyperstimulation in fertility treatment, effects on bone metabolism should be investigated.

Study design, size, duration: In this prospective observational study blood samples of 95 patients were taken on four time points during 106 long protocol stimulation cycles (FSH n = 16, HMG n = 54): T1 – luteal phase of the previous cycle/start of GnRH analogues; T2 – start of stimulation; T3 – oocyte retrieval; T4 – luteal phase of stimulation cycle.

Participants/materials, setting, methods: Inclusion criteria were FSH <20mIU/ml at T1, no disease or medication with influence on bone metabolism, like anorexia or systemic glucocorticoids. At every time point bone resorption markers C-terminal telopeptide (CTX) and tartrate-resistant phosphatase (TRACP) as well as formation markers osteocalcin (OC) and bone specific alkaline phosphatase (BAP) and sex hormones follicle stimulating hormone (FSH), luteinising hormone (LH) and 17ß-estradiol (E2) were measured. At T1 calcium and vitamin D3, at T4 progesterone were additionally analysed.

Main results and the role of chance: Parameters of bone resorption CTX and TRACP as well as the bone formation marker OC were – in part significantly* – lower at T4 (CTX: 0.24 mg/ml, TRACP: 1.79U/l*, OC: 16.6 ng/ml*) than T1 (CTX: 0.26 mg/ml, TRACP: 2.09U/l*, OC: 18.5 ng/ml*). Only the bone formation marker BAP was slightly, but not significantly, higher at T4 (9.95 µg/l) than at T1 (9.75 µg/l).

Parameters of bone metabolism did not significantly differ after HMG- compared to FSH-stimulation. BAP, OC and TRACP were slightly but not significantly higher in the FSH group at T4 (FSH group: BAP 10.23 µg/l, OC 18.39 ng/ml, TRACP 2.08U/l; HMG-group: BAP 9.87 µg/l, OC 16.22 ng/ml, TRACP 1.71U/l), CTX was equal for both groups (0.24 mg/ml).

Limitations, reasons for caution: This study included a limited number of patients. Especially in the FSH subgroup data of 16 patients only were analysed. Only short term effects are investigated in this study. Further studie are needed for more evidence concerning bone safety of ovarian stimulation.

Wider implications of the findings: Ovarian hyperstimulation seems to have a beneficial effect on bone, so there is no reason not to treat patients with an elevated risk for osteoporosis.

There is no evidence for a difference between FSH and HMG. Nevertheless one should keep in mind that pregnancy itself is connected with bone loss.

Trial registration number: As this study is not an interventional study there is no trial registration number.

P-794 Morbidity and mortality associated with assisted reproduction tecniques (ART) in our hospital since 2011 to 2016

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Study question: To analyze the morbidity and mortality associated with the techniques of fertility carried out since the start of our service in 2011 until 2016.

Summary answer: We analyzed the rates of complications including mortality in our patients in five years, and we evaluate if the data are similar to the literature

What is known already: The incidence of hemoperitoneum is 0.06-0.08% and is caused by trauma to ovarian or intrafolicullar vessels, pelvic organs (uterus, bladder, colon) or iliac vessels.

The infection is an exceptional complication, with a frequency of 0.007% according to SEGO. There are several theories to explain infection: direct inoculation from the vagina, a history of pelvic inflammatory disease or endometriosis, or puncture of an intestinal loop.

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of unknown etiology, with an incidence of 0.6-10% of patients undergoing ovarian stimulation. Severe SHO occurs in 0.52% of IVF cycles and may compromise the lives of patients.

Study design, size, duration: We performed a retrospective observational study of the 8301 patients treated using assisted reproduction techniques, both through artificial insemination (AI) and in vitro fertilization (IVF) in our hospital between 2011 and 2016 and we analyzed the morbidity and mortality derived from the fertility treatments

Participants/materials, setting, methods: We analyzed a total of 8301 patients, who underwent ART during the years 2011 and 2016 in the same hospital, analyzing the rates of complications during the cycles. The morbidity analized were: hemoperitoneum with or without income, mild ovarian hyperstimulation syndrome (no admission) or moderate-severe (with admission), infection, embryonic reduction and other complications. We also look for cases of maternal mortality associated with ART.

Main results and the role of chance: From 2011 to 2016, a total of 8301 patients diagnosed with infertility were treated in our hospital (1,084 in 2011, 1,413 in 2012, 1,589 in 2013, 1,450 in 2014, 1,401 in 2015 and 1,364 in 2016). The complications derived from the treatments were: 11 hemoperitoneum, 9 of them (0.1%) required admission, 3 (0.2%) in 2012, 1 (0.1%) in 2013 and 4 (0.3%) in 2014. Two patients did not required admission, one in 2014 and one in 2015.

In total we had 78 cases of hyperstimulation syndrome, 34 (0.4%) were moderate and severe cases that required hospitalization, 6 cases (0.6%) in 2011, 5 (0.4%) in 2012, 7 (0.4%) in 2013, 9 (0.6%) in 2014, 5 (0.4%) in 2015 and 2 (0.1%) in 2016. 44 cases (0.5%) were mild cases with outpatient management: (0.2%) in 2011, 4 (0.3%) in 2012, 11 (0.7%) in 2013, 9 (0.6%) in both 2014, 2015 and 2016.

Both cases of infection (0,02%) required hospital treatment, one in 2013 and one in 2014.

15 patients (0,2%) requested embryo reduction, 6 (0.6%) in 2011, 2 (0.1%) in 2012 and in 2014, 1 in 2013 (0.1 %) and 4 (0.3%) in 2015

We had no mortality cases.

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Limitations, reasons for caution: It should be taken into account that this is a descriptive and retrospective study to analyze the complications associated with our sample, but we do not have a comparison group. The large sample size helps us get our data.

Wider implications of the findings: The rates of complications associated with ART are similar in patients treated at our service than data published in the literature, and remain stable over the years. Fortunately, we have not mortality cases associated with these treatments.

Trial registration number: It's not a trial.

P-795 Triplet-to-twin fetal reduction may increase risk of demise in the remaining fetuses

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Study question: Does fetal reduction (FR) improve the neonatal outcomes of the remaining twins in triplet pregnancies following ART?

Summary answer: FR was associated with a reduced risk of premature delivery <32 weeks but increased risk of subsequent fetal demise.

What is known already: ART has increased the incidence of triplet pregnancies which are associated with potentially severe obstetrical complications. Triplet-to-twin FR is frequently proposed to women with these higher-order pregnancies, in the expectation that such a technique may reduce the subsequent risks of preterm delivery and neonatal death. Nonetheless, given the scarcity of studies evaluating the benefits of this approach, the ideal management strategy of triplet pregnancies remains rather elusive.

Study design, size, duration: We performed a United Kingdom population-based retrospective analysis of all twin and triplet intrauterine pregnancies following ART cycles included in the anonymized database of the Human Fertilisation and Embryology Authority (HFEA) between 2000 and 2011. We subdivided these pregnancies in the following 3 study groups: twin (Group A), triplet-expectant-management (Group B) and triplet-to-twin (after FR, Group C) pregnancies. Pregnancies in which a fetal termination for reasons other than elective FR was performed were excluded.

Participants/materials, setting, methods: A total of 27005 intrauterine twin/triplet pregnancies were included in the analysis (26124 twin, 777 triplet-expectant-management and 104 triplet-to-twin pregnancies, respectively). Our main outcomes measures were preterm delivery <32 weeks and the number of live birth deliveries. Given the effect of female age on miscarriage rates, we performed multivariable logistic regression in all these analyses to account for this potential confounder, performing pairwise comparisons whenever warranted.

Main results and the role of chance:

	Twin (Group A) n = 26124	Triplet-expectant-management (Group B) n = 777	Triplet-to-twin (Group C) n = 104	p-value
Female age				<0.001
18-34	61.7%	57.9%	53.9%	
35-37	24.6%	25.5%	31.7%	
38-39	9.4%	8.9%	7.7%	
40-42	4.1%	7.3%	6.7%	
>42	0.2%	0.4%	0.0%	
Type of ART treatment				<0.001
Artificial insemination	1.4%	3.9%	8.7%	
IVF/ICSI	98.6%	96.1%	91.3%	
Preterm delivery				
<32 weeks	8.4%	24.0%	9.8%	<0.001*‡
Live birth delivery				
Of at least 1 fetus	94.9%	92.2%	90.5%	<0.001*†
Of at least 2 fetuses	82.9%	82.9%	46.8%	<0.001†‡
Of 3 fetuses	-	61.1%	-	-

*The predicted preterm and live-birth delivery rates were adjusted for female age using multivariable logistic regression; pairwise comparisons p<0.05 for the *A-versus-B, †A-versus-C and ‡B-versus-C groups, respectively.*

Twin and triplet-to-twin pregnancies had similar preterm delivery rates <32 weeks (8.4% and 9.8%, respectively), both of which were significantly lower than the risk observed in the triplet-expectant-management group (24.0%). Conversely, triplet-to-twin pregnancies had the lowest chance of delivering at least two live borns, when compared to both the twin and triplet-expectant-management groups. Specifically, women who performed FR had a 46.8% chance of delivering both of the remaining twins, which was significantly lower than the 82.9% odds of triplet-expectant-management pregnancies.

Limitations, reasons for caution: Owing to the use of this particular registry, we could not account for other potential confounding factors such as the timing and specific methods used for FR, which both may have varied substantially among the treatment centers contributing data.

Wider implications of the findings: While our results confirm that FR may indeed reduce the risk of preterm delivery, they also show that this may come at the cost of an increased risk of fetal demise. Such conclusions stress the utmost importance of considering triplet pregnancies as an ART complication to be avoided.

Trial registration number: Not applicable.

P-796 The rate of elective terminations in second trimester due to fetal malformation is similar in pregnancies conceived after assisted reproduction compared with spontaneous conception

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Study question: Are women pregnant after assisted reproduction less likely to terminate a pregnancy after detection of a fetal malformation compared with spontaneously pregnant women?

Summary answer: We found similar rates of second trimester pregnancy terminations, regardless of mode of conception.

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What is known already: Numerous studies have found an increased risk of congenital malformations among live-born children conceived after assisted reproduction compared with spontaneously conceived children. The underlying subfertility matters, as children born by couples taking a long time to conceive spontaneously also have an increased risk of congenital malformations. Still the assisted reproductive technology may also play a role. Little is known concerning the prenatal detection rate of fetal malformations in pregnancies conceived after assisted reproduction and it is unclear if women pregnant after assisted reproduction are more or less likely to terminate a pregnancy due to a fetal malformation.

Study design, size, duration: A 6-year Danish population-based cohort study including 20 723 pregnancies conceived after assisted reproduction and 280 287 spontaneously conceived pregnancies. Assisted reproduction included IVF, ICSI, frozen-embryo-transfer, intrauterine insemination and ovulation induction. All data were retrieved from the Danish Fetal Medicine Database, which includes information on combined first trimester screening for aneuploidies, anomaly scans and data on pregnancy outcome from the Danish Cytogenetic Central Register, the Danish National Patient Register and the Danish National Birth Register.

Participants/materials, setting, methods: Pregnant women who opted for the Danish National prenatal screening program from 2008 to 2013. Data on fetal malformations were based on ultrasound findings at the combined first trimester screening for aneuploidies (week 11 to 14) or at the anomaly scan (week 18 to 21). In Denmark termination of pregnancies is legal until 12 weeks of gestation. Late termination can be performed until 22 weeks of gestation but requires specific permission from the Health Authorities.

Main results and the role of chance: Fetal malformations were detected among 1.95% (n = 404) of the fetuses conceived after assisted reproduction versus 1.73% (n = 4860) of the spontaneously conceived fetuses, p = 0.02. When adjusting for known confounders such as BMI, smoking, maternal age and parity the adjusted odds ratio was 1.18 [1.03-1.35] for fetal malformations. The rate of termination after week 12 was 0.9% among women pregnant after assisted reproduction and 0.8% among spontaneously pregnant women, p = 0.02. Still, the indications for terminating the pregnancy differed between the two groups. In women pregnant after assisted reproduction 87.7% of the second trimester terminations were on fetal indications, where the fetus was diagnosed with a malformation or severe illness, compared with only 71.2% of the second trimester terminations in the group of spontaneously pregnant women, p<0.0001. As most pregnancies after assisted reproduction are planned, this explains why very few second trimester terminations were performed on other indications, than fetal malformations or severe fetal illness.

Limitations, reasons for caution: Information on mode of conception is self-reported in the Danish Fetal Medicine Database and may therefore be insufficient. Furthermore the study included only fetal malformations detected by ultrasound and were not confirmed by karyotypes or postnatal pediatric examinations.

Wider implications of the findings: The higher risk of malformations in live-born children conceived after assisted reproduction versus spontaneous conception cannot be explained by a higher tendency to continue a pregnancy despite a fetal malformation in women pregnant after assisted reproduction. Therefore a prenatal selection-bias cannot explain the increased malformations rates after assisted reproduction.

Trial registration number: Not apllicable

POSTER VIEWING SESSION
STEM CELLS

P-797 Effects of VEGF+ adipose tissue derived mesenchymal stem cells with platelet rich plasma on inbred rat ovarian functions in premature ovarian insufficiency model

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Study question: Does the single or co-transplantation of VEGF$^+$ mesenchymal stem cells (MSCs) and platelet rich plasma (PRP) improve the ovarian function of cyclophosphamide treated inbred rats?

Summary answer: MSCs supported the ovarian stroma improving the development of functional follicles by interacting with theca and granulosa cells, while PRP-treatment demonstrated anti-inflammatory and anti-apoptotic effects.

What is known already: Premature over insufficiency (POI) is an infertility problem that occurs in females under 40 years of age and is currently limited in treatment possibilities. The in vivo and in vitro studies including stem cell treatment-attempts to recover the ovarian function and fertility are at very early-stage.

Study design, size, duration: In this study, 72 inbred rats (Fisher 344) were used, which were divided in 5-groups (control, sham, VEGF$^+$MSC, PRP and VEGF$^+$MSC+PRP). The POI model was developed by injecting 100 mg/kg cyclophosphamide in the abdominal area of the rats. MSCs were isolated from adipose tissue of Fisher-344 rats (n = 10) and the blood was collected from the same animals to isolate PRP by serial centrifugation. MSCs were labeled with GFP and improved with VEGF by gene transfection.

Participants/materials, setting, methods: After two-months following the injection of VEGF$^+$-MSCs and/or PRP, the blood samples were analyzed with respect to the AMH and Estrodiol (E_2) levels. The ovarian tissues were collected and the follicles were counted and categorized following the staining with haematoxylin-eosin. The gene expression analyses were performed to estimate the degree of inflammation and apoptosis beside the regenerative status of the tissue. The results were confirmed by immune-fluorescence staining and evaluated by blind-analyses.

Main results and the role of chance: After two months following the transplantation of cells and/or PRP into the disease model rats, the total follicle numbers of VEGF$^+$MSC and VEGF$^+$MSC+PRP groups were significantly improved compared to the sham-group, P < 0.01 and P < 0.01 respectively. The recovery level of follicle number for these two groups corresponded to 63% of that in control group. The fractions of primordial follicles within the total follicle count in VEGF$^+$MSC and VEGF$^+$MSC+PRP groups were estimated as high as those in the control. PRP-group samples showed weak recovery with high variance within itself. In all experimental groups, AMH levels were increased with respect to sham-group, and quantitatively reached to half of the control group level. The E_2 level was increased to the highest in the VEGF$^+$MSC+PRP group (P:0.002, P:0.035 and P:0.014 respect to sham, VEGF$^+$MSC and PRP). The BMP4, TGF-beta and IGF-1 expressions in VEGF$^+$MSC+PRP group were increased 6-, 5-, 7-fold compared to control-group, and significantly higher than in sham-group. Additionally, the MSCs were localized in the stroma and around the primary, secondary, pre-antral and antral follicles. In the follicles of VEGF$^+$MSC and VEGF$^+$MSC+PRP groups, DDX-4 positive cells were also determined.

Limitations, reasons for caution: During the study, the number of animals in sham group was reduced to its half of initial number due to the side effect of cyclophosphamide treatment. However the remaining number of animals in this group was still sufficient for a proper statistical analysis.

Wider implications of the findings: MSCs application was shown to be important for the recovery of ovarian function and folliculogenesis. The alone-administered PRP was not sufficient for the recovery, but it was crucial for inflammation suppression. However, when MSCs and PRP were given together, the highest recovery in functional follicles was obtained on this model.

Trial registration number: none.

P-798 Estrogen enhances endothelial differentiation and angiogenic activity in rat adipose-derived stromal cells

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Study question: Whether estrogen regulates endothelial differentiation of mesenchymal stem cells (MSCs), and may be helpful in cytotherapy against ischemic organ injury.

Summary answer: The endothelial differentiation of adipose-derived MSCs could be enhanced by the estrogen treatment.

What is known already: Menopause women has been reported poorer prognosis after cardiovascular event than man at the same age. The rapidly deteriorating endothelial function after menopause has been suggested the major cause. Cytotherapy with MSCs has been proved effective to recovery ischemic organ injury,however, the efficacy of cytotherapy depends on the endothelial differentiation of MSCs. To reverse the poorer prognosis of menopause women in cardiovascular event, estrogen may play an important role.

Study design, size, duration: Animal study, total 12 Sprague Dawley (SD) rats were used in this study through September to December 2016.

Participants/materials, setting, methods: Adipose-derived MSCs were isolated form abdominal adipose tissues of male SD rats and cultured in endothelial cell growth medium (EGM-2) with or without estrogen treatment (l00 nM of 17α-estradiol) for 14 days to induce endothelial differentiation. After culturing, Flow cytometric analysis, Matri-gel and trans-well examination, and immunofluorescent stainings were performed to examine the endothelial differentiation and angiogenic activity.

Main results and the role of chance: Restuls from immunofluorecent stainings showed the nuclear translocation of estrogen receptor β (ER-β) in adipose-derived MSCs after culturing with estrogen. Expression levels of endothelial progenitor cell markers (CXCR4/CD34, Sca-1/KDR, c-Kit/CD31) were increased with estrogen treatment in flow cytometric analysis. The migratory and *in vitro* angiogenic activity of adipose-derived MSCs were also enhanced by estrogen treatment. In addition, abundant expression level of angiogenic factors VEGF was found in adipose-derived MSCs treated with estrogen.

Limitations, reasons for caution: Our data was limited to animal study, further study will be needed to examine the regulatory mechanism and signalling pathway of estrogen in MSCs.

Wider implications of the findings: Estrogen regulating endothelial differentiation of MSCs could be applied in cytotherapy against ischemic organ injury in rats.

Trial registration number: nil.

P-799 Galectin-1 Regulates Mouse Trophoblast Stem Cell Migration and Invasion

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Study question: What is the role of Galectin-1 in trophoblast stem cells?

Summary answer: Galectin-1 could regulate mouse TS cell migration and invasion.

What is known already: Galectin-1 is highly expressed in mouse blastocysts and trophoblast giant cells during implantation and dysregulated Galectin-1 is associated with many pregnancy-related abnormalities. Elevated Galectin-1 contributes to certain cancer cells invasion.

Study design, size, duration: in vitro cell culture and in vivo embryo study.

Participants/materials, setting, methods: The expression of Galectin-1 in mouse oocyte, pre-implantation embryos (zygote, 2, 4, 8 cell stages, and blastocyst), and mouse TS cells were examined by RT-qPCR, Western blot, immunofluorescence. Endogenous Galectin-1 expressions in mouse TS cells were manipulated by adenoviral infection or small interfering RNA transfection, and the expressions of downstream genes were assayed by RT-qPCR and Western blot. The migratory and invasiveness of mouse TS cells were examined by trans-well migration and invasion assay, respectively.

Main results and the role of chance: Galectin-1 is expressed in mouse oocyte, all stages of pre-implantation embryos, and mouse TS cells. Peak levels of Galectin-1 mRNA and protein are detected in day 4 and 5 after induction of TS cells differentiation, respectively. Overexpression of Galectin-1 increases TS cell migratory and invasive capability, and knockdown of Galectin-1 attenuates these effects. Additionally, expressions of matrix metalloproteinase (MMP) 2/9 as well as several epithelial mesenchymal transition (EMT) and TGFβ related molecules were altered after manipulating Galectin-1 expression in TS cells.

Limitations, reasons for caution: Experiments in embryos and TS cells are limited in mouse only.

Wider implications of the findings: Our study revealed the importance of Galectin-1 in trophoblast stem cell differentiation and trophoblast cell migration as well as invasion, which may provide us a better understanding of the complexity of molecular dynamics during implantation and placentation.

Trial registration number: We do not need trial registration number.

P-800 Higher restoration of liver fibrosis by stem cells derived from Menstrual Blood compared to those of Bone Marrow in mice model

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Study question: Could menstrual blood stem cells (MenSCs) be considered as an appropriate substitute of bone marrow stem cells (BMSCs) for the treatment of acute liver failure?

Summary answer: Menstrual blood stem cells have the more therapeutic effect than bone marrow stem cells in the restoration of acute liver failure.

What is known already: Cell-based therapies have focused on the use of human bone marrow-derived mesenchymal stem cells for liver regeneration, but their main limitation is limited availability, invasive sample collection, and low proliferation capacity.

Menstrual blood-derived stem cells have advantages including high proliferation rate, high accessibility, renewability, and sustainability in culture without genetic abnormalities and non-ethical concerns. In previous studies, we showed the higher in vitro transdifferentiation ability of these cells into hepatocyte- like cells in comparison with BMSCs.

Study design, size, duration: After in vitro differentiation of both stem cells into hepatocyte progenitor-like (HPL) cells, MenSCs and BMSCs in undifferentiated or differentiated state were separately transplanted intravenously 48 hours after induction of liver fibrosis in Balb/C mice (each group = 5 mice). For in vivo tracking, the cells were labeled by GFP expression. All mice were sacrificed at 7 and 30 days post-transplantation to examine biochemical and molecular markers and evaluate pathological appearances.

Participants/materials, setting, methods:

- GFP transfection was done by Electroporation and tracking of GFP-transduced MenSCs was done with an in vivo imaging system.
- H&E, PAS and Masson's trichrome staining was done to evaluate liver tissue restoration, glycogen storage ability, and collagen appearance.
- The mRNA expression of hepatic markers including tyrosine aminotransferase, albumin, cytokeratin-18 and cytochrome P450 family 7 Subfamily A member 1 (CYP7A1) and inflammatory markers including IL-6 and COX-2 was examined using Real-time PCR.

Main results and the role of chance:

- Tracking of GFP-labeled MenSCs showed the migration of cells into injured areas of the liver during 1 hour after transplantation.
- MenSCs and differentiated MenSCs succeeded to engraft into the host liver.
- Transplantation of both type stem cells could improve glycogen storage ability and decrease the collagen fibers deposition in the liver sections.
- Assessment of serum parameters at day 7 exhibited significant reductions (all $P \leq 0.01$), such this downward trend continued until day 30.
- The restoration of liver biochemical markers and changes in mRNA levels of hepatic markers like albumin and CYP7A in the MenSCs treated group was more significant compared with the BMSCs treated group.
- MenSCs were more efficient than BMSCs in the suppression of mRNA expression of inflammatory markers such as interleukin-6 and cyclooxygenase-2 ($P \leq 0.01$, $P \leq 0.05$, respectively).
- HPL cells in reference to undifferentiated cells had the better effectiveness in the treatment of the acute liver injury.

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Limitations, reasons for caution: The possibility of animal mortality after liver fibrosis induction: the induced animals are so susceptible due to the disorder of liver functionality.

Wider implications of the findings: MenSCs can be introduced as a new promising replacement for BMSCs for liver fibrosis treatment.The evaluation of these cells in standardized clinical trials is required to prove the future clinical application of these cells. Moreover, more studies on possible mechanisms govern on liver tissue restoration by MenSCs should be defined.

Trial registration number:

P-801 Efficient production of trophoblast lineage cells from human induced pluripotent stem cells

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Study question: there may be different optimal conditions for trophoblast differentiation of human induced pluripotent stem cells (hiPSCs).

Summary answer: there are optimal conditions for BMP4 (Bone morphological protein 4) -induced trophoblast differentiation from hiPSCs.

What is known already: Embryonic stem (ES) cells and hiPSCs can differentiate into extra-embryonic cells in the presence of BMP4.

Different differentiation protocols are often used in different studies, and varying culture conditions may have resulted in conflicting observations.

Furthermore, given that the genetic background of human induced pluripotent stem cells is predominantly responsible for transcriptional and epigenetic differences.

In this study, in order to define molecular features of BMP4-mediated differentiated cells and optimal conditions for efficient production of trophoblast cells, we conducted a large-scale, high-fidelity gene expression assay following treatment of differentiated cells with various concentrations of BMP4.

Study design, size, duration: Three iPS cell lines (menstrual blood, uterine endometrium and placental artery) established in our laboratory were used in this study.

hiPSCs were subcultured 7.5×10^4 cells per well to the animal and human-drived substitues free medium, supplemented with Bone morphogenetic protein 4 (BMP4 0,5,10,20,50,100 ng/mL) up to 10 days.

Participants/materials, setting, methods: hiPS cells used in this study were established by retroviral infection of 4 reprogramming factors (OCT-3/4, SOX2, c-MYC, and KLF4), and transgene silencing was confirmed.

Samples were harvested from 1, 3, 5, 8, and 10 days for analysis of differentiation.

We analyzed population doublings (PDs), chemiluminescence enzyme immunoassay and electrochemiluminescence immunoassay of placental hormones in the culture medium, Immunofluorescence, gene expression assay, and flow cytometery.

Main results and the role of chance: The morphologic changes occurred after about 5days.

High dose of BMP4 (100 ng/mL) induce apotosis in some hiPSC.

The concentration of HCG, P4 and the expression levels of various trophoblast marker genes are upregulated by BMP4 in dose-dependent manner in all types of hiPS cells.

HCG and HLA-G positive cells were different in immunohistochemistry in day10.

High dose BMP4 differentiation formed cluster in array card analysis. However, miRNA expression states were not upregulated by BMP4.

Furthermore, single-cell analysis using flow cytometry revealed that the proportions of cells expressing HCG were increased by treatment with a high dose of BMP4, but not similar results were identified in KRT7 (cytotrophoblast marker) and HLA-G (extravilloustrophoblast marker)

It was demonstrated that hiPS cells could differnttiate to trophoblast lineages in animal and human-drived gradient free conditions, since the expression states of trophoblast-related genes and proteins were detected on BMP4 supplementation. Non-coding RNA expression states were affected by donor cell types rather than dose effect of BMP4 and single cell analysis reveal there is tendency to differentiate into syncytiotrophoblast or extravilloustrophoblast. It indicates the optimal conditions for trophoblast differentiation from hiPS cells differs among parental cell lines.

Limitations, reasons for caution: Our result indicated there are optimal conditions for trophoblast differentiation from hiPSCs.

However, We constituted only in vitro cell differentiation model.

It is difficult to reproduce early placentation, because of lacking in vivo model.

Our results will aid in the production of patient-specific trophoblast cells from hiPSCs.

Wider implications of the findings: Establishment of differentiation protocol into trophoblast lineage become powerful tool to research early human placentation.

Trial registration number: Nothing.

P-802 ChIP-seq reveals a different genomic distribution of epigenetic mark H3K27me3 at ICR/DMRs of imprinted genes induced by morphine in mouse Embryonic Stem Cells (mESCs)

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Study question: To elucidate if the chronic treatment of morphine could have an impact on imprinted genes at transcriptional an epigenetic level.

Summary answer: Morphine modifies the expression of the imprinted genes and increases the enrichment of H3K27me3 at the ICR/DMR of imprinted genes in mESC.

What is known already: Normal mammalian development requires maternal and paternal contribution, attributed to imprinted genes. Those are controlled by cis-acting regulatory elements, termed imprinting control regions (ICRs) and have parental-specific epigenetic modifications. ICRs are CG-rich sequences, differentially methylated in a region called DMRs. We have found that the epigenetic regulator complex polycomb/H3K27me3; involved in processes such as cellular memory, genomic imprinting, X-inactivation, and the transgenerational epigenetic memory; can play an important role in the epigenetic memory induced by morphine in mESC. Therefore, our aim is to analyze, if the impact of morphine or other external stimuli, could have important consequences at genomic imprinting.

Study design, size, duration: Chronic morphine treatment was performed incubating mESC during 24 h. Gene expression and epigenetic analysis was carried out by qRT-PCR and ChIP-sequencing approaches.

Participants/materials, setting, methods: The global expression of H3K27me3 was quantified by immunoblotting analysis. Imprinted gene expression was carried out by qRT-PCR. The genomic distribution of the epigenetic mark H3K27me3 was explored by the chromatin immunoprecipitation with subsequent High Throughput Sequencing (ChIP-seq). Following the sequencing, the bioinformatic resources allowed to study the imprinted genes.

Main results and the role of chance: qRT-PCR showed that morphine changes the gene expression of imprinted genes, confirming that those genes are the targets of morphine in mESC. For example, we observed that paternally expressed Rasgrf1 and Peg1 showed a significant decrease in their expression while paternally expressed Peg5 and Peg10 presented an increased expression. Moreover, immunoblotting analysis indicated a significant down regulation in H3K27me3 at protein level. ChIP-Seq analysis confirmed the previous results, showing a decrease in the number of peaks and genes enriched by H3K27me3 after morphine treatment. The alterations induced by morphine at H3K27me3

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gene enrichment were not restricted to promoter. In fact, they were more frequent in the gene body and transcription start site regions and, mostly, they matched with CpG island sites. Analyzing the distribution of the H3K27me3 along the landscape of imprinted genes, surprisingly, we observed an increase of H3K27me3 enrichment at ICRs/DMRs, providing an epigenetic mechanism that can be modified by morphine. Maternally expressed Meg3 and paternally expressed Peg1 were affected in the DMRs, and maternally expressed H19 and paternally expressed Peg3 and Peg10 in the ICRs, all of them colocalizing with CpG island regions.

Limitations, reasons for caution: Further methylation analysis would be of interest to complete and support our study.

Wider implications of the findings: Morphine modifies the epigenetic status of imprinted genes at ICRs/DMRs providing an epigenetic mechanism that can be modified by external environmental agents. The effect of morphine on imprinting could have an impact on the cellular memory, genomic imprinting, X-inactivation, embryionic development and transgenerational epigenetic inheritance, worthy to be study.

Trial registration number: N/A.

P-803 Study of the reparative effects of human mural granulosa iPSCs-derived granulosa cells on premature ovarian failure in mice

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Study question: Can graulosa-like cells which derived from human mural granulosa iPS(GiPS) restore ovarian dysfunction associated with premature ovarian failure (POF)?

Summary answer: Graulosa -like cells derived from human GiPS, may restore damaged ovarian function and offer a suitable clinical strategy for regenerative medicine.

What is known already: Young female patients who receive chemotherapy frequently face premature ovarian failure (POF),for which there are currently no ideal treatments or medications. Furthermore, apoptosis of ovarian granulosa cells is an important mechanism underlying the decline in ovarian reserve and function.

Study design, size, duration: Mice ovaries were injured with cyclophosphamide to create a damaged ovary mice model. Transplanted GiPS-derived granulosa like cells were injected into the tail vein of sterilized mice (n = 20), or culture medium was injected into the sterilized mice via the tail vein as chemoablated group (n = 20). Non-sterilized mice were untreated controls (n = 20). ovarian tissue weight,plasma E2 level,and the number of follicles was compared in all groups.

Participants/materials, setting, methods: Human mural granulosa cells were collected from egg follicles retrieved from women undergoing infertility treatment.After short-term culture, the granulosa cells were infected with Sendai viral vectors. GiPSCs were subjected to further determin their pluripotent characteristics. We used two step approaches comprising in vitro treatments with cocktails of growth factors (Wnt 3a,ActivinA,BMP4,ect) for 12days. Expression of granulosa cell markers were analyzed using immunohistochemistry,FACS,and quantitative PCR.

Main results and the role of chance: Ovarian tissue weight,plasma E2 level,and the number of follicles were all significantly higher in cell treated group compared with chemoablated group.These results suggest that GiPS-derived granulosa like cells may not only effectively enhance granulosa cells growth and repair damaged ovarian tissue, but may also maintain the ovarian tissue niche, promoting follicular development and maturation.

Limitations, reasons for caution: Although GiPSCs-derived granulosa cells did not induce teratoma formation in vivo in the POF mice,the undifferentiated iPSCs are still potential risk in clinical trial in future.

Wider implications of the findings: Our research results showed that GiPSCs-derived granulosa cells can repair ovarian injury, stimulate regeneration, and improve ovarian function. GiPSCs-derived granulosa cells transplantation may provide an effective and novel method for treating POF.

Trial registration number: not applicable.

P-804 Identification of integrin heterodimers expressed on the surface of spermatogonial stem cells in inbred mice

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Study question: What is type of integrin heterodimers expressed functionally on the undifferentiated spermatogonial stem cells (SSCs) derived from mouse with inbred strain?

Summary answer: The inbred (C57BL6) mouse-derived SSCs have integrin $\alpha_4\beta_1$ and $\alpha_V\beta_1$, $\alpha_V\beta_3$ and/or $\alpha_V\beta_5$ on plasma membrane.

What is known already: Microenvironments surrounded with various extracellular matrix (ECM) components can decide specifically the fate of SSCs and integrin heterodimers recognizing directly ECM proteins play an important role in transporting ECM-derived signals into cytoplasm, resulting in inducing a variety of biological functions such as cell attachment, self-renewal and differentiation. However, to date, studies on type of integrin heterodimers expressed functionally on the undifferentiated SSCs derived from mouse with inbred strain with reproducibility of conclusions remain unclear.

Study design, size, duration: We tried to investigate systematically what kind of integrin heterodimers are expressed transcriptionally, translationally and functionally in the SSCs derived from testis of inbred (C57BL/6) mouse.

Participants/materials, setting, methods: Magnetic activated cell sorting (MACS) using Thy1 antibody was used for isolating SSCs from testis, and real-time PCR or fluorescence immunoassay was conducted for measuring transcriptional or translational level of integrin α and β subunits in the isolated SSCs. Subsequently, antibody inhibition assay was conducted for confirming functionality of presumed integrin heterodimers.

Main results and the role of chance: In quantifying transcriptional levels of genes encoding total 25 integrin subunits, 4 integrin α (α_3, α_4, α_V and α_L) and 5 integrin β (β_1, β_3, β_4, β_5 and β_7) subunit genes showed significantly increased transcriptional up-regulation, compared to the other integrin subunit genes. When translational levels of the integrin α subunits showing high transcription level (α_3, α_4, α_V and α_L) were measured, significantly strong translational up-regulation of integrin α_V and α_L subunit genes were detected, whereas integrin α_3 and α_4 subunit genes were translated weakly. Moreover, integrin β_1 and β_4 subunit genes showed significantly stronger translational up-regulation than integrin β_3, β_5 and β_7 subunit genes. Based on these results, we speculated that the undifferentiated SSCs derived from C57BL/6 mouse might express integrin $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_V\beta_1$, $\alpha_V\beta_3$ and/or $\alpha_V\beta_5$ on plasma membrane. Subsequently, attachment of integrin $\alpha_4\beta_1$ and $\alpha_V\beta_1$, $\alpha_V\beta_3$ and/or $\alpha_V\beta_5$ in SSCs to fibronectin and vitronectin showed significantly higher levels of adhesion, whereas attachment of integrin $\alpha_3\beta_1$ to laminin didn't showed significantly adhesion. Moreover, functional blocking of integrin $\alpha_4\beta_1$ and $\alpha_V\beta_1$, $\alpha_V\beta_3$ and/or $\alpha_V\beta_5$ in SSCs significantly inhibited attachment to fibronectin and vitronectin, respectively.

Limitations, reasons for caution: This results were derived from only mouse with inbred strain. Therefore, studies on the expression of integrin heterodimers in hybrid strain mouse, other species and human should be conducted in the future.

Wider implications of the findings: This information will greatly contribute to constructing non-cellular niche supporting self-renewal of SSCs derived from inbred mouse in the future.

Trial registration number: N.A.

P-805 A Simple Method to Differentiate Mouse Embryonic Stem Cells into Male Germ Cells

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Study question: We evaluated several methods for creating and maintaining differentiating cells in order to induce primordial germ cell (PGC) appearance.

Summary answer: Creation of embryoid bodies (EB) in hanging droplets (HD) supplemented with differentiating factors yielded PGCs more consistently and at a higher proportion.

What is known already: Differentiation of embryonic stem cells (ESC) often proceeds through the formation of EBs, three-dimensional aggregates of stem cells which have the ability to spontaneously form ectodermal, mesodermal, and endodermal layers. EBs can be formed in HDs or in suspension. After formation, EBs can be cultured with growth factors in order to obtain PGCs. Development of PGCs has also been reported without the need to go through EB formation by simply adding growth factors to cells cultured in wells.

Study design, size, duration: From April 2016 to January 2017, 260 EBs were formed from mouse embryonic stem cells (mESC) via the hanging drop method. After formation, EBs were maintained with media supplemented with differentiating factors in either HDs or in culture dishes. ESCs were also separately cultured with differentiating factors in well-plates. PGC differentiation was assessed by germ cell-alkaline phosphatase (GCAP) and immunofluorescence staining.

Participants/materials, setting, methods: mESCs were maintained on mouse embryonic fibroblast (MEF) cells. Confluent colonies were passaged by trypsinization. 1000 feeder-free mESCs were placed in 25uL HDs. After 2 days, EBs were either transferred to HDs containing RA or cultured in dishes with RA. On day 8, EBs were treated with collagenase and stained for AP/OCT4/DAZL. A separate cohort of mESCs were maintained in well-plates supplemented with Activin A/bFGF/KSR. Cells were re-plated after 3 days and maintained with LIF/BMP4/BMP8b/SCF/EGF.

Main results and the role of chance: After the development of 260 EBs in their respective HDs, 100 EB were re-plated on day 2 to corresponding HDs containing RA while the remaining 160 EBs were transferred to RA-supplemented Petri dishes. On day 8, EBs cultured in HDs reached approximately 150um in diameter, while EBs in dishes reached 280-300um. All EBs were frozen in a DMSO solution at day 8 for later analysis. After thawing, EBs were treated with collagenase IV to isolate individual cells for staining. GCAP staining of EBs cultured in HD yielded an average of 77% GCAP positive cells, while those in dishes only presented 22% GCAP postivity. EBs were also assessed for OCT4 activity to determine loss of stemness. In HD-cultured EBs, 40% of isolated cells were strongly positive for OCT4, compared to 60% of cells isolated from EBs cultured in dishes, indicating a higher level of cell differentiation in the former. DAZL staining was also performed to undoubtedly detect male germ cell appearance. EBs cultured in HDs yielded 5% DAZL-positive cells, whereas EBs cultured in dishes only yielded 1% DAZL-positive cells. Because of the peculiarity of the multi-well culture, assessment for PGD appearance is still being carried out.

Limitations, reasons for caution: This is a preliminary study assessing only the early stages of differentiation with somewhat limited efficiency. Once confirmed that HD culture in the presence of differentiating factors is the best method to expeditely generate PGCs, the next step would be toward enhancing efficiency and coaxing towards post-meiotic stages.

Wider implications of the findings: ESC differentiation offer the possibility to generate gametes in vitro. Mouse ESCs provide a great inexpensive and ethically acceptable model to experiment with these procedures. These cells, however, are not genotyped to the individual. Using pluripotent stem cells would ultimately generate genotyped gametes useful for patient treatment.

Trial registration number: Not applicable.

P-806 Differentiation of primordial germ cells from premature ovarian insufficiency-derived induced pluripotent stem cells under 3i culture system

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Study question: Can the induced pluripotent stem cells (iPSCs) derived from patient with primary ovarian insufficiency (POI) differentiate into germ cells for potential disease modeling *in vitro*?

Summary answer: POI-iPSCs under 3 inhibitors (3i) culture system have potential of germline competence and could differentiate into primordial germ cells (PGCs) with potential for meiotic progression.

What is known already: iPSC technology is an approach for generating patient-specific stem cells for disease modeling and for developing novel therapies. It has also been confirmed that iPSCs differentiate into germ cells under routine culture. But we did not know if the iPSCs derived from POI patients had the potential of germline competence, and if PGCs differentiated from POI-iPSCs could initiate the meiotic progression.

Study design, size, duration: We compared the differentition ability of PGCs, the gene expression level of germ cell-related genes, and the potential of initiating the meiotic progression in iPSCs derived from POI patients with an iPSCs derived from normal fibroblasts, and in 3i culture system with routine culture system.

Participants/materials, setting, methods: We established 12 POI-iPSCs by retroviral transduction using defined factors under 3i culture system. The morphology, growth characteristics, gene expression profiles, and differentiation *in vitro* and *in vivo* of POI-iPSCs were analyzed. Then, POI-iPSCs were pre-induced in N2B27 medium, then were transfer to PGCs medium for further induction. We analyzed some critical determinant for human PGC gene induction, such as sox17, BLIMP1, CD38, and meiotic progression assay.

Main results and the role of chance: Our results showed the POI-iPSCs were successfully generated from POI patients' adult cells under 3i culture system. The POI-iPSCs can be induced to differentiation into PGCs with normal karyotypes. This study proved that disease special iPSC lines derived from POI patients could be generated and successfully differentiated into PGCs with the potential for meiotic progression.

Limitations, reasons for caution: In this study, the differentiated PGCs could progress further to meiosis, and form follicles remains to be determined in the study of POI.

Wider implications of the findings: This provide a new biological cell model and approach for studying pathogenesis of POI with genetic tendency and discovering potential drugs for POI.

Trial registration number: No clinical trial.

P-807 Systemic administration of bone marrow derived stem cells or uterine derived stem cells is superior to local injection in a mouse uterine injury model

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Study question: It is unknown whether uterine-derived cells (UDCs) may confer an advantage over bone marrow derived cells (BMDCs) by local intrauterine injection or systemic administration

Summary answer: BMDCs is greater than UDCs, and systemic administration results in better recruitment to the uterus than local injection

What is known already: Stem cells have been shown to undergo recruitment into the uterus where they can differentiate into endometrial cells

Study design, size, duration: Eight-week-old C57BL/6J wild-type mice underwent local uterine injury to the left horn following laparotomy. Mice were divided into four treatment groups (n = 14 in each group) as BMDCs-iv, BMDCs-iu, UDCs-iv and UDCs-iu groups. BMDCs ($1x10^7$cells), UDCs ($5x10^5$ cells) from green fluorescent protein (GFP transgenic donors) or saline (control) were injected either intravenously or locally (uterine lumen) into wild-type recipients. Additionally, groups PBS-iv and PBS-iu received saline injection serving as controls.

Participants/materials, setting, methods: Mice were sacrificed following 2 or 3 weeks after BMDCs or UDCs injection, and uteri tissues were collected for FACS analysis and immunohistochemistry/immunofluorescence studies.

Main results and the role of chance: FACS analysis showed that mice injected intravenously with BMDCs had increased recruitment of GFP^+ cells at

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2 weeks and 3 weeks compared to those injected locally (0.264% vs.0.042%, P = 0.0075,2 weeks; 0.217% vs. 0.03%,P = 0.004, 3weeks, to the non-injured uterus) (0. 261% vs.0.0475%, P = 0.0024,2 weeks; 0.22% vs. 0.058%, P = 0.029, 3 weeks, to the injured uterus). Moreover, mice injected intravenously with UDCs demonstrated greater recruitment of GFP^+ cells to the injured uterus at 3 weeks compared with those injected locally (0.048% vs. 0.022%) (P = 0.0029). In addition, systemic injection BMDCs led to significantly greater recruitment of GFP^+ cells at 2 weeks and 3 weeks to the uterus compared with UDCs (0.252% vs.0.022%, P = 0.0042, at 2 weeks; 0.217% vs. 0.0225%, P = 0.0237, at 3 weeks, to the non-injured uterus) (0.288% vs.0.044%, P = 0.0053, at 2 weeks; 0.22% vs. 0.048%, P = 0.0162, at 3 weeks, to the injured uterus). For local injection, there were no significant differences between BMDCs or UDCs Cells at 2 weeks or 3 weeks (P > 0.05). Immunohistochemical staining of uterine tissues demonstrated that GFP^+ cells were found in stroma but not in epithelium or blood vessels. Immunofluorescence results revealed that GFP^+ cells were mostly CD45 negative, and were negative for CD31 and cytokeratin, confirming their stromal identity.

Limitations, reasons for caution: Further studies are necessary to investigate the differences in therapeutic potential and mechanisms between BMDCs and UDCs in repairing the uterus.

Wider implications of the findings: These findings may inform investigators developing stem-cell based therapies targeting the uterus.

Trial registration number: not applicable

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