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ABSTRACT BOOK

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SHORT PAPER PRESENTATIONS TUESDAY

SRFPD SRF POST-DOCTORAL PRIZE SESSION

PD.1 Paternal under and over-nutrition alters sperm RNA profile and in vitro embryo development in mice

Hannah L Morgan; Nadine Holmes; Sonal Henson; Victoria Wright; Adam J Watkins
University of Nottingham

Background: While sub-optimal paternal diet at the time of conception has been linked to perturbed offspring health, the underlying mechanisms are undefined. One proposed mechanism is a change in sperm RNA content (coding and non-coding) impacting subsequently on early embryo development. This study aimed to examine how sub-optimal paternal diet alters sperm RNA profiles and their impact on early embryo dynamics in mice.

Methods: Male C57/BL6J mice were fed either a control (CD: 18% casein, 10% fat, 21% sugar), low-protein (LPD: 9% casein, 24% sugar, 10% fat), or Western diet (WD: 19% casein, 34% sugar, 21% fat) for 8 weeks before being mated naturally with CD fed females. One-cell embryos were cultured individually in EmbryoMax-KSOM media (37C; 5% CO2) in an EmbryoScope time-lapse incubator and analysed for morphometric and cell division dynamics. After mating, mature CD and WD epididymal sperm were collected for sequencing of total (mRNA and non-coding) RNA by Illumina HiSeq. Differential expression was determined using the DeSeq2 package.

Results: We observed 41 differentially expressed RNAs (with 32 protein coding, 2 long non-coding, 1 miRNA) in LPD sperm and 142 (with 53 protein coding, 11 lncRNA, 36 miRNA and 4 other small-nuclear RNA) in WD sperm compared to CD sperm. Embryos from WD and LPD males took a significantly longer time to progress from the stage of pronuclear fading to blastocyst expansion. Furthermore, LPD and WD derived embryos displayed a reduced cleavage time between the 2-cell and 4-cell stages.

Conclusion: Our observations indicate that both paternal undernutrition (LPD) and overnutrition (WD) slow the rate of early embryo cleavage, however it did not affect their ability to reach the blastocyst stage. These early morphokinetic changes may be related to differences in sperm RNA profile, and could have implications for the future health of the offspring.

PD.2 Beneficial effects of melatonin on canine oocyte nuclear maturation through reduction of oxidative stress

Fataneh Ghafari; Richard J Piercy; Ali A. Fouladi-Nashta
Royal Veterinary College

Maturation of canine oocytes in vitro is very low accompanied with high degeneration. High fat content of predisposes them to oxidative stress and production of high levels reactive oxygen species (ROS). These studies have tested the antioxidant effect of melatonin (MTN) mediated through its receptors (MTNR-A1and B1) on dog oocytes during culture. Ovaries of spayed dog were used for isolation and culture of cumulus oocyte complexes (COCs). Experiment1 analysed expression of MTNRs by immunofluorescence staining. MTNR-A1 was highly expressed evenly scattered within the oocytes and with lower intensity in the cumulus cells. Experiment 2: COCs (n=300) were cultured in the absence (control) or presence of 1nM, 100nM, and 10uM MTN for 72h. Nuclear stage in meiosis was determined after denuding and fixing the oocytes and Hoechst staining. Lowest percentage of oocytes remained at GV stage (6.7% ± 4.2, highest MII maturation rate (32.3% ± 6.4), minimum degeneration (20.5% ± 3.2) and maximal meiotic resumption (56.2% ± 8.6) were all result of 100nM supplementation of melatonin in the basic maturation medium ($P < 0.05$). Experiment 3 analysed the effects of melatonin on ROS production using DCHFDA staining. Densitometry using ImageJ software showed that the overall intensity of fluorescence was lower in oocytes treated with 100nM melatonin ($p<0.05$). Experiment 4 analysed impact of melatonin supplementation on expression of ROS repairing enzymes (GPX1, SOD1, SOD2, GSR & CAT). Fresh and in vitro matured COCs were snap-frozen (25 COCs per group/repeat) after in vitro maturation and used for RNA extraction and qRTPCR. Melatonin reduced SOD-2 ($p<0.05$) and catalase ($P<0.01$) expression. These data suggests melatonin protection of oocytes from oxidative stress results in reduced degeneration and increased nuclear maturation. The beneficial effect of melatonin supplementation during in vitro maturation of dog oocytes may result in production of developmentally competent oocytes which requires further investigation.
Acknowledgement: Duchenne UK.

PD.3 SMAD4 within granulosa cells promotes adhesion of transzonal projections to the oocyte in the mouse

Sofia Granados Aparici; Qin Yang; Hugh Clarke
Research Institute, McGill University Health Centre

Development of the oocyte requires physical contact with the surrounding granulosa cells of the follicle, which provide nutrients and regulatory signals. Contact is achieved through specialized filopodia termed transzonal projections (TZPs)

that emanate from the granulosa cells. Growth-differentiation factor (GDF) 9, a TGFβ family member produced by the oocyte, increases the number of TZPs, but how it does so is unknown. We examined the effect of depleting SMAD4, an essential element in the canonical TGFβ signaling pathway, on the generation and stability of TZPs. We deleted Smad4 from granulosa cells of mice using Amhr2-Cre in vivo and estrogen receptor (ER)-Cre in vitro, both on a background where Cre-mediated recombination generates green fluorescent protein (GFP), allowing us to monitor Cre activity within individual cells. Amhr2-Cre induced recombination in only a small fraction of the granulosa cells; we observed no reduction in TZP-number in these cells. ER-Cre generated a high percentage of GFP-positive granulosa cells and a ~70% reduction in SMAD4. However, we observed no reduction in TZP-number, and the granulosa cells generated new TZPs when reaggregated with granulosa cell-free oocytes. In contrast, when the granulosa cell bodies were physically stripped from the oocytes, leaving the TZPs embedded within the zona pellucida, TZP-number was reduced by 50% in the SMAD4-depleted group. Strikingly, N-cadherin and Notch2 transmembrane proteins, which are specifically expressed in granulosa cells, were reduced by up to 50% in the SMAD4-depleted group. We suggest that GDF9-SMAD signaling modulates a molecular network of cell adhesion proteins that helps to stabilize the attachment of TZPs to the oocyte surface.

PD.4 Dynamics of extracellular vesicle based embryo-maternal communication in pre-implantation microenvironment

Kasun Godakumara; Alireza Fazeli

Estonian University of Life Sciences

Background: One of the most critical steps in mammalian reproduction is Implantation. Embryos with impaired capacity of embryo-maternal cross talk are thought to have reduced potential for implantation. One agent of embryo-maternal communications are extracellular vesicles (EV). EVs are lipid-bilayer bound biological nanoparticles implicated in intercellular communication between many of the known cell types.

Methods: In the current study, we have isolated EVs from trophoblast analogue JAr spheroids and supplemented the EVs to receptive endometrium analogue RL95-2 cells to simulate pre-implantation embryo-maternal dialogue. The transcriptome of the endometrial cells were examined at 30 min, 4h, 24h and 48h intervals using nanopore technology. Resulting datasets were analyzed for differential expression (DE) and gene set enrichment analysis (GSEA) to explore the altered physiology of the endometrial cells.

Results: At the time points 30 min, 4h and 24h the endometrial cells showed a significantly altered transcriptome compared to unsupplemented controls. The degree of DE was highest in 24h. In the 48h time point, endometrial cells exhibited a much-reduced level of DE. Biological pathways crucial in embryo implantation such as focal adhesion were highly enriched in endometrial cells in 30min, 4h and 24h time points. The enrichment level was reduced in 48h time point.

Conclusion: Trophoblast EVs induce a swift and drastic effect on endometrial transcriptome. The effect peaks around 24h of EV supplementation, indicating a generalized effect on cell physiology. Alterations are especially apparent in biological pathways critical in embryo implantation such as focal adhesion, extracellular matrix receptor interactions and cytokine receptor interactions. The altered state of the endometrial transcriptome seems to return to pre-EV treatment level after 48h indicating that the EV induced transcriptomic changes are non-permanent and may need continues dosing to maintain. These observations can be helpful in elucidating the dynamics of embryo-maternal communication in the pre-implantation period.

PD.5 The repair of DNA double-strand breaks is deficient in human preimplantation embryos indicating that genome editing performed at early developmental stages may carry additional risks

Nada Kubikova[1]; Marga Esbert[2]; Shiny Titus[3]; Clement Coudereau[4]; Munuse Savash[1]; Richard Scott[3]; Dagan Wells[4]

[1]University of Oxford; [2]IVI Barcelona; [3]IVI RMA; [4]Juno Genetics

Background: The emergence of genome editing (GE) technologies, such as CRISPR, offers the possibility of converting mutant genes to the wild-type (normal). The application of GE at the preimplantation stage is controversial due to uncertainty over safety and concerns about the alteration of the human germline. However, from a technical perspective editing at this stage is attractive since it is the only developmental stage where delivery of GE reagents to every cell can be guaranteed. To better understand aspects of safety and potential efficacy, we evaluated the cellular response to CRISPR in human preimplantation embryos.

Methods: GE reagents were injected into donated human oocytes at the same time as fertilisation with donor sperm using ICSI. The CRISPR components targeted a non-coding site, inducing a double strand DNA break (DSB). An artificial DNA fragment was provided, which cells could potentially use for DSB repair via homology directed repair (HDR).

Embryos were grown for 60 hours, then disaggregated. Individual cells underwent whole genome amplification and next generation sequencing, revealing chromosome abnormalities and allowing interrogation of the targeted site.

Results: Alteration at the targeted site was detected for 24/25 (96%) embryos. The dominant repair pathway was non-homologous end joining (NHEJ) (81%). HDR occurred in 19% of the edited cells. In 17 embryos (68%) failure of DSB repair led to abnormalities affecting the chromosome containing the target site.

Conclusions: To correct a mutation, a DSB is created by CRISPR then repaired by HDR using an artificial wild-type template. However, our results show that the great majority of DSBs are repaired by NHEJ, a pathway that tends to introduce additional mutations. Furthermore, in some embryos DSBs were not resolved, leading to chromosomal abnormalities. These findings illustrate that early human embryos are deficient in DSB repair, and caution against the application of traditional CRISPR strategies at this stage.

SHORT PAPER PRESENTATIONS WEDNESDAY

SP1A ARCS PRE-REG SHORT PAPERS JEAN PURDY PRIZE

1A.1 Does number of sperm used at in vitro fertilisation insemination impact rate of normal and abnormal fertilisation events?

Bethany Muller; Hannah Newby; Rachel Gregoire

Hewitt Fertility Centre

Background: The number of sperm used in IVF is typically between 100,000 and 500,000 motile sperm/ml[1], and commonly calculated based on the concentration and the proportion showing progressive motility. Due to historic inclusion of morphology in our centre's insemination calculation, IVF was routinely locally performed with concentrations between 500,000 and 1.67 million motile sperm/ml. This is despite sperm morphology being associated with a high degree of subjectivity and sampling error, and its application to clinical decision-making having been cautioned[2]. Although fertilisation and implantation rates met accepted performance indicators (PIs), polyploidy rate exceeded the agreed PI of 6%[2]. In this work, sperm morphology was removed from the calculation to reduce sperm concentration.

Methods: Insemination calculation was modified from 100,000 normal motile sperm/ml to 150,000 motile sperm/ml. The impact of the change was assessed by comparing the fertilisation, polyploidy, and implantation rates between 240 vs 279 IVF cycles using Chi-Squared test. Data were split into female age groups: 35, 36-39, 40, and 'all ages'. Additionally, Pearson's correlation was used to retrospectively investigate trends between insemination concentration and fertilisation and implantation PIs for 4,053 IVF cycles.

Results: No significant differences were found between fertilisation and implantation rates in any age group. Polyploidy rates were significantly reduced (35: 10.2% (125/1230) to 5.3% (82/1536) ($p<0.0001$); 36-39: 11.7% (75/640) to 7.0% (64/915) ($p<0.01$); all ages: 10.3% (222/2519) to 6.2% (169/2716) ($p<0.0001$)), except in older women (40: 7.6% (22/289) to 8.7% (23/265) ($p=0.76$)). A positive correlation between sperm concentration at insemination and polyploidy rate was observed at concentrations between 150,000 and 1 million motile sperm/ml ($p<0.05$). No other relationships were noted.

Conclusion: Reduction in sperm concentration at insemination reduces polyploidy events, whilst maintaining fertilisation and implantation rates. The absence of an accepted standard for IVF insemination concentration is likely leading to suboptimal clinical outcomes for IVF patients.

1. ESHRE Guideline Group on Good Practice in IVF Labs (2015). Revised guidelines for good practice in IVF laboratories. Human Reproduction, 31 4, 685-686.

2. ESHRE Special Interest Group of Embryology & Alpha Scientists in Reproductive Medicine (2017). The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. Reproductive Biomedicine Online, 35 5, 494-510.

1A.2 Computer aided semen analysis (CASA) in therapeutic semen analysis improves embryologist efficiency and accuracy

Tamanda Timvere; Rachel Gregoire

Hewitt Fertility Centre

Background: Manual semen analysis (SA) is the gold standard of SA (1). Aspects of manual SA impact the accuracy of analysis. Morphology and motility rely on subjective estimation of percentage. Concentration assessment is more

objective but remains subject to sources of error (calculations, dilution, pipetting and placing of coverslip on counting chambers), limiting the efficiency and accuracy of practitioners.

Aims: Introduce and validate CASA in therapeutic sperm preparation laboratories. Observe differences in efficiency and accuracy.

Methods: 65 semen samples were produced at a fertility clinic for routine SA or use in treatment and assessed in triplicate: Embryologist using local methods (Method 1), Embryologist using CASA (Method 2) and Andrologist following WHO 2010 guidelines current at the time (Method 3). Each method was timed to assess efficiency. IVF insemination volume was calculated using parameters from each assessment to compare parity.
Statistical analysis:Friedman Test & repeated measures ANOVA (significance level of $p<0.05$)

Results: Method 2 was quicker than Method 1 ($p<0.001$).
Mean insemination volume calculated from Method 2 was higher than Method 1 & 3 ($p<0.001$).
Fast-progressive motility was higher in Method 3 than Method 1 & 2 ($p<0.001$).
Slow-progressive motility was higher in Method 1 than Method 2 & 3 ($p<0.001$).
Non-progressive motility was higher in Method 1 than Method 2 & 3 ($p<0.001$).
Non-progressive motility was higher in Method 2 than Method 1 & 3 ($p<0.001$).
No difference observed in concentration assessments between all 3 methods ($p=0.181$).

Conclusion: Manual Andrologist and CASA are more efficient than Embryologists assessments. No difference was observed in concentration between all methods. Embryologist and Andrologist manual methods observed higher progressive motility than CASA, causing reduced insemination volumes calculated from progressive motility and concentration. Unpublished in-house data suggests reduced insemination volumes negatively impact IVF fertilisation for patients with 'borderline' samples. Embryologists adopting CASA improves efficiency when performing sperm preparations and accuracy in IVF insemination.

(1) WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

1A.3 Have online consent forms impacted the likelihood of patients consenting to training?

Morven Dean; Amy Wanless; George Hughes; Anne Quinn

ACU Dundee

It is essential that ART staff are appropriately trained to ensure competency to undertake clinical procedures to the highest standard of technical proficiency. This can only be achieved when gametes and embryos, surplus to treatment requirement, are available for training purposes with appropriate consent. There has been some concern that this availability has declined since the implementation of online consent forms.

The aim of this audit was to identify whether completion of consent forms online has reduced the likelihood that patients will consent to training with their gametes and embryos. Online and paper consent forms of 100 patients per format were evaluated prior to and after the introduction of online consents. The consent to training with gametes and embryos within the relevant HFEA forms were recorded and compared, using a two-way ANOVA test.

When completing paper consents: 71% of female patients consented to training with their gametes, and 63% to training with their embryos; 70% of male patients consented to training with their gametes, and 64% with their embryos. When completing consents online: 57% of female patients consented to training with their gametes, and 49% to training with their embryos; 59% of male patients consented to training with their gametes, and 49% with their embryos. There was a significant decrease in the number of patients consenting to training following conversion to online consent completion.

These preliminary findings create concern over the understanding of online consents, and whether patients are making informed decisions when completing these forms. Reduced consent for training purposes has significant consequences for the development of staff training and skills. Improved patient awareness is critical to prevent further decline in availability of gametes and embryos for training. Finally, it would be of interest to establish whether these results are consistent across all UK clinics.

1A.4 Equilibration of media aliquots - an advisory note

Georgia Manley; Stephanie Gadd
CARE Fertility

Research focus: How constant is osmolality when culture medium is aliquoted to equilibrate prior to use?

The importance of the study: Clinics may aliquot culture media for pre-equilibration for convenience or to minimise wastage. Temperature, container surface area and humidity can increase the evaporation of water from the medium, and subsequently osmolality. Increased osmolality (>300 mOsm/l) has been proven to affect embryo development and viability through increased DNA damage, cell shrinkage and oxidative stress (1). Therefore, measures should be taken to ensure osmolality remains within safe limits.

Study design: Culture media ranging from 1.5ml to 5ml were aliquoted in duplicate directly from the refrigerator in their original containers and into various tube types, then immediately loose capped and weighed. Aliquots were placed into humidified and non-humidified incubators at 37C, mimicking IVF laboratory scenarios. Tubes were reweighed following 24h and 48h incubation, with water evaporation used to calculate changes in osmolality.

Findings: Aliquots incubated in the non-humified environment demonstrated a linear increase in osmolality, which was inversely proportional to the volume aliquoted. This was pronounced in tubes with a two-position cap compared to screw-lid tubes. After 48h in a non-humidified environment, for a 1.5ml aliquot, osmolality increased by 26mOsm/kg in a 10ml screw-top tube and by 36mOsm/kg in a 5ml tube with a two-position cap. In a humidified environment the osmolality increases were 5mOSm/kg and 7mOsm/kg respectively.

Implications: Results from the non-humidified incubator suggest factors such as aliquot volume and humidity must be considered, to prevent osmolality increasing above safe working ranges, especially in extended culture. Aliquot volumes should be considered carefully, and aliquots should be equilibrated in a humidified incubator for a maximum 24h before use.

Limitations: Osmolality was calculated from water loss rather than by direct measurement.

1. Romanova N, Schmitz J, Strakeljahn M, Grünberger A, Bahnemann J, Noll T. Single-Cell Analysis of CHO Cells Reveals Clonal Heterogeneity in Hyperosmolality-Induced Stress Response. Cells. 2022;11(11). establish whether these results are consistent across all UK clinics.

1A.6 Is ICSI really necessary in cases of poor sperm morphology?

Emma Atkinson; Karen Thompson; Laura McArthur
Care Fertility (Leeds)

Objectives: ICSI is mainly used in treatments where the sub-fertility is attributed to a male factor, determined by a pre-treatment semen analysis. The impact of sperm morphology on fertilisation is controversial but there are some studies which demonstrate that poor morphology may contribute to poor or failed fertilisation in these patients (1). The current WHO criteria states that normal morphology of less than 4% is classed as abnormal, and many clinics use this cut-off to decide whether to perform ICSI or conventional IVF. With sperm selection becoming stricter and more in-depth semen analyses being performed, are we performing ICSI unnecessarily for patients with a low morphology score, particularly when other semen parameters are normal?

Methods: This was a retrospective study of IVF cycles that took place at our clinic throughout 2021. Cycles were split into two groups according to the normal morphology score from the most recent semen analysis; scores of less than 4% (group 1), and scores of 4% and above (group 2). Normal fertilisation rates were calculated per cycle, and an average was taken for each group. Results were compared using an independent T-test to determine statistical significance.

Results: The data from 501 IVF cycles were included in the study: 114 cycles in group 1 and 387 cycles in group 2. Average fertilisation rates for each group were 66.00 % and 67.30 %, respectively. The incidence of complete fertilisation failure was 2.6% in group 1 and 4.4% in group 2. Statistical analysis confirmed no significant difference between the two groups.

Conclusions: Our analysis showed no difference in fertilisation rates between patients with poor or normal morphology scores. The results are comparable with previous studies, including a recent meta-analysis (2). It may be time to disregard poor sperm morphology as an indicator for ICSI when all other sperm parameters are normal.

1. Effects of the normal sperm morphology rate on the clinical and neonatal outcomes of conventional IVF cycles Andrologia 2020 Jun;52 (5):e13568 Chen L, Li D, Ni X, Zhu L, Zhang N, Fang J et al 2. The association of impaired semen quality and pregnancy rates in assisted reproduction technology cycles: Systemic review and meta-analysis. Andrologia. 2022 Mar 3:e14409 Del Guidice F, Belladellie F, Chen T, Glover F, Mulloy EA, Kasman AM et al

SP1B BFS EARLY CAREER CLINICIAN SHORT PAPERS

1B.1 A comparison of artificial intelligence (AI) models to predict ploidy status using a morphokinetic and meta-datset of 8147 blastocysts

Thomas Bamford[1]; Christina Easter[2]; Sue Montgomery[1]; Rachel Smith[1]; Rima Dhillon Smith[2]; Amy Barrie[1]; Alison Campbell[1]; Arri Coomarasamy[2]
[1]Care Fertility; [2]University of Birmingham

Objective: This study aims to compare model performance metrics for different AI models built to predict ploidy status using morphokinetic, clinical and embryological data.

Materials and methods: Data for this research was obtained from the treatment of 1725 couples from 2012 to 2021 at nine independent IVF clinics in the UK, using 8147 biopsied blastocysts. All embryos were cultured in a time lapse system. A total of 3004 euploid embryos and 5023 aneuploid embryos were included in the final verified dataset. We developed a total of 12 AI models using four different approaches: binary logistic regression (LR), random forest classifiers (RFC), extreme gradient boosting (XGB) and deep learning (DL). For each AI method three datasets were used to create three separate models, a large dataset of 22 predictive variables and 8027 embryos (model 1), a second smaller dataset of 2373 embryos and 26 predictive variables (model 2) and a final model where the target outcome measure was switched from euploid to aneuploid (model 3). All models were robustly validated using internal-external cross validation.

Results: All morphokinetic variables were significantly delayed in aneuploid embryos. The likelihood of euploidy was significantly increased the more expanded the blastocyst ($p<0.001$) and the better the trophectoderm grade ($p<0.01$). Univariate analysis showed no association with ploidy status for morula or cleavage stage fragmentation, morula grade, fertilisation method, sperm concentration or progressive motility. Male ageing did not correlate with the percentage of euploid embryos when stratified for female age. The best performing model was LR built using the larger dataset with 22 predictors and coding to predict probability of euploidy (AUC 0.71 (95% CI 0.67-0.73). RFC, XGB and DL had an AUC of 0.68, 0.63 and 0.63, respectively.

Conclusion: This study has highlighted the ability to harness morphokinetics as a tool for ploidy prediction using one of the largest datasets known.

1B.2 The effect of cyclophosphamide and ifosfamide in childhood on subsequent male fertility and reproductive function: A systematic review

Camilla Roberts; Kathleen Duffin; Rod Mitchell
University of Edinburgh

Background: Cancer treatment in childhood can impair future fertility, and alkylating agents (e.g. cyclophosphamide and ifosfamide) are considered high risk to fertility(1,2). Currently, there are no established fertility preservation options for pre-pubertal male patients. As such, it is essential to understand effects of chemotherapeutic agents on the pre-pubertal testis. This systematic review aims to investigate the effects of cyclophosphamide and ifosfamide on the pre-pubertal testis.

Methods: PubMed and Embase were searched for articles reporting reproductive outcomes for males treated with cyclophosphamide or ifosfamide when pre-pubertal. Data was extracted on the following outcomes: pregnancy/fatherhood, semen analysis, reproductive hormones, puberty, and testicular volume. Study was conducted as per PRISMA guidelines(3).

Results: Literature search yielded 1,745 studies; after abstract screening, 147 full text papers met the inclusion criteria. This study reports on 35 papers which contained patient specific data. Data was extracted on 236 participants, all of whom had received cyclophosphamide. Median age at treatment and analysis was 9.0 and 16.4 years, respectively. At analysis, 107/126 (84.9%) participants had normal pubertal progression, and 6/57 (10.5%) participants sired a pregnancy. 59/95 (60.2%) were azoospermic. 69/154 patients had elevated FSH levels, with 53/154 >10.4IU/L, predictive of azoospermia(4). 55/170 patients had reduced testosterone, and 10/32 had reduced inhibin B. Cumulative cyclophosphamide dose was available in 95 patients. Increasing doses were associated with reduced sperm count ($p=0.0028$) and elevated FSH ($p=0.0028$), with most occurring at doses >8mg/m2. There was no significant correlation between dose and testosterone, LH, inhibin B, or testicular volume.

Conclusion: Pre-pubertal cyclophosphamide exposure is associated with dose-dependent reduction in sperm count and FSH, indicating impaired spermatogenesis. There was limited patient specific data about cyclophosphamide dosage and reproductive outcomes, and no such data for ifosfamide. Incorporating reproductive outcomes into cancer follow-up will facilitate further understanding of the effects of these drugs administered in childhood.

1. Mulder RL, Font-Gonzalez A, Green DM, Loeffen EAH, Hudson MM, Loonen J, et al. Fertility preservation for male patients with childhood, adolescent, and young adult cancer: recommendations from the PanCareLIFE Consortium and

the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Oncol. 2021;22(2):e57-67. 2. Chow EJ, Stratton KL, Leisenring WM, Oeffinger KC, Sklar CA, Donaldson SS, et al. Pregnancy after chemotherapy in male and female survivors of childhood cancer treated between 1970 and 1999: A report from the Childhood Cancer Survivor Study cohort. Lancet Oncol. 2016 May 1;17(5):567-76. 3. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Vol. 10, Systematic Reviews. 2021. 4. Kelsey TW, McConville L, Edgar AB, Ungurianu AI, Mitchell RT, Anderson RA, et al. Follicle Stimulating Hormone is an accurate predictor of azoospermia in childhood cancer survivors. PLoS One. 2017;12(7):1-11.

1B.3 Draft national legislation on assisted human reproduction (AHR): Determining opinions and perceptions among fertility patients

Sorca O Brien[1]; Laurentina Schaler[1]; Louise Glover[2]; Mary Wingfield[1]
[1]Merrion Fertility Clinic; [2]Merrion fertility Clinic/UCD/TCD

Objective: Ireland remains one of five European countries lacking dedicated legislation on Assisted Human Reproduction (AHR).(1) Draft legislation, first introduced in 2017, was recently revised with a view to enacting in 2022.(2) Challenges can arise when healthcare legislation is enacted without key stakeholder involvement or input, as highlighted by recent controversial legislative decisions in the US (Roe-vs-Wade). Having previously assessed HCP opinion of the proposed Irish AHR legislation, we sought to ascertain the views of fertility patients prior to its implementation.

Study design: A survey questionnaire based on all clinically relevant aspects of the Irish draft AHR Bill 2017 was previously distributed to relevant HCPs using an online platform. An amended version was circulated to all patients who had a doctor consultation at our fertility clinic from 2020-2021 inclusive.

Results: Over 1000 respondents completed the survey (response rate 25%). Most (99.7%) supported the establishment of a regulatory authority. Similar to our previous study of HCPs, over 80% of patients support access to varied techniques, with >70% expressing support for treatment availability regardless of relationship status or gender identity. Views of patients are at variance with several proposals surrounding surrogacy, with 86% favouring a pre-birth order to assign parentage from birth, rather than the proposed birth order 6 weeks after birth. The majority (89%) also support legislation around international surrogacy. Contrary to the draft Bill, 72% of patients believe that men, as well as women, should be able to use posthumously any stored gametes or embryos belonging to the deceased partner or the couple.

Conclusion: This study has uniquely ascertained the views of fertility patients to help inform the AHR national legislation as it nears completion. Similar studies could help other countries, and policy bodies such as ESHRE, to frame good legislation in this specialised and complex field.

1. Atlas. European Atlas of Fertility Treatment Policies (Fertility Europe in conjunction with the European Parliamentary Forum for Sexual and Reproductive rights): https://fertilityeurope.eu/european-atlas-of-fertility-treatment-policies/, 2021. 2. DoH. Health (Assisted Human Reproduction) Bill 2022, 2022.

1B.4 Male fertility: Impact of SARS-CoV-2 vaccines

Laurentina Schaler[1]; Jordi Guardiola[2]; Magda Ghanim[3]; Julia Kaulsay[4]; Niamh Cantwell[2]; Vincent Kelly[3]; Mary Wingfield[1]; Louise E Glover[1]
[1]Merrion Fertility Clinic/National Maternity Hospital/ University College Dublin; [2]Merrion Fertility Clinic; [3]Trinity College Dublin; [4]Merrion Fertility Clinic/National Maternity Hospital

Background: The SARS-CoV-2 pandemic posed an unprecedented global challenge. Expedited vaccine development led to public concerns regarding potential unknown impacts of the novel vaccine on gametes in people of child-bearing age. Objectives: The aim of this study is to investigate possible impacts of the SARS-CoV-2 vaccine on sperm parameters and markers of inflammation in semen and sperm samples of men who have received the vaccine.

Methods: Semen and matched peripheral blood samples were assessed in males pre-vaccine, within 46 + 18.9 hours of vaccine completion (acute) and at 88.4 + 12 days (3 month) post vaccination. Symptom scores, sperm parameters, serum and seminal plasma immune factors (IL-6, IL-8, IL-10, IFN-, TNF-, IP-10; CXCL10, MCP-1, CCL2) and seminal and serum anti-SARS-CoV-2 spike isotypes IgA, IgM and IgG1 were analysed.

Results: Paired serum and seminal plasma samples were acquired at all three timepoints for 17 subjects in total. No overall change from baseline in mean volume, pH, sperm concentration, motility, morphology or DFI in the acute or long phase was found. Two men showed a clinically relevant reduction in sperm motility in the acute phase that returned to normal after 3 months. All seminal samples were found to be seronegative for anti-SARS-Co-V2 antibodies, indicating that systemic antibodies are precluded from transport to seminal plasma. Seminal plasma MCP-1 levels

showed an acute but transient elevation post-vaccine, while IL-8 was marginally increased 3 months after vaccine completion. Our findings also indicate a modest, positive correlation between serum levels of the anti-inflammatory cytokine IL-10 and self-reported symptoms post-vaccine.

Conclusion: Along with international consensus of reproductive medicine organisations, we fully endorse vaccination against COVID-19 with consideration of a short delay in treatment following completion of vaccination regime to avoid acute phase changes in sperm parameters (1,2).

1. BFS. Covid-19 vaccines and fertility. 2021. Available from: https://www.britishfertilitysociety.org.uk/wp-content/uploads/2021/07/Covid19-Vaccines-FAQ-2.1.pdf. 2. ESHRE. COVID-19 vaccination and assisted reproduction. 2021. Available from: https://www.eshre.eu/Europe/Position-statements/COVID19.

1B.5 Young oocyte donors do not negatively impact live birth rates in their recipients

Tim Bracewell-Milnes[1]; Sara Aziz[2]; James Nicopoullos[1]; Shabana Bora[1]; Raef Faris[1]; Jaya Parikh[1]; Yau Thum[1]
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Background: Older women have a decreased number and quality of oocytes and are encouraged to utilise oocytes from younger donors to increase in vitro fertilisation (IVF) success. A recent study by Humphries et al. reported that oocytes from donors aged <25 produced somewhat decreased live birth rates compared to those aged ≥26. We therefore, investigated the effect of donor age on live birth rates and evaluated oocyte recipient preferences of age.

Methods: Data from 2010-2021 was used to assess effects of donor age on the primary outcome of live birth rate, and the secondary outcomes, including positive pregnancy rate and miscarriage rate. A questionnaire was distributed to oocyte recipients to ascertain age preferences and if the Humphries' study modified preferences.

Results: 1182 donors were included in analysis. Donors aged ≤22 showed increased live birth rates in fresh cycles compared to donors aged 26-28 ($p<0.0136$), 29-31 ($p<0.0044$), and 32-34 ($p<0.0003$). Increased positive pregnancy rates were reported in fresh cycles for those ≤22 compared to all other groups. Furthermore, our questionnaire revealed that recipients preferred donors aged 26-34 over those aged <26 ($p<0.0001$). The preference towards the 26-34 age group increased after Humphries' results were presented ($p<0.0001$).

Discussion: The 26-34 donor age preference was due to recipients valuing donor maturity and the increase after study results were presented demonstrates how patients are influenced by information from healthcare professionals. Our findings of improved LBR and positive pregnancy rates in those aged 18-22 provide evidence to support 18 as the lower age limit of oocyte donation within the UK.

Conclusions: The results of this study provide evidence to improve treatment success for patients requiring donor oocytes, however further studies would be required to validate our findings and their applicability due to superior fertility outcomes at the LFC compared to the UK average.

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1B.6 A rapid improvement event: Progesterone prescribing in prevention of miscarriage

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A Rapid Improvement Event (RIE) is a standard operational excellence technique that uses team-based problem solving to improve processes. In this study, an RIE was undertaken to improve progesterone prescribing rates for those with a history of miscarriage experiencing vaginal bleeding in early pregnancy. NICE guidelines on the prescription of progesterone in these instances changed in November 2021 after Cochrane meta-analysis and the PRISM randomized control trial which demonstrated a higher incidence of live births in those prescribed vaginal micronized progesterone for threatened miscarriage. A RIE involves a team approach and a standard sequence of events allowing analysis and improvement of a process. Analysis in the form of audit revealed a low progesterone prescribing rate for eligible patients in our unit. Dissection of this problem into its elements revealed a low level of staff knowledge regarding the change in guidelines and a lack of confidence in prescription of progesterone. A plan of actionable events to improve prescribing rates was devised. The updated guidance and local recommendations on appropriate micronized progesterone formulations were presented at hospital grand rounds. Infographics were displayed in areas visible to stakeholders within the hospital and on the hospital's social media pages. The validity of these educational measures to improve the process was reaudited after two months. Progesterone prescribing improved by 48%. Those comfortable with prescribing as per the new guidelines improved from 43% to 78%. A RIE proved to be an effective and efficient approach to collaboration, decision-making and action.

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SP1C NURSING AND PSYCHOSOCIAL INC BFS NURSE PRIZE

1C.1 Test it, know it, plan it: Exploring the perceived usefulness of a digital platform to remotely assess infertile patients ahead of treatment abroad

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Enhanced Fertility

Infertility is a life crisis. It takes 3.2 years to be diagnosed, 1.6 years to access a specialist, and 2.2 years to complete treatment (7 years total). This study aims to explore patients' experience with pre-treatment care and the perceived usefulness of a digital platform designed to speed up the mentioned 7-year average.

A cross-cultural sample of 59 women (M=37.2, SD=4.9, Mo=33 years old) answered an online questionnaire. The majority were in a heterosexual relationship (88.1%) and had been trying to conceive for more than 24 months (69.5%). Although, in most cases, more time trying to conceive meant more testing, some basic tests (e.g., physical examination) were still missing in cases of long-term infertility. Plus, people who took more than four weeks to complete tests (group 1) scored significantly higher values than people who took less than four weeks (group 2), in terms of the struggle to find where to have the tests done (U=294, z=-2.598, p=.009, r=.338), expensive tests (U=302, z=-2.123, p=.034, r=.276), difficulty managing emotions (U=243, z=-3.241, p=.001, r=.422), and preference for scheduling of the tests to be handled by their doctor (U=287, z=-2.062, p=.039, r=.268). This means it's harder for group 1 to cope and move forward with treatment.

Furthermore, 61.02% reported the testing to have been very disruptive (above 5 on a scale of 0-10) to their lives. When asked about the perceived usefulness of the digital platform, 58.1% to 74.2% of respondents found it to be extremely useful (5 on a scale of 1-5) on eleven variables. There were no significant differences between age-based groups, or between groups based on the time spent trying to conceive. Hence, no matter how old a woman is or for how long she's been trying to conceive, she would like to have available a platform like the one developed.

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1C.2 A hermeneutic phenomenological study of parental expectations and the meaning of transition to early parenting in couples with a pregnancy conceived using IVF

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University of Greenwich

Background: Increasing numbers of couples are becoming parents through In vitro fertilisation (IVF) after finding that their decision to commence their family is thwarted by a diagnosis of infertility. Those who have undergone assisted pregnancy may have faced greater psychological, physical and often financial demands in becoming parents which may heighten expectations of parenthood for this group.

Method: A hermeneutic phenomenological study using in-depth data analysis. Couples expecting their first child, a singleton non-donor pregnancy conceived using IVF, were purposively selected and interviewed on three occasions: at 34 weeks pregnant, six weeks following birth and at three months post birth. The study design enabled a unique combination of both time point and longitudinal data analysis.

Findings: 'Returning to the Path' was identified as the point at which couples felt they were where they had anticipated being several years earlier, drawing on three over-arching themes: Seeking the Way, Returning to the Path and Journeying On. These focussed upon the meaning parents attributed to their experiences. This included a tentative path of pregnancy, the influence of gendered responsibility, the role of technology and relationships with others.

Conclusion: Infertility is a deviation from the life path that a couple anticipated; the point of and influences on returning to that path occurs at different times for different couples and is influenced by differing factors. The pregnancy may be experienced as a 'tentative' progression, however following birth, parenthood was embraced with an instinctive, baby-led style. Transition to parenthood was aided by social support and reliance on the couple relationship. Consideration of potential siblings was an issue which arose in early parenthood, as couples recognised ongoing implications of the path they had travelled.

1C.3 The implementation and pathway for semen analysis via an app based fertility service - preliminary findings

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Semen Analysis is recognised by NICE 2017 (1) as a first line test when assessing male fertility. Our aim is to ensure that we take a holistic and inclusive approach for fertility care to include male partners when reviewing our fertility care offering. By providing semen analysis testing on a trial basis via an app based fertility service, our aim was to reach out to men who are trying to conceive and begin the process of initial investigation earlier. This enabled our clients to have a clearer understanding of their basic fertility by having access to the test and interpretation and guidance by fertility nurse specialists. Following assessment of 10 male clients against a selection criteria, a semen analysis testing kit was sent to the patient in partnership with a UKAS (2) accredited third party provider. The result was then interpreted against WHO (3) Semen Analysis guidelines and provided to the client by competent fertility practitioners, and a consultation was offered to explain the result and next steps. The trial took place in March 2022 and showed the following: 10 semen analysis tests were completed with 6 showing normal results against WHO semen analysis criteria, 2 results showed reduced motility and 2 results showed a low sperm count. These results (although small numbers) suggest that by alerting clients to these results we have been able to advise on recommendations including lifestyle changes and interventions, to potentially improve sperm health and access further investigations sooner than perhaps originally planned. Our recommendation following the review of this trial, has been to partner with a third party UKAS Accredited organisation to offer semen analysis testing, interpretation and guidance as part of the service to promote greater access to reproductive choices and earlier interventions.

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1C.4 The international fertility education initiative - a global collaboration to improve fertility awareness

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Background: Research shows that people's knowledge about fertility is generally low. For those who want children, more awareness about the factors that affect fertility and chance of pregnancy might improve their chance of achieving their desired family size.

Methods: The International Fertility Education Initiative (IFEI) was established in 2020 and is a multidisciplinary global collaboration dedicated to improving fertility awareness through research and education. The group includes health professionals, researchers, and representatives from patient organisations, professional societies, and industry. Countries represented are Australia, Belgium, Czech Republic, Denmark, France, Greece, Japan, Portugal, Sweden and UK. IFEI's strategic goals are to: 1. Generate evidence about people's knowledge, attitudes and behaviours relating to fertility and family formation in different countries. 2. Develop educational resources for the public and health and education professionals that are inclusive of all communities. 3. Evaluate the educational resources to establish their acceptability, salience, comprehensibility and effectiveness in improving knowledge and inclusivity. 4. Promote the inclusion of fertility education in health and education policy and advocate for a government sponsored fertility health education program in every country.

Results: As an example of impact, the IFEI founders, in collaboration with members of ESHRE and Fertility Europe, developed a fertility education poster, which was launched in the EU Parliament. The poster is free to download and translated into 35 languages. It targets the general public of diverse age groups, with the aim of facilitating conversations around fertility education by the poster being displayed in e.g. family planning clinics and schools. Evaluation of the public's perception of the poster is underway.

Conclusions: We welcome people with an interest in fertility education to join us to help establish national fertility education programmes in as many countries as possible. This is presented on behalf of IFEI. For further information, please visit IFEI (eshre.eu).

1C.5 Exploring women's perspectives on dismissal of reproductive health symptoms by medical professionals

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Hertility Health

Background: Women make up 51% of the United Kingdom (UK) population, yet literature shows that they experience poorer health outcomes due to a lack of support by the healthcare system resulting in a drop in life expectancy. Our study investigated women's experiences accessing healthcare for their reproductive health symptoms in the UK.

Methods: A cross-sectional survey was conducted on 1511 UK-based women aged 20-40 in March 2022. It assessed general demographic information and individual perspectives on managing reproductive and women's health.

Results: Almost all (93.6%) participants agreed that women's health problems weren't taken as seriously as men's health problems. Most participants (86.4%) reported that either they or someone in their life had encountered occasional to constant dismissal of reproductive health-related issues by medical professionals. It was noted that participants aged 20-30 were more likely to be dismissed and not seek help for women's health problems than those aged 30-40 (78.6% vs 69.8%). In most cases (74.1%), this dismissal was by a doctor, 47.0% of whom were male, and 30.0% were female. The most commonly dismissed symptoms were period-related, namely period pain (71.0%), irregular periods (51.0%), heavy periods (49.0%) and PMS symptoms (33%). Of people aged 20-30, 22.8% reported menopause-associated symptoms being dismissed. Other topics of dismissal included fertility-related concerns (33.0%) and miscarriage/baby loss (32%), with 7.0% saying they struggled to get pregnant with no GP support.

Conclusion: Our findings support the presence of gender bias within reproductive healthcare in the UK. Dismissal of symptoms by doctors is common, and younger people appear more likely to be dismissed. Further research is needed to understand the potential consequences of this. This highlights the need for improved medical training around women's health-related issues.

1C.6 Why I left my fertility clinic for another ART provider - it's not what you think: A RealTalk patient survey

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Objective: This large study aimed to assess why patients chose to switch ART provider[s] before they had exhausted all their treatment options.

Methods: A 12-question online questionnaire was disseminated to patients (N=1060) who had undergone 1 fertility treatment through 40 patient advocates (February-April 2019). The confidential online survey consisted of four demographic questions, four closed-ended treatment-history questions, and three open-ended questions (answers of unlimited length) about experiences with ART providers. Long form responses were modeled using Natural Language Processing (NLP) techniques, and grouped into topics, from which several "themes" emerged. Methodology, results and conclusions were reviewed and endorsed by a committee of fertility specialists.

Results: The reasons given for leaving their clinic fell into six clear themes: a treatment/approach not tailored to the patient and their needs (23%); poor bedside manner (communication style, the patient not feeling "heard") (18%); logistical challenges e.g., doctor, clinic or patient had moved (14%); cost/access issues (9%); lack of efficacy e.g., treatment unsuccessful (8%); provider was "all business" e.g., patient felt like a number (8%). Four clear themes emerged in the reasons provided for staying with their clinic: good access/cost/insurance coverage (29%); already enrolled in next steps at the clinic (e.g., purchased IVF bundle, embryo storage, etc.) (26%); had a connection with staff (25%); felt optimistic (20%). Relationships were also a major theme when respondents were asked to provide further comments about their experiences and/or what they value in a fertility doctor: a good fit (24%); provider has an interest in learning about them and/or exploring tailored treatment options (13%); feels connected and is being treated like a human being (11%); provider communicates and shows compassion (10%).

Conclusions: The patient-provider relationship was the most common reason cited for leaving an ART provider, and also the most common reason for staying.

SP1D SRF PhD STUDENT PRIZE SESSION

1D.1 At fertilization in mouse eggs a sperm induced secondary rise in ATP levels is independent of Ca2+ oscillations

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Background: Egg activation at fertilization in mammalian eggs is caused by a series of cytosolic Ca2+ oscillations triggered by sperm PLCzeta. These Ca2+ oscillations are associated with a two-phased increase in ATP concentrations driven by increased mitochondrial activity (1,2). The initial ATP increase starts with the first Ca2+ transient. A second increase in ATP occurs about 1 hour later and is not evident after PLCzeta injection (2).

Methods: ATP was measured by the luminescence of luciferase (2). Intracellular Ca2+, NADH or FAD were measured in by fluorescence as described previously (1). Thapsigarin, thimerosal, or Sr2+ medium were used as artificial methods for elevating Ca2+ and BAPTA-AM was used to buffer Ca2+. Eggs and sperm from CD-1 mice were fertilized in vitro.

Results: The second rise in ATP at fertilization occurred in 56/60 eggs at 56.0 ± 22.0 (SD) minutes after the start of Ca2+ oscillations. This secondary ATP increase occurred before an increase in the frequency of Ca2+ oscillations by 15.0 ± 13.3 minutes. Fertilization induced NADH and FAD changes tracked Ca2+ transients and did not show a clear secondary rise. The second rise in ATP is sperm specific and did not occur during Ca2+ oscillations stimulated by thimerosal (n = 17 eggs) or Sr2+ medium (n = 26 eggs). In eggs loaded with low concentrations of BAPTA the sperm caused a single Ca2+ increase, but a two-phased ATP increase similar to control IVF still occurred in 23 eggs at 66.3 ± 15.2 minutes. A singular Ca2+ increase caused application of thapsigargin only caused a single increase in ATP levels (n = 16 eggs).

Conclusions: The secondary rise in ATP in fertilizing mouse eggs is not explained by Ca2+ dependent stimulation of mitochondria. Sperm may introduce a factor into eggs that directly promotes mitochondrial ATP production at fertilization.

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1D.2 A common flame retardant (polybrominated diphenyl ether: PBDE-47) detectable in testicular tissue may adversely affect sperm quality in humans and dogs

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Background: Declining sperm quality in humans and dogs has been linked to exposure to environmental contaminants. Flame retardants (polybrominated diphenyl ethers: PBDEs) are ubiquitous and are associated with altered sperm quality. Since we have previously detected the congener PBDE-47 in a range of reproductive tissues, we hypothesized that testicular PBDE-47 may induce chronic exposure effects on adult sperm in both dogs and humans.

Methods: PBDE-47 was measured in dog testes (n=35) removed at routine neutering. Sperm was collected for routine reproductive examination of eight stud dogs and incubated with PBDE-47 at 2x, 20x, and 200x mean dog testis concentrations. Exposure effects on progressive motility were tested at 0h and 1h (iSperm). Human sperm samples were obtained from HFEA donors (n=10: Andrology clinic at Nottingham University Hospitals). Samples were incubated with PBDE-47 across an extended concentration range (2x, 20x, 100x, 200x, 1000x) and motility was measure at 0 and 3h (CASA & SAMi diagnostic software). Ethical approvals were provided by the Committee for Animal Research and Ethics for Dog and Human studies and owner/donor consents obtained.

Results: Mean testicular PBDE-47 of 0.376 ug/kg used as baseline. In the dog, a significant PBDE-47 dose ($P<0.001$) and dose by time interaction ($P=0.05$) was observed. Sperm incubated with PBDE were less motile at 1h than those incubated with vehicle alone. In the human, a significant dose ($P<0.001$) and time ($P<0.05$) effect were observed with reduced sperm motility at 3h. In contrast to the dog, there was no significant dose by time interaction.

Conclusion: Short term exposure to PBDE-47 at testicular relevant concentrations, reduces sperm motility in both the dog and in the human. These data suggest that testicular PBDE-47 may perturb sperm function directly in adult dogs and humans.

1D.3 Regulation of primordial follicle quiescence by TGFB signalling

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Background: The reproductive capacity of the ovary is determined by a finite population of primordial follicles (PFs), each consisting of an arrested oocyte surrounded by a single layer of somatic granulosa cells (GCs). However, the molecular mechanisms that regulate quiescence remain unresolved. The Transforming Growth Factor Beta (TGFB) signalling mediator and transcription factor, SMAD3 has been previously identified in the GCs of PFs (1). Upon initiation of follicle growth, SMAD3 is excluded from the nuclei of GCs. This suggests TGFB signalling is actively regulating target genes involved in maintaining PF arrest (2). Therefore, in this study we aimed to examine the impact TGFB signalling has on gene regulation within this context.

Methodology: Ovaries densely populated with PFs were dissected from 4-day old wild-type C57B16 mice and maintained in culture for 2 or 24 hours, with or without TGFB1 supplementation (10ng/ml). Following incubation, RNA was extracted and processed for next generation sequencing. Raw data was processed in Galaxy and count files were generated for each control (2H,n=3) (24H,n=5) and TGFB-treated sample (2H,n=3) (24H,n=10). This workflow was repeated with (n=6) or without (n=6) supplementation of the TGF-BRI inhibitor A83-01 (1M) for 24 hours.

Results: A total of 6676 genes were present in the 2H dataset, with 38 differentially expressed genes (DEG) (FDR<0.05). Of the 6526 genes in the 24H dataset, there were 744 DEGs, with eight 'common genes' identified in both DEG cohorts. There were 6969 genes in the inhibitor dataset, with 343 DEGs. Preliminary ontological assessment of the TGFB datasets, appears to show an upregulation of structural/developmental systems and downregulation of processes involved in the Cell Cycle, with the inhibitor dataset displaying the inverse result.

Conclusions: These results are consistent with a role for TGFB within the PFs of the ovary, regulating and maintaining these follicles in a relatively quiescent state.

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1D.4 Receptive phase simulated endometrial extracellular vesicles proteome differs from non-receptive simulated periods

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Background: Successful pregnancy requires a receptive endometrium and a good quality embryo. Locally, this process involves mutual communication between the embryo and the endometrium in the uterine milieu. Recently it's been suggested that extracellular vesicles (EVs) regulate early embryo development as it reaches the uterus, in addition to priming the endometrium for embryo implantation. However, a complete understanding of EVs and their cargo's role in mediating implantation has not been demonstrated.

Method: We explored purified endometrial-derived EVs, using RL95 cells a receptive endometrium model, influenced by menstrual cycle hormones estrogen (E (10nM); proliferative phase) progesterone (P (10nM); secretory phase) and estrogen plus progesterone (EP (10nM+100nM); receptive phase). EVs were isolated from conditioned media after 24h of hormonal stimulation by differential centrifugation and size exclusion chromatography. Nanoparticle tracking analysis was used to examine the concentration and size of particles and proteomic analysis performed by shotgun label-free mass spectrometry.

Results: Results showed that endometrial EVs were secreted in numbers independent of hormonal stimulation (mean concentration of EVs, 5.63e+08/ml E, 4.993e+08/ml P, 5.343e+08/ml EP and 4.306e+08/ml Control.N=3). There was a significant difference in EV sizes produced in response to hormones (the P group compared to E and EP in the 135nm and 165nm size range). Proteomics analysis showed that hormonal changes affected the endometrial EVs proteome, with EVs from EP group showing significantly altered proteins involved in embryo implantation, endometrial receptivity, and embryo development. KEGG and GO analysis identified pathways in the EP group involved in immune modulation and pathways known to support implantation including mTOR signalling pathway and VEGF signalling pathway.

Conclusion: These results support the concept of a communication system between the embryo and the maternal endometrium is via EVs. Application of this knowledge may allow EVs to be exploited as an endometrial tissue biopsy alternative and have a range of applications in understanding infertility.

1D.5 Does cigarette smoking increase the risk of ectopic pregnancy by activation of epithelial to mesenchymal transition?

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Background: An ectopic pregnancy occurs when an embryo implants and develops outside the uterus. 95-98% occur in the Fallopian tube (1). Cigarette smoking doubles the risk of ectopic pregnancy, but the molecular mechanisms behind this association remain unclear (1). Recent research has identified that the epithelial-mesenchymal transition (EMT) process plays a role in intra-uterine embryo implantation but whether EMT affects ectopic pregnancy is unknown (2). We hypothesised that systemically absorbed chemicals from cigarette smoke induce EMT in the Fallopian tube, leading to ectopic pregnancy.

Methods: An in-vitro model of ectopic pregnancy was utilised to assess the effects of the cigarette components Benzo(a)Pyrene [B(a)P] and nicotine nitrosamine ketone (NNK) on blastocyst attachment, invasion, and EMT protein expression. Blastocysts were flushed from the uteri of female CD1 mice (n=12 per run, n=6 runs performed) at embryonic day 3.5 following super-ovulation and timed mating. Blastocysts (10 per well) were added to monolayers of immortalised human endometrial epithelial cells (control -- uterine implantation) or Fallopian tube cells (ectopic implantation). Co-cultures were then incubated for 48hr with 10µM B(a)P, 100µM NNK or vehicle (DMSO) before blastocyst attachment was assessed. Cultures were fixed after 72hr to examine trophoblast invasion and immunofluorescence for Twist1 (EMT master-regulator) expression.

Results: Blastocyst attachment was significantly lower in Fallopian tube cells compared to endometrial epithelial cells ($p<0.0001$). B(a)P and NNK increased attachment of embryos over vehicle controls, in both Fallopian tube epithelial cells and endometrial epithelial cells ($p<0.0001$ for both). No change in trophoblast invasion was observed after exposure to B(a)P or NNK. B(a)P, but not NNK, increased Twist1 expression in both blastocyst and adjacent cell layers ($p<0.05$).

Discussion: B(a)P and NNK, two systemically absorbed chemicals from cigarette smoking, increase the chance of embryo attachment and therefore may increase the likelihood of ectopic pregnancy. However, B(a)P and NNK appear not to affect the invasion process.

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1D.6 Maternal spleen-liver axis inflammation during obese pregnancy and placental nutrient sensing

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Introduction: The mechanisms through which maternal obesity contributes to adverse offspring outcomes via placental dysfunction are unclear. The spleen and liver function between immunity and metabolism and their dysfunction could exacerbate maternal inflammation already present during obesity. The present study will determine whether obesity induces inflammation in the maternal spleen and liver in association with changes in placental nutrient sensing.

Methods: Mice were fed a high fat high sugar (HFHS) diet for a minimum of 9 weeks prior and during pregnancy (n=10) whilst control mice were fed chow (n=10). Tissues were collected on day 19 of pregnancy. Maternal plasma cytokines were measured via multiplex assays and via qPCR in maternal spleen and liver. Splenic and hepatic gene expression and western blots of NLRP3 inflammasome and receptor for advanced glycation end products (RAGE) were measured as well. Comparisons were conducted via T-tests or Mann-Whitney depending on data distribution. RNA-sequencing was conducted on the placental labyrinth zone and differentially expressed genes were analyzed for cytokine and growth factor-related pathways.

Results: Fetal weight was decreased by ~10% in HFHS pregnancies ($p=0.002$). IL1A was elevated in HFHS plasma, spleen, and liver ($p=0.012$, $p<0.01$, $p<0.001$). Further, splenic and hepatic protein levels of RAGE and NLRP3 were significantly elevated in HFHS animals (RAGE: $p=0.026$, $p=0.004$; NLRP3: $p=0.005$, $p=0.026$). Total hepatic NFkB was significantly higher in HFHS dams ($p=0.008$). Phosphorylated-p44 MAPK was significantly increased in HFHS livers ($p<0.001$) and total levels were increased in both spleen and liver ($p<0.001$ for both). Phosphorylated-STAT5 was increased in HFHS spleen and liver ($p=0.002$, $p<0.001$). Placental IL1A and insulin-like growth factor binding-protein genes were significantly increased in HFHS male placentas only.

Conclusion: Diet-induced obesity results in a pro-inflammatory phenotype in the maternal spleen, liver, and placenta, and fetal growth restriction. Work is underway to assess nutrient transport in the placenta.

SP1E MALE FERTILITY SHORT PAPERS including Iwan Lewis-Jones Prize

1E.1 Optimising the alkalinisation protocol for patients with retrograde ejaculation

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The Hewitt Fertility Centre

Retrograde ejaculation (RE) is caused when the internal urethral sphincter at the neck of the bladder, which usually contracts to move seminal fluid to the prostatic urethra and prevent its retrograde flow into the urinary bladder, does not contract properly. Patients with RE are still able to achieve an erection and reach orgasm, but with no, or very little semen ejaculated. RE can be diagnosed by assessing the post-orgasmic urine for the presence of sperm. This sperm can subsequently be cryopreserved or processed for use in treatment. A reduction in sperm motility is seen in urine with low pH and high osmolality but alkalinisation of the urine present in the bladder prior to masturbation can facilitate the retrieval of motile sperm from the post-orgasmic urine (1). Careful preparation on the day leading up to, and on the morning of analysis should be followed by the patient, as indicated by the clinic. A literature review showed that the WHO issued a vague recommendation of 'drinking water with sodium chloride and sodium bicarbonate' (2) and published clinical practice ranged from the ingestion of multiple doses of 50mg-5g of sodium bicarbonate (3,4), the use of the Liverpool solution (5), multiple doses of 60mg of pseudoephedrine (6) and urination into a specimen pot containing culture medium (7). This presentation details the literature and suggests a safe and practical protocol that is tolerant to the patient and can optimise motile sperm recovery, working in close partnership with a local Medicines Management Review Board for safe clinical practice. National and/or international guidance on the optimal alkalinisation method, including the required dosage and exact timing of the treatment, to best prepare patients with RE safely is needed. Clinics must review clinical outcomes and work with local pharmacies to deliver safe and effective treatment to RE patients.

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1E.3 The impact of an AI sperm selection tool on ICSI outcomes - a validation study

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Manchester Fertility

Background: A number of sperm selection technologies have been developed in recent years to improve outcomes following intracytoplasmic sperm injection (ICSI) (1). SiD (SiD V1.0; IVF 2.0, UK) is a novel individual-sperm identification software, using artificial intelligence to assist embryologists in sperm selection during ICSI procedures. Using a digitizer attached to an ICSI microscope, the algorithm detects motile spermatozoa, and assesses the movement of each individual sperm in the visual field (2).

Objectives: This study aims to validate SiD software for clinical use, and assess its potential in improving embryology outcomes, and clinical pregnancy rate.

Methods: ICSI cases with 8-16 mature oocytes and sperm concentration of >0.3Million/ml were incorporated into the study. Half of the oocytes for each patient were injected with SiD selected sperm, and half by manual selection. 43 cycles were included in the study. Fertilisation, blastocyst utilisation (suitability for transfer or freezing) and pregnancy rates were analysed using Paired t-tests and Chi-squared tests.

Results: No significant differences were observed between Non-SiD and SiD groups in fertilisation rates (74.10% vs. 77.46%; p=0.3990) and blastocyst utilisation rates (33.99% vs. 44.72%; p=0.4456). 36 embryos (61% Non-SiD, 39% SiD) were transferred, including both fresh and frozen embryos. Positive pregnancy test rates for Non-SiD and SiD embryos

were 54.50% and 71.43%, respectively, this also showed no significant difference (p=0.4609). Clinical pregnancy data is yet to be confirmed at the time of writing.

Conclusion: This preliminary data identifies no significant difference between fertilisation rate and blastocyst utilisation rate of Non-SiD vs. SiD selected sperm. However more Non-SiD embryos were selected for embryo transfer based on day 5 blastocyst quality, and SiD embryos showed a higher positive pregnancy rate. Due to the minimal data set, it is difficult to conclude whether the use of SiD is beneficial in improving outcomes. More data is needed to assess clinical significance.

1. Novel Techniques of Sperm Selection for Improving IVF and ICSI Outcomes. Oseguera Lopez, Ivan, et al. November 29, 2019, Frontiers in Cell and Developmental Biology, Vol. 7
2. Computer software (SiD) assisted real-time single sperm selection associated with fertilization and blastocyst formation. Mendizabal-Ruiz, G, et al. April 09, 2022, Reprod Biomed Online.

1E.4 Cost-effectiveness comparison of 95,034 cycles of intrauterine insemination (IUI) and 30,667 cycles of in-vitro fertilisation (IVF) using donor sperm

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[1]University of Dundee; [2]School of Medicine, University of Dundee; [3]TFP GCRM Fertility

Background: NICE guidelines recommend six intrauterine insemination (IUI) cycles with donor sperm before in-vitro fertilisation (IVF), as IUI is considered the less expensive and less invasive option (1). This fails to fully consider the patient's age, the relative decline in success rates with additional IUI cycles and the increasing donor sperm cost.

Methods: The Human Fertilisation and Embryology Authority database (1991 to 2018) yielded data on 50,661 unstimulated IUI (uIUI) cycles, 44,373 stimulated IUI (sIUI) cycles and 30,667 IVF cycles with donor sperm. The live birth rate (LBR) and cost per live birth (CLB) [which included direct (treatment costs)] for IVF and 1st, 2nd, 3rd, 4th, 5th and 6th (or above) cycles of IUI were calculated.

Result: Among women aged <35 years, the LBR for IVF was 33.6% (CLB £20,669). The LBR for the 1st uIUI was 15.8% (TCLB £13,950), the 3rd uIUI was 13.2% (CLB £16,638) and the 6th uIUI (or above) was 11.2% (CLB £19,570). For women aged 38-39 years, the LBR for IVF was 23.0% (CLB £30,277). The LBR for the 1st uIUI was 9.5% (CLB £23,228), and the 6th uIUI (or above) was 7.3% (CLB £30,250). In contrast, for women aged 40-42 years, the LBR for IVF was 14.2% (CLB £48,858) and the LBR for the 1st uIUI was 2.9% (CLB £75,130). Therefore, IVF was cost-favourable compared with IUI in this age range.

Conclusion: For women aged <35 years, CLB of 6th uIUI(or above) was £1,099 cheaper than IVF and the CLB of 1st cycle of uIUI was £7,049 cheaper than IVF among women aged 38-39 years. However, for women aged 40-42 years, the CLB of IVF was £26,272 cheaper than uIUI. This data should assist objective counselling on cost-effectiveness.

1. National Institute for Health and Care Excellence (NICE). Fertility: assessment and treatment for people with fertility problems, 2013

1E.5 The impact of paternal diet on late gestation fetal heart gene expression in mice

Afsaneh Khoshkerdar[1]; Hannah Morgan[2]; Marcos Castellanos Uribe[3]; Iqbal Khan[3]; Adam Watkins[1]
[1]University of Nottingham; [2]Faculty of Medicine & Health Sciences, University of Nottingham; [3]Faculty of Science, University of Nottingham

Background: The association between poor maternal diet and offspring cardiovascular ill-health is well defined. However, the impact of paternal over- and undernutrition on fetal cardiovascular development has been overlooked.

Methods: Male C57/BL6J mice were fed either a control (CD: 18% casein, 10% fat, 21% sugar), a low-protein (LPD: 9% casein, 24% sugar, 10% fat), a Western diet (WD: 19% casein, 34% sugar, 21% fat) or an LPD or WD supplemented with methyl donors (termed MD-LPD and MD-WD respectively) before mating with 8--12-week-old females. On embryonic day 17.5, dams were culled for the analysis of fetal growth. Fetal hearts were snap frozen, prior to RNA isolation and analysis of global gene expression using the Clariom S Assay Mouse GeneChip array (ThermoFisher Scientific) and analyzed using the Partek Genomics Suite and the online WebGestalt tool.

Results: Paternal diet had no significant impact on litter size or fetal growth dynamics. However, we observed over 4000 differentially expressed genes (FDR <0.1; P<0.05) between CD, LPD, and WD fetal hearts. Gene ontology and pathway (KEGG) analysis identified the downregulation of central lipid, amino acid, and carbohydrate metabolic processes in LPD hearts while genes involved in angiogenesis and embryonic organ development were upregulated in WD hearts. Interestingly, in MD-WD fetal hearts, only 2000 genes were differentially expressed involving calcium signaling, embryonic organ development, and immune responses. In MD-WD hearts, genes involved in lipid metabolism and angiogenesis were upregulated.

Conclusion: The current study indicates that while poor paternal diet at the time of conception has minimal impacts on his fertility, the expression of multiple genes involved in central cardiovascular metabolic and morphological pathways are altered in the fetal heart. Interestingly, each diet resulted in unique gene expression profiles. Further studies are required to define the impacts of these changes on offspring's cardiovascular health.

1E.6 Using SiD software to associate single spermatozoa motility patterns with ICSI outcomes, a multicentric retrospective study

Alejandro Chavez Badiola[1]; Adolfo Flores Saiffe Farias[1]; Gerardo Mendizabal-Ruiz[1]; Roberto Valencia-Murillo[1]; Denny Sakkas[2]; Olcay Ocali[2]; Pavlo Mazur[3]; Xavier Vinals-Gonzalez[4]; Fernando Meseguer Estornell[5]; Marcos Meseguer Escriva[5]; Darren Griffin[6]; Andrew Drakeley[1]; Jacques Cohen[7]

[1]IVF 2.0 Ltd; [2]Boston IVF; [3]IVMED fertility center; [4]Aria Fertility; [5]IVI RMA; [6]School of biosciences, University of Kent; [7]IVF2.0 ltd

Purpose/Background/Objectives: Sperm selection for intracytoplasmic sperm injection (ICSI) is key to its success. Artificial Intelligence-assisted sperm selection is a promising data-driven alternative to the inherent subjectivity of current practice. SiDTM (IVF 2.0 Limited, UK) software is able to assess all spermatozoa in a visual field and compute motility patterns for each sperm in real time [1]. Here we retrospectively assess the association between SiDTM extracted motility patterns with ICSI outcomes.

Methods: 670 ICSI videos and their outcomes were retrospectively collected from six fertility clinics, between July to December 2021, and analyzed. Ethical approval was obtained. Motility variables for the injected sperm were computed from ICSI videos using SiDTM software, as defined elsewhere [1,2]. Testing groups were defined as AG1 (< 38 yo) or donated; and AG2 (≥ 38 yo). Mann-Whitney U test was used to compare groups.

Results: Using all the datasets (AG1+AG2), spermatozoa that developed euploid embryos (n=21) showed increased linearity (LIN) in comparison with aneuploids (n=31) (U(52)=210, p=0.03). This was replicated in AG1 (U(31)=160, p=0.04) but not in AG2. Spermatozoa that successfully fertilized an oocyte in AG1 (n=218), showed higher values of straightness (STR) than those that did not fertilize (n=55) (U(273)=4628, p=0.01). Analysis of AG2 suggested that spermatozoa that successfully developed a usable blastocyst (n=45) had an increased Average Path Velocity (AVP) (U(n=142)=1640, p=0.02), increased Straight Line Velocity (VSL) (U(n=142)=1714, p=0.04), and increased HMP (U(n=142)=1723, p=0.04) compared against those that did not develop a usable blastocyst.

Conclusions: Our dataset suggests that motility patterns have a modest correlation with ICSI outcomes. Machine learning techniques might be applied to assist embryologists during sperm selection for ICSI, and probably, increase success rates.

[1] Mendizabal-Ruiz G, Chavez-Badiola A, Aguilar Figueroa I, et al. Computer software (SiD) assisted real-time single sperm selection associated with fertilization and blastocyst formation. Reprod. Biomed Online; 2022. In press. [2] World Health Organization. WHO laboratory manual for the examination and processing of human semen, 6th ed. ed. World Health Organization, Geneva. 2021.

SHORT PAPER PRESENTATIONS THURSDAY

SP2A ARCS POST REG SHORT PAPERS

2A.1 Lean management in the IVF clinic: Using technology to eliminate wasted time in IVF lab processes whilst maximising value to patients

Cristina Hickman; Yael Kfir; Michelle Tran; Noam Bergelson; Adriana Brualla; Meryem Bousfiha; Eran Eshed

Fairtility

Introduction: To assess the amount of time embryologists spend during an average IVF cycle and explore how technology can be used to lean processes whilst improving standards of care.

Methods: 6 lab directors from 6 clinics from three countries (2 UK, 1 Spain, 3 USA) were interviewed to quantify the steps in a typical IVF cycle by following their current procedures. The lab directors were then asked to estimate the time required if they were to implement the following technologies fully integrated with CHLOE-EQ: time-lapse, electronic witnessing, electronic medical record. The total amount of time before and after CHLOE-EQ integration was compared, and the savings extrapolated to estimate their value in hourly, cycle capacity and monetary terms.

Results: Overall, the average time required per cycle before CHLOE-EQ was 15.9 hours and after CHLOE EQ was 9.4 hours, an average 41% reduction in time required per cycle (p<0.001). Before CHLOE EQ, the fastest clinic needs an average of 7.7hours per cycle, whilst the slowest needed an average of 31.5 hours per cycle. On average, cycles in the

USA were more time consuming than those in Europe (mean+-st dev: 20+-10 vs. 12+-5 hours, p<0.001). After CHLOE-EQ, the fastest clinic needed an average of 6.2hours per cycle, whilst the slowest needed an average of 13.1 hours per cycle. CHLOE-EQ integrations reduced the variation in time per cycle between clinics compared to before CHLOE-EQ implementation (p<0.001). CHLOE-EQ implementation had a direct association with reduction in cost per cycle, reduction in risk, increase in capacity of cycles per embryologist. The amount saved was associated with the size of the clinic and the average salary of embryologists.

Conclusion: Introducing fully integrated digitised technologies into clinical practice can increase efficiencies, reduce risk, reduce cost and improve standards of care.

2A.2 Are vitrified donor oocytes an option for patients with high sperm DNA fragmentation?

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Background: Sperm DNA damage is a contributor to male infertility [1] and poor outcomes following standard IVF [2]. ICSI is recommended for patients with high DNA damage as the effect upon ICSI outcomes appears to be lower [3], possibly due to morphological sperm selection, decreased exposure to laboratory stressors or extended exposure to the oocyte environment, which has some DNA-repair capacity. Oocytes from younger women may have increased DNA-repair capacity, minimising the effect of DNA damage upon donor oocyte cycles [4,5]. Donor oocyte vitrification is increasingly common, but differences have been identified after oocyte warming, including in gene expression [6]. It remains unclear whether these changes affect oocyte DNA-repair capacity.

Objective: To identify the effects of sperm DNA fragmentation upon fresh and frozen oocyte donation cycles.

Materials and methods: A retrospective study of 239 donor-recipient cycles, including 6 IVF cycles, 120 frozen ICSI cycles and 113 fresh ICSI cycles. All donors fulfilled HFEA eligibility criteria. All donors were stimulated via antagonist protocol and a minimum of 6 oocytes were allocated. ICSI was recommended for all recipients, 6 opted for IVF. The SpermComet test (Examen) was used to quantify sperm DNA fragmentation.
Results associated with various values of average comet score (ACS) were analysed. Chi squared testing (Graphpad) was used to assess the contribution of cryostatus of oocytes and ACS upon outcomes including live birth rate.

Results: We identified no significant effect of ACS or whether oocytes had been frozen prior to ICSI upon outcomes including live birth rate after donor treatment.

Conclusions: Similar rates of clinical success were seen for fresh and frozen donor eggs, across various levels of sperm DNA fragmentation (detected by SpermComet). Patients with high DNA fragmentation need not be deterred from vitrified donor oocytes. Although all oocytes utilised were collected from women fulfilling HFEA donor eligibility criteria, findings may also reassure patients considering elective oocyte cryopreservation.

1) Kathryn C. Humm. Role of increased male age in IVF and egg donation: is sperm DNA fragmentation responsible? Fertil Steril. 2013 Jan;99(1):30-6.
2) Simon et al. Sperm DNA damage has a negative association with Live birth rates after IVF. Reproductive Biomedicine online (2013) 26, 68-78L
3) Hee-Jun Chi. ICSI significantly improved the pregnancy rate of patients with a high sperm DNA fragmentation index. Clin Exp Reprod Med. 2017 Sep; 44(3): 132-140.
4) Gat I. Sperm DNA fragmentation index does not correlate with blastocyst euploidy rate in egg donor cycles. Gynacol Endocrinol 2018 Mar: 34(3) 212-216
5) T Antonouli S, The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. Arch Gynecol Obstet 2019 Jul; 300(1) 207-215
6) Barberet J, Barry F, Choux C, Guilleman M, Karoui S, Simonot R et al. What impact does oocyte vitrification have on epigenetics and gene expression?. Clinical Epigenetics. 2020;12(1).

2A.3 Utilisation of monopronucleated (1PN) embryos and potential clinical benefits for patients

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Studies have shown that embryos categorised at fertilisation check as 1PN may still have potential to create viable, successful pregnancies (1)(2). Based on these studies a 1PN policy has been put into clinical practice. 1PN embryos derived from IVF and ICSI are to be cultured to blastocyst (day 5/6) for potential utilisation within the lab for all patients. This includes vitrification, PGT-A and transfer. Patients were only allowed to transfer a 1PN embryo if the only available blastocyst was a 1PN embryo derived from IVF. Since implementing the policy in April 2022 till now, August 2022, we have cultured 126 1PN embryos (IVF and ICSI derived). 37 of these have shown signs of blastulation (29.4%). Three IVF derived 1PN embryos have been transferred, two have resulted in ongoing pregnancies (one being a twin

pregnancy) and the third patient is yet to test. 10 embryos were biopsied and tested for ploidy status. Three of these were ICSI derived and were all were found to be haploid. Seven were IVF derived and were all found to be diploid (with three being euploid and four aneuploid). From our data and experience so far, we conclude that 1PN culture has and will be beneficial for our patients. It has been a promising start, especially within our low prognosis patients who would otherwise have had failed cycles. This policy is ongoing within our clinic and more data is to be collected.

1. Nuria Soler, Rosa Bautista-Llacer, Laura Escrich, Andrea Oller, Noelia Grau, Raquel Tena, María Fernanda Insua, Paloma Ferrer, María-Jose Escrib, Xavier Vendrell. Rescuing monopronucleated-derived human blastocysts: a model to study chromosomal topography and fingerprinting. Fertility and Sterility 2021; 116(2): 583-596. 2. Ming Li, Yujiao Dang, Ying Wang, Junsheng Li, Ping Liu. Value of transferring embryos derived from monopronucleated (1PN) zygotes at the time of fertilisation assessment. Zygote

2A.4 Poor quality blastocysts diagnosed as euploid have a higher chance of achieving a live birth than untested poor-quality blastocysts

Balsam, Al Hashimi[1]; Nick Macklon[1]; Darren Griffin[2]; Elena Linara-demakakou[1]; Kamal Ahuja[1]
[1]London Women's clinic; [2]University of Kent

Many IVF clinics only consider for transfer blastocysts with a minimum morphology grading of 4BC or CB based on NEQAS grading system. Blastocysts graded CC/CD/DC/DD are considered of suboptimal quality and may be discarded due to their expected lower chances of implantation, associated in part with their higher likelihood of being aneuploid. This practice may reduce the number of embryos available for transfer and could, potentially, lead to chromosomally normal embryos not being considered for treatment. This retrospective single centre observational study analysed cycle outcomes in which suboptimal quality blastocysts were transferred between 2016-2021. Two groups of patients were identified: Group A underwent transfer of single untested frozen thawed embryos (147 cycles), and Group B underwent transfer of single frozen thawed embryos that were reported as euploid after biopsy at the blastocyst stage and testing by PGT-A (36 cycles). The mean maternal age was 36.0±5.4. in group A and 38.9±3.8 . in group B. 81% of patients in group A had previously undergone embryo transfer with high quality embryos from the same cohort while this percentage in Group B was 67%. Clinical pregnancy (CP) rate in Group A was 17.6% (26/147) and live birth rate (LB) was 14.7% (21/147). In Group B 776 suboptimal quality embryos were subjected to PGT-A testing. The euploidy rate was 13% (101/776). The embryos transferred (n=36), resulted in a CP of 33% and LB rate of 28%. The difference in outcomes between two groups were statistically significant p= .00046 These data indicate that checking euploid status of suboptimal embryos can improve clinical outcomes when only such embryos are available transfer. Applying PGT-A in this context may therefore increase (rather than decrease) the number of embryos available for transfer as well as chances of achieving a live birth.

E Reshef, A Robles, J Hynes, J Turocy, and E Forman. A review of factors influencing the implantation of euploid blastocysts after in vitro fertilization. Fertil Steril. VOL. 3 NO. 2 pp 105-120, MAY 2022. H Zhu, Zhang H, Fadlalla E, Wang R, Geng D, R Liu. Culturing surplus poor-quality embryos to blastocyst stage have positive predictive value of clinical pregnancy rate. Iran J Reprod Med. Vol. 12. No. 9. pp: 609-616, September 2014.

2A.5 Assessment of the power of single variables to predict blastulation, embryo utilisation and livebirth and how this may help both time-lapse algorithm development, and standard selection practices

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Background and aims: Assessment of the power of time-lapse imaging (TLI) to predict outcomes is based mainly on data selected from transferred embryos. This creates selection bias due to lack of representation of the whole embryo cohort. We aimed to reveal the power of single morphokinetic variables and to consider if timing is more important than morphology.

Methods: 31,323 embryos underwent TLI and comprehensive annotation from time of pronuclear fading (tPNf) until blastulation. Fate was recorded as vitrified, transferred or discarded. For live birth analysis, 4805 single embryo transfers were included. Receiver operating characteristic analyses determined the predictive ability of singular variables, using Area Under the Curve (AUC).

Results: Analysing the entire cohort, prediction of embryo utilisation was high, with AUC values up to 0.80 (expanded blastocyst; tEB). Whether an embryo will reach the full blastocyst stage (tB), could be predicted early, using either time of pronuclear fading (tPNf)(AUC=0.63) or time to two-cells (t2)(AUC=0.65). Considering transferred embryos, later morphokinetic variables were most predictive of live birth, especially time of starting blastulation (tSB)(AUC=0.59). Trophectoderm (TE) and inner cell mass (ICM) morphologies were less predictive of live birth than morphokinetics (AUC 0.55 and 0.53 respectively). Using time of insemination (IVF or ICSI), rather than time of pronuclear fading, gave higher predictive capabilities overall.

Conclusions: Most TLI, morphokinetic algorithms are built on transferred embryos with known outcomes. To maximise predictive power, timepoint of insemination (rather than pronuclear fading) should be included. Even single morphokinetic variables can have profound predictive value. Later variables are predictive of embryo utilisation and live birth, supporting the use of blastocyst over early cleavage stage selection, and morphokinetics over morphology alone. This study highlights the relative predictive power of morphokinetics over blastocyst morphology and the pitfalls of focusing on a preselected population of embryos for anything other than live birth selection.

2A.6 Analysis of cumulative PGT-M data from embryos for primary ciliary dyskinesia suggests gene duplication in consanguineous reproductive partners

Valerie Shaikly[1]; Roy Pascal Naja[2]; Ana Cervero Cervero[2]; Vladimir Ovsyankin[1]; Ben Raybould[3]; Ahmed Amer[3]; Shadi Khalil[3]; Mohamed Taranissi[1]
[1]ARGC; [2]Igenomix (Vitrolife); [3]London Fertility Centre

PGT-M was performed for a consanguineous couple believed to be carriers of the same mutation (c.5503C>T) in the DNHA5 gene previously identified by exome sequencing and associated with primary ciliary dyskinesia. The patients had four treatment cycles over six months, from which 12 trophectoderm biopsies were sent to a specialist genetics laboratory, and PGT-M was performed using linkage analysis in addition to direct mutation detection. Analysing samples of cycle 4, the genetics laboratory observed contradictory results when linkage analysis was compared to direct mutation detection, leading to inconclusive diagnoses. Additional MLPA analysis of genomic DNA showed four copies of DNHA5 for the female partner and three copies for the male. The contradictory PGT-M results and further MLPA suggest the female partner has one mutant and one wildtype allele on each chromosome, and the male partner has one mutant allele on one chromosome and one wildtype and one mutant allele on the other chromosome. This implies that only the male partner is a carrier, and that PGT-M was not necessary. The patients were counselled that the risk for an embryo having no functional copies of DNHA5 could not be ascertained but would likely be lower than 25%. Exome sequencing also identified both partners as unlikely carriers for congenital adrenal hyperplasia due to duplication of the CYP21A2 gene (C.955C>T+CYP21A2dup), a frequently observed and elucidated duplication. This case demonstrates that PGT-M for consanguineous couples for recessive conditions may benefit from extended family studies at the pre-PGTM stage. It may also serve as evidence for advising consanguineous patients that embryo testing technologies for some genes may yield higher false negative rates than expected where fewer embryo samples and family studies are not available for review. Family segregation analysis is required to show that artefacts are not responsible for the inconclusive embryo results observed.

SP2B BFS YOUNG SCIENTIST SHORT PAPERS

2B.2 Assessing chromosome ageing in mammalian oocytes

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University of Edinburgh

Background: The decline in fertility with maternal age is associated with increased oocyte aneuploidy that gives rise to trisomic conceptions and congenital conditions such as Down syndrome. Existing data from mature mouse oocytes show that cohesin protein levels decrease with maternal age, resulting in reduced chromosome cohesion and increased aneuploidy (1, 2). However, it is unclear when in oogenesis these age-dependent changes in chromosome structure are occurring, or whether this phenomenon also occurs in humans.

Methods: We used Fluorescence In-Situ Hybridisation (FISH) in archived mammalian ovary wax sections to quantify sister chromatid separation in oocytes during folliculogenesis. Mouse samples were collected from C57BL/6J mice at 3, 6 and 9 months. FISH was carried out for chromosome 1 at peri-centromeric and peri-telomeric regions. Human samples were collected from 7 obstetric patients undergoing Caesarian sections, and FISH performed for the peri-telomeric region of chromosome 21.

Results: In mouse oocytes, age-dependent increases in chromatid separation can be detected in primordial, primary and secondary follicles. On chromosome 1, chromatid separation at the peri-telomeric region was more sensitive to age-dependent changes than the peri-centromeric region, with inter-chromatid distances increasing more than 2-fold between 3 and 9 months. Notably, the increase in chromatid separation over this time-course is more consistent with oocyte ageing representing progressive changes in the primordial oocyte pool rather than a production line or selection process that is based on oocyte quality. Preliminary data from obstetric patients indicates that chromatid separation increases with age in human dictyate oocytes at the primordial follicle stage.

Conclusions: The increase in inter-chromatid distances with maternal age at the primordial follicle stage suggests that chromosome ageing occurs during the stage of prolonged oocyte arrest in both mice and humans. This work has

implications for the development of therapies to interfere with chromosome ageing and reduce age-dependent oocyte aneuploidies.

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2B.3 The uterine microbiome and the microbial metabolite butyrate stimulate pro-inflammatory responses in endometrial epithelial cells, suggesting a possible impact on female fertility

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The maternal endometrial microenvironment, including the microbiome, is crucial for embryo implantation. The composition of the endometrial microbiome has not been fully characterised, therefore we aimed to identify which microbes are present in endometrial biopsies taken from a cohort of women undergoing assisted reproductive technologies (ART) with either successful or unsuccessful outcomes. Bacterial dysbiosis is often associated with variation in microbial-derived metabolites composition, such as short chain fatty acids (SCFAs). We thus wondered what effect SCFAs have on endometrial epithelial cells and on female fertility. Through 16S analysis we identified a more diverse microbiome in the women with unsuccessful pregnancy outcome, with a lower abundance of Lactobacillus spp. and an increased abundance of Corynebacterium spp. and Prevotella spp., which correlated with the elevated levels of IL-17A identified in the serum. Butyrate induced increased expression of antimicrobial peptides, cytokines, and chemokines in both tumoral and primary endometrial cells. Among the cytokines induced, we could also observe increased production of IL-17A protein in endometrial epithelial cells. The effect of butyrate on endometrial receptivity and stromal decidualisation was assessed by treating cells with progesterone with or without butyrate. Butyrate seems to enhance stromal cells decidualisation and to drive the expression of endometrial receptivity markers. Women who are not able to conceive show a different microbiome, which may alter local metabolites, including butyrate. We showed that this SCFA has a pro-inflammatory activity in endometrial epithelial cells and that it impacts on fertility by increasing stromal decidualisation and epithelial receptivity markers, leading to possible changes in the window of implantation.

2B.4 Exploring the experiences of women who have moved to Israel and subsequently used Israeli fertility treatment services: A qualitative study

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Background: Israel's pronatalist culture results in a social expectation to have children. This culture drives Israel's high fertility rate of 2.9. Israel's health policy reflects this expectation by providing unlimited state funded fertility treatment until the birth of two live children. Challenges of fertility treatment are exacerbated by the societal pressure to have children. Furthermore, the lack of financial burden creates a culture of perseverance following treatment failures. Whilst the experiences of Israeli women who use fertility treatment have been studied, no research exists into the experiences of women who immigrated to Israel and were therefore raised in a different culture. This study aims to address this gap in knowledge.

Methods: This is a qualitative study using semi-structured interviews to investigate the experiences of 13 women who utilised Israeli state funded fertility treatment. Participants were located across Israel and were recruited using purposive sampling through social media. Data was analysed using framework analysis.

Results: Three themes were identified: 1. Systemic factors: The lack of financial burden was positive, however, participants struggled to navigate the bureaucratic healthcare system, especially when experiencing a language barrier. 2. Influence of others: Encountering a cold bedside manner alongside contending with the cultural expectations of a pronatalist society was challenging. Understanding of healthcare professionals regarding Jewish values and receiving support from immigrants who appreciated the same culture shock as participants improved treatment experiences. 3. Impact of journey: Participants often withdrew socially and the treatment process had implications on participants' lives, jobs and relationships.

Conclusion: Undergoing fertility treatment in Israel had both positive and negative implications on the participants' experiences. The barriers that these women face should be better anticipated with improved provision of resources in English and further research into how women can be supported whilst navigating Israel's pronatalist culture.

2B.5 Quantifying the daily variability following a single estimation of reproductive hormone levels

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Background: Due to practical limitations, the diagnosis of hypogonadism is predominantly based on a single measure of reproductive hormones, often with confirmation on a second occasion[1]. Factors associated with reproductive hormone variation include: pulsatile secretion[2], diurnal rhythm[3], and food intake[4], which can affect the accuracy of diagnosis of reproductive disorders. There is limited data quantitatively estimating the variability of reproductive hormone levels over the day. Hormonal sampling data collected over several hours allowed quantification of how representative a single morning measure of reproductive hormones (often used for diagnosis in the clinic) is of the daily hormonal profile.

Methods: Data from 13 research studies (including 267 participants) conducted at Imperial College London were used to quantify the variability in reproductive hormones in both healthy men and women (n=142), and those with reproductive disorders (n=125). The impact on hormone levels of pulsatile secretion, diurnal variation, feeding, and overall variability (Coefficient of Variation (CV)) was quantified.

Results: The initial morning value of reproductive hormones was higher than the mean value throughout the day (percentage decrease from morning to daily mean: LH 18.5%, FSH 9.8% and Testosterone 9.6% and Oestradiol 2.7%). FSH was the least variable reproductive hormone (CV 9%), followed by sex-steroids (testosterone 12%, oestradiol 13%), whereas LH was the most variable (CV 28%). In healthy men, testosterone fell between 9am and 5pm by 14.9% (95%CI -4.20%, -25.5%), although morning levels correlated with (and could be predicted from) evening levels in the same individual (r2=0.53, P<0.0001). Testosterone was reduced more following a mixed meal (34.3%) than after an intravenous bolus of glucose (7.4%; P<0.0001).

Discussion: Quantification of the variability of a single measure of reproductive hormones enables more precise estimation of the hormonal profile during the day, with relevance for the diagnosis of hypogonadism and its aetiology.

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2B.6 When to stop freezing? The impact of embryo quality at cryopreservation on thaw survival and FET outcomes (2015-2021)

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Introduction: The efficacy of frozen embryos, increasing indications for freeze-all cycles (agonist trigger use/high progesterone) and increasing prevalence of pre-implantation genetic testing for aneuploidy (PGT-A) despite HFEA guidance has led to increasing embryo freezing. Accurate data on outcomes by grade of embryo at freezing is essential to counsel patients as to the appropriateness of routinely freezing embryos of lower quality.

Methods: Retrospective analysis of 2844 consecutive frozen cycles between 2015-2020 where a single blastocyst was thawed for transfer. Primary outcomes were thaw survival and live birth rate (LBR)/transfer stratified by embryo expansion and embryo quality (inner cell mass (ICM) and trophectoderm (TE)) using the Gardner's scoring system at freezing. Cycles were further analysed by Day of freezing (5 or 6), age at freezing (<38 and ≥ 38).

Results: Thaw survival of 97.8%, 97.6%, 97.3% and 89.7% was demonstrated for embryo expansion at freezing of 3-6 respectively. Thaw survival of 99.3%, 98.5%, 96.9%, 95.6% and 98.2% was demonstrated for embryo grades at freezing of AA, AB/BA, BB, BC/CB and CC respectively. No difference was noted by day of freeze. In both age groups LBR decreased with embryo quality. In the <38 group for embryos of expansion 3-5 a LBR of 47.8%, 43.7%, 41.9%, 40.1%, 30.4%, 23.3% and 25.0% was demonstrated for grades AA, AB, BA, BB, BC, CB and CC respectively.

Conclusions: Thaw survival is not significantly affected by day of freeze or embryo expansion, unless fully hatched, nor by quality down to CC. However, LBR in both age groups declines as embryo quality declines. Although "CC" embryos, often deemed unsuitable to freeze achieve a not insignificant 25% and 15% LBR in the <38 and ≥ 38 groups respectively.

This data is essential in establishing freezing practice and counselling patients to allow informed choice balancing against economic and emotional burdens of cycle failure.

SP2C PROGRESS IN REPRODUCTIVE GENETICS

2C.1 New data on the incidence and impact of DNA contamination affecting embryo samples used for PGT-A highlights the importance of diagnostic methods that allow contamination to be detected

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Background: Since the introduction of molecular techniques, such as next generation sequencing (NGS), for preimplantation genetic testing for aneuploidy (PGT-A), standard methods have been unable to detect when an embryo biopsy specimen is contaminated with non-embryonic DNA. This potentially serious problem has been largely ignored. We determined the frequency and diagnostic impact of contamination affecting PGT-A samples for the first time.

Methods: We assessed 27,720 trophectoderm biopsies from 22 clinics. Biopsy specimens underwent PGT-A using a highly validated NGS method (PGT-Seq). As well as giving a quantitative indication of the copy number of each chromosome, thousands of single nucleotide polymorphisms (SNPs) are genotyped, providing a qualitative strategy for aneuploidy detection and also revealing any instances of DNA contamination. Most embryos with a contaminated sample undergo re-biopsy, revealing the true chromosomal status of the embryo. Comparing results from the first (contaminated) biopsy and the re-biopsy allows calculation of false positive (FP) and false negative (FN) rates associated with contamination.

Results: 115 samples were shown to be contaminated using PGT-Seq (0.4%). Contamination rates in individual clinics ranged from 0% to 0.78 %. 88 of the associated embryos underwent re-biopsy and yielded an uncontaminated PGT-A result. Had contamination not been detected, and the initial biopsy result had been used to classify the embryos, 26.7% of these would have had a FP and 23.3% a FN .

Conclusions: ~1 in 250 biopsy specimens were contaminated, although the incidence was almost double this in some clinics. Half of the affected samples would have been misdiagnosed if we had not been able to detect contamination. Viable embryos would have potentially been discarded due to FPs and aneuploid embryos transferred as a consequence of FNs. These results emphasize the importance of precautions to avoid DNA contamination, and the value of PGT-A methods capable of detecting it when it occurs.

2C.2 Gamete donor conception and direct-to-consumer genetic testing: How are donor conceived people, their parents and donors using direct-to-consumer genetic testing?

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Purpose: The ConnecteDNA project is an ongoing qualitative research study (March 2021-February 2024). It investigates how people in the UK, involved in donor conception, are impacted by DTCGT and considers the implications for policy and practice. It aims to contribute to evidence on how donor conceived people, as well as parents through donor conception and donors, are using DTCGT.

Methods: This presentation focusses on in-depth interviews with parents through donor conception, donors and donor conceived adults who have used, or considered using, DTCGT.

Results: Our findings show that people involved in UK donor conception are using DCTGT to access and manage information about donor conception. This includes donor conceived people, previously unaware of the circumstances of their conception, discovering they are donor conceived after using DTCGT. We have found that donor conceived people, already aware that they are donor conceived, may also use DTCGT in search of information about 'donor relatives' and/or their genetic make-up, including understandings of their ethnicity and health risks. Furthermore, some donors are using DTCGT to make themselves contactable to people conceived via their donation(s). Parents through donor conception may use, or consider using DTCGT, to trace people related to their children through donor conception. This may be done when the child is very young, with the intention of finding relatives (particularly half siblings) who they can then grow up with. Others take a more responsive approach, only using DTCGT to support a child's request or interest in more information. DTCGT is also used to trace genetic relatives alongside, or instead of, 'official' routes for accessing information about donor conception, as well as social media and public records.

Conclusions: DTCGT is transforming how people involved in donor conception seek information about genetic relatives. Professionals who work with people using, or considering, donor conception, should make their clients

2C.3 Systematic review and meta-analysis: Does pre-implantation genetic testing for aneuploidy at blastocyst stage improve live birth rate?

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Objective: To establish if preimplantation genetic testing for aneuploidy (PGT-A) at blastocyst stage improves the composite outcome of live birth rate and ongoing pregnancy rate per embryo transfer compared to conventional morphological assessment.

Methods: A systematic literature search was conducted using PubMed, EMBASE and the Cochrane database from 1st March 2000 until 1st March 2022. Studies that compared reproductive outcomes following in-vitro fertilisation using comprehensive chromosome screening (CCS) at blastocyst stage with traditional morphological methods were evaluated.

Results: Of the 1307 citations identified, five randomised control trials (RCTs) and 11 cohort studies met the inclusion criteria for the study. The pooled data identified a benefit between PGT-A and control groups in the composite outcome of live birth rate and ongoing pregnancy per embryo transfer in both the RCTs (RR 1.10, 95% CI 1.03-1.18) and cohort studies (RR1.43, 95% CI 1.22-1.68). Euploid embryos identified by CCS were more likely to be successfully implanted (RCT: RR 1.20, 95% CI 1.10-1.31 and cohort: RR 1.48, 95% CI 1.22-1.79), with a lower chance of miscarriage (RCT: RR 0.76, 95% CI 0.57-1.00 and cohort: RR 0.59, 95% CI 0.44-0.79) and compared to morphological assessment alone.

Conclusions: CCS-based PGT-A at blastocyst biopsy stage increases the composite outcome of live births and ongoing pregnancies per embryo transfer compared to morphological assessment alone. In view of the limited number of RCTs and cohort studies, future reviews and analyses are required to confirm these findings.

2C.4 Accurate mitochondrial DNA quantification clarifies the clinical value of measuring mtDNA in trophectoderm biopsy specimens

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Background: Individual mitochondria contain one or more copies of their own genome (mtDNA). Changes in the amount of mtDNA in blastocysts has been reported in association with implantation potential, leading to proposals that mtDNA quantification might serve as a useful biomarker of embryo viability. However, results from clinical studies to explore this possibility have yielded contradictory data, due in part to deficiencies of the methods used. We seek to clarify the truth about mtDNA using a quantification strategy that we believe to be the most accurate ever devised.
Methods: 651 blastocysts derived from 133 couples underwent biopsy for PGT-A on day-5 or day-6. A highly validated real-time PCR method was used to quantify three distinct sites in the mtDNA. These were normalised against 221 independent loci in the nuclear genome - a vital step when evaluating samples where the cell number is unknown, such as a trophectoderm biopsy.
Results: Lower mtDNA quantities were observed in association with day-6 biopsy ($p<0.0001$), extent of blastocyst expansion ($p<0.0001$), and superior trophectoderm (TE) morphology, although the latter was not statistically significant ($p=0.09$). On average, mtDNA levels were higher in aneuploid embryos ($p<0.0001$). There was no association between mtDNA level and the chances of implantation in this dataset.
Conclusions: Reduced mtDNA in embryos of excellent morphological grade likely reflects the increased TE cell numbers of such embryos. Little if any mtDNA replication occurs during preimplantation development, and consequently, the mtDNA content is divided amongst an ever-growing number of cells. In this context, mtDNA quantification of blastocyst biopsy specimens provides a sensitive measure of TE cell number, but probably provides little additional benefit for embryo selection beyond conventional morphological grading. However, the fact that increasing mtDNA was observed in aneuploid embryos, may indicate that subtle differences in TE cellularity exist, not fully captured by traditional morphological assessment.

2C.5 Polymorphic rearrangements of human chromosome 9: Correlation with male infertility

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Background and objectives: Chromosomal polymorphisms are structural variations in a chromosome defining the genomic variety of a species. Chromosome 9 variations occur in 1-2% of the general population. Although it is classified as a minor chromosomal rearrangement with no phenotypic effects, there is growing evidence that chromosome 9 rearrangements are associated with recurrent miscarriages, abnormal clinical phenotype, and subfertility. Hence the aim of this research was to study the presence of chromosome 9 variations in male infertile patients, and its association with advanced semen and molecular parameters.

Methods: A total of 96 male patients referred to our laboratories for infertility and underwent cytogenetic and chromosomal microarrays analysis. In addition, they were also tested for semen analysis, sperm fluorescence in situ hybridization (FISH) for the presence of aneuploidies, TUNEL assay for the analysis of sperm DNA fragmentation, and Y microdeletion detection.

Results: Cytogenetic analysis revealed chromosome 9 anomalies in 6.2 % of patients: two of them showed a pericentric inversion 46,XY,inv(9)(p11q12), while one patient showed 46,XY,inv(9)(p11q13). The remaining three patients showed a polymorphic variant 9qh (qh+, qh++, qh-). Sperm analysis revealed a moderate or severe oligospermia associated with teratozoospermia in the same patients, and pathological value of sperm DNA fragmentation (≥ 27%) in patients with chromosome 9 pericentric inversion. Furthermore, FISH technique revealed sperm aneuploidy (above 9%) in patients with chromosome 9 pericentric inversion and in patient with chromosome 9 polymorphic variant 9qh++.

Conclusion: Our data suggests that chromosome 9 polymorphic rearrangements could be associated with abnormalities in sperm quality due to a defect in normal meiotic segregation, contributing to male infertility. Our data agree with recent studies concerning couples who are candidates for assisted reproduction due to infertility or recurring abortions. In this cohort, previous studies have reported a higher incidence of chromosome 9 polymorphic variants than the general population.

2C.6 Preimplantation genetic testing for monogenic disease (PGT-M): Reliance on analysis of linked polymorphisms risks serious diagnostic errors

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Juno Genetics

Background: PGT-M has helped thousands of couples at risk of transmitting an inherited condition to have healthy children. Tailoring methods to the unique genetics of each couple requires significant effort, increasing patient waiting times and costs. To minimise patient-specific work-up, PGT-M methods have been developed that focus on the examination of polymorphisms in the vicinity of the gene of interest. Such polymorphisms are present in the general population, providing a more generic approach for PGT-M, applicable to multiple families. DNA samples from the couple are tested along with additional family members, revealing which alleles accompany the mutant copy of the gene. While PGT-M based upon analysis of linked polymorphisms generally works well, we demonstrate the importance of also testing for the disease-causing mutations.
Methods: Trophectoderm biopsy was followed by multiple displacement amplification and karyomapping, genotyping ~300,000 polymorphisms scattered across the genome. In each case, particular attention was given to the polymorphisms in close proximity to the gene causing the condition. Additionally, DNA fragments encompassing the patients' mutation sites were amplified and sequenced.
Results: We report four PGT-M cases where the genetic reports used to assign the status of patients were incorrect, meaning that the assumptions upon which linkage analysis were based would have been wrong. We also describe three cases where meiotic recombination separated the mutant gene from its associated polymorphisms, greatly reducing diagnostic accuracy. Finally, we discuss consanguineous couples, whose shared ancestry results in a lack of distinct alleles on their chromosomes, compromising linkage-based approaches. In all cases, serious diagnostic problems were averted because of our policy of including direct mutation testing in PGT-M cases.
Conclusions: Where possible, direct detection of the disease-causing mutations carried by the couple should be undertaken in PGT-M cycles. Basing a diagnosis on linked polymorphisms alone is suboptimal and increases the risk of misdiagnosis.

SP2D FERTILITY CHALLENGES ACROSS THE LIFE SPAN

2D.1 Fertility preservation and realignment in transgender women

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Background: Medical care for transgender people is multi-faceted and attention to individual reproductive aspirations is an essential yet often overlooked aspect of care. Given the impact of hormonal therapy and other gender affirmation procedures on reproductive function, extensive counselling and consideration of fertility preservation is recommended prior to their commencement. This comprehensive review provides an in-depth exploration of the reproductive aspirations of transgender women and discusses utilisation of fertility preservation services.

Methods: An extensive literature search of major databases (Pubmed, Medline, PsychInfo, GoogleScholar, Web of Science) was performed on the topic of fertility preservation in transgender women. The literature was critically appraised with focus on reproductive aspirations, fertility preservation options, barriers to utilisation and future techniques.

Results: This review highlights that among transgender individuals, fertility aspirations vary. Fertility preservation counselling is a determinant for uptake of fertility preservation. However, despite adequate counselling, uptake may remain low, thus, highlighting a mismatch between desire and uptake. Barriers to fertility uptake include: an unwillingness to delay gender transition, cost, social stigma and concerns that fertility preservation would pronounce feelings of gender dysphoria. Current techniques for fertility preservation are discussed (sperm cryopreservation, embryo cryopreservation, testicular tissue cryopreservation and assisted reproductive technology) as well as future options (testicular tissue cryopreservation and uterine transplantation) which remain experimental at present.

Conclusions: Taking transgender women's reproductive aspirations seriously is an important aspect of care. This review demonstrates the disparity between reproductive aspirations and fertility preservation uptake. We highlight that further studies exploring the attitudes of transgender women to fertility are required. With a deeper understanding of their perspectives, barriers to accessing services can be more readily overcome and services more effectively designed. With future scientific progress, the desires of transgender women should be approached with considerations of equality and wellbeing at the forefront of clinicians' minds.

2D.2 Drug screening to identify protectants against chemotherapy-induced male infertility

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Background: Due to advancing treatment modalities, the number of patients surviving childhood cancer has increased dramatically in recent years. However, as childhood cancer treatment can often result in infertility, this in turn has led to an increased need to protect testicular tissue from the damage induced by treatments, such as chemotherapy. We aimed to identify FDA-approved compounds that could be repurposed as a fertoprotectant of spermatogonial stem cells against the side effects of chemotherapy.

Methods: We performed an unbiased drug screen of >1200 compounds in vitro in rat GC6-spg cells (Type A spermatogonia) to identify potential protectants of spermatogonial stem cells from the detrimental effects of cisplatin, a commonly used chemotherapeutic agent. The cells were tracked by means of live cell imaging in an IncuCyte, while changes in apoptosis were also assessed using a caspase-3 substrate. Z-scores were calculated based on multiple parameters, such as changes in proliferation, total cell number and total apoptotic cells. Compounds with a Z-score >2 were identified as 'hits'.

Results: We identified >30 'hit' compounds which could potentially act as protectants against the detrimental effects of cisplatin. Validation by means of an 8-point dose response live cell imaging, reduced the number of hits to 9. However, 7 of these compounds were identified as being within the same family of drug compounds, indicating a potentially important mechanism of protection.

Conclusions: Further studies assessing survival following cisplatin-treatment in combination with the 'hits' will be carried out to further confirm the 'hits' as chemoprotectants in spermatogonia. Furthermore, DNA damage response markers will be assessed to shed light on the mechanism behind the protection within the cells. Identifying (and confirming) compounds that can be repurposed as chemoprotectants of spermatogonial stem cells could have important consequences for the fertility of long-term survivors of childhood cancer.

2D.3 Luteinizing hormone is able to protect reproductive health in cancer patients

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Objective: Cancer therapies cause premature ovarian insufficiency (POI) by inducing a severe reduction of primordial follicles (PMFs). We investigated if luteinizing hormone (LH) can protect ovarian follicles against cyclophosphamide (CPM)-induced damage in women.

Materials and methods: Ovarian cortical tissues were collected from nine patients (ageSD: 15.334.50) who had cryopreserved their tissue before receiving anticancer treatment. Ovarian cortical strips were thawed and randomly assigned to Control (CTRL), phosphoramide mustard (PM), and PMLH (LH added 1 hour before PM). Samples were analyzed after 8, 16, 24 and 48 hrs of treatment. The cultured ovarian cortical samples were processed for: Histology for PMFs and primary follicles (PFs) analysis; Immunohistochemistry; and Real-Time PCR. To investigate the mechanism underlying the observed effects, the expression of markers involved in DNA damage (gH2AX), apoptosis (Cleaved caspase 3, NOXA, PUMA), follicle activation (p-AKT, FOXO3a), cell cycle arrest (p21, Ki67), and inflammation (IL1b, TNF) were analysed.

Results: The follicle density in the untreated group varied from 233.03 to 3420.27 PMFs/mm3 (n=9). Relative PMFs density (%) was significantly reduced in PM vs CTRL either after 24 or 48 hrs, which was significantly counteracted by LH (24 hrs: CTRL=76.192.12; PM=44.955.06; PM+LH=73.9711.64. 48 hrs: CTRL=66.473.96; PM=36.763.96; PMLH=55.748.72). At 16 hrs, LH did not prevent DNA damage induced by PM as evidenced by gH2AXoocytes in both PM and PH+LH groups; at 24hrs, gH2AX expression was downregulated in the PM+LH group (PM@ 100% vs PMLH@35%). LH also reduced the levels of pro-apoptotic factors such as NOXA, PUMA and CC3, and the follicle activation lowering AKT-FOXO3a signaling axis. PM treatment creates a proinflammatory microenvironment, as shown by increased IL1b and TNF gene expression, partially counteracted by LH pretreatment.

Conclusions: LH significantly reduces PMF loss in ovarian cortical strips cultured in vitro with PM, the active metabolite of CPM.

2D.4 Identification and characterisation of novel follicle-stimulating hormone receptor antagonists

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Follicle-stimulating hormone receptor (FSHR) is a Class A G protein-coupled receptor (GPCR) that controls many crucial reproductive physiological processes. The FSHR interacts with its heterodimeric glycoprotein hormone (FSH) and primarily activates Gαs/cAMP/PKA signalling pathway to support the growth and survival of ovarian follicles, leading to steroidogenic activity and follicular maturation. Recent studies have proposed age-related extragonadal roles of FSH/FSHR with elevated FSH linked to post-menopausal osteoporosis, deposition/changes in adipose tissue, and cognitive defects. Together, these findings have identified FSH/FSHR inhibition as an approach to therapeutically target and combat these menopause-related co-morbidities, and as a potential non-steroidal mechanism of contraception. Therefore, we aimed to screen AI-generated FSHR-targeting small molecule compounds for their ability to inhibit FSH-dependent cAMP production. 84 AI-generated small molecule compounds (provided by Atomwise, San Francisco) were screened in HEK293 cells transiently expressing FSHR, and the ability to inhibit FSH-dependent cAMP-production was analysed by live cre-luc analysis. 3 inhibitors were identified that displayed >90% inhibition at high FSH concentration, with their IC50 value ranging between 31-39 µM. Further investigations on the concentration-dependent effect of the inhibitors (0-100µM) on FSH-dependent cAMP-production was assessed. Results suggest that these inhibitors displayed non-competitive antagonism, as there were no obvious changes in potency. Our findings present 3 new small molecule pharmacological non-competitive FSHR inhibitors, which may present new pathways for non-steroidal contraceptives or treatment of menopause-related co-morbidities.

2D.5 Determining the impact of FSH glycosylation variants on the pre-antral follicle transcriptome in the ageing ovary

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Ovarian ageing is a naturally occurring physiological process, marked by dynamic changes in ovarian function and hormone secretion. A key of ovarian regulator is follicle stimulating hormone (FSH). FSH is secreted as two glycosylation

variants: partially glycosylated FSH (FSH21) and fully glycosylated FSH (FSH24). Analysis has shown that the ratio of FSH21:FSH24 changes with age, with FSH21 predominant during reproductive prime, and FSH24 predominant around menopause. How FSH glycoforms modulate follicle function in the ageing ovary remains unknown. This study aimed to determine the effects of FSH21 and FSH24 on follicle growth and survival in young versus ageing mice. Mouse ovarian follicles were isolated from 12-16wk-old (reproductive prime), 6-8-month (ageing) and 11+ month old (approaching ovaria senescence) C57/BL6 mice and treated -/+10ng/ml, FSH21, FSH24. Mimicking changes in ratios of FSH21:FSH24 that occur with ageing, follicles were additionally treated with 80:20 FSH21:FSH24 (mimic reproductive prime), or 20:80 FSH21:FSH24 (mimic menopause). Follicles were cultured for up to 96hrs and imaged daily to evaluate follicle morphology, with follicles snap frozen at 24hr intervals, for RNA sequencing. Morphological assessment revealed that age impacted follicle response to FSH glycoforms, with FSH21 and 80:20 FSH21:FSH24 increasing follicle growth across all time points in 12-16wk, while 80:20 FSH21:FSH24 increased 6month follicle growth, from 48-to96hrs. 20:80 FSH21:FSH24 increased 11+month follicle growth from 48hrs. Treatment of follicles with FSH24 or 20:80 FSH21:FSH24 resulted in decreased survival rates in the 12-week follicles, whereas 80:20 FSH21:FSH24 decreased survival rates in 11+month follicles. RNASeq analysis revealed both FSH glycoform and age-dependent differences in gene expression in size-matched pre-antral follicles isolated from 12-week and 11+month mice. These data suggest that FSH glycosylation distinctly modulate the follicular microenvironment to control follicle growth and survival, in an age-specific manner.

SP2E FACTORS IMPACTING REPRODUCTIVE OUTCOMES

2E.1 Infertile human endometrial organoid apical protein secretions are dysregulated and impair trophoblast progenitor cell adhesion

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Embryo implantation failure leads to infertility and remains a challenging problem for IVF. As an important approach to regulate implantation, endometrial glands produce and secrete factors apically into the uterine cavity in the receptive phase to prepare the initial blastocyst adhesion and implantation. Organoids were recently developed from human endometrial glands and show long-term expandability, genetic stability and maintenance of their hormone responsiveness. Importantly, organoid exhibits similar apical-basal polarity compared to endometrial gland making it an ideal model to study glandular secretions. We established organoids using endometrial biopsies from women with normal fertility and primary infertility (referred to as fertile and infertile organoids). Organoids from both groups were treated with hormones to model the receptive phase of the endometrial glands and intra-organoid apical fluid (IOF) was collected to compare the apical protein secretion profile. Our data show that infertile organoids were dysregulated in their response to estrogen and progesterone treatment. Proteomic analysis of IOF identified 131 decreased and 19 increased proteins in infertile group compared to fertile (>1.5-fold change). Many of the proteins were similarly differentially regulated in organoid cells at the mRNA level. To determine the effect of organoid apical secretion on blastocyst adhesion, we first developed epithelial monolayers using fertile organoids and compared them with previously established primary human endometrial epithelial monolayers. Using miR-29c as an example, we confirmed that both models respond similarly to microRNA overexpression. IOF was collected after hormone stimulation to treat trophoblast progenitor spheroids (blastocyst surrogates) and their adhesion on the organoid-derived endometrial cell monolayer determined. Incubation of infertile IOF significantly reduced trophoblast spheroid adhesive capacity compared to fertile IOF ($P < 0.0001$) and medium control ($P < 0.01$), respectively. Together, this study paves the way to determine the molecular mechanisms by which endometrial glandular apical released factors regulate blastocyst initial attachment.

2E.2 The impact of consent for disclosure (CD) in the HFEA ART register: Possible implications for treatment outcome research

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The Human Fertilisation and Embryology Authority (HFEA) ART data is used in clinical research to explore health outcomes at birth and later in life. After the introduction of Consent for Disclosure (CD) for the use of patient identifying information in October 2009, only ART cycles with CD are available for research using bespoke datasets. It is unclear if cycles with CD are representative of the entire patient population and treatment cycles, making research on children born since October 2009 problematic. If CD and non-CD groups differ, there is a possibility of biased results when using only CD data in outcome and linkage studies. Thus we set out to understand what drives CD and whether it is associated with treatment outcomes. Fully anonymised bespoke data from 1991 to 2018 was obtained from the HFEA, comprising CD and non-CD ART cycles. Logistic regression was used to identify patient characteristics associated

with CD over time since 2009. We explored differences in live birthrate trends and birthweight outcomes for CD and non-CD cycles. The cohort comprised all 561,052 registered ART cycles between October 2009 and 2018, of which 55% provided CD and 45% did not. Maternal Age, ethnicity and year of treatment were associated with CD. Older women (43-44 years) were 35%[95%CI(32,37)%] less likely to give CD compared to 18-34 year-olds. Black and Asian ethnicities were less likely to give CD compared to whites. Live birth rates were higher in the CD group, while low birth weight was more prevalent in the non-CD group. CD rates have improved from 39% in 2010 to 66% in 2018; however, the association with treatment outcomes persists in recent years. These data indicate that excluding the non-CD cycles may bias the results of linkage and other studies; therefore, careful attention is recommended when analysing and interpreting this data.

2E.3 The association between dietary patterns and risk of miscarriage: a systematic review and meta-analysis

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Background: The evidence on the association between diet and miscarriage risk is scant and conflicting.

Objective: A systematic review and meta-analysis was conducted to summarise the evidence on the association between peri-conceptual diet and miscarriage risk in healthy women of reproductive age.

Methods: Electronic searches were carried out in PubMed, MEDLINE, Embase, CINAHL Plus and CENTRAL from database inception to August 2022 without restriction of regions, publication types or languages.

Results: We included 20 studies (11 cohort and 9 case-control), of which 7 presented data suitable for meta-analysis (3 cohort and 4 case-control, n = 14,341 women). No experimental studies were identified.
Diet data were grouped for meta-analysis based on the food categories that were outlined in the Eatwell model by Public Health England. Summary effect sizes (odds ratio [OR] with 95% confidence interval [CI]) were calculated for each food category

Our primary analyses suggest a reduction in miscarriage odds with high intake of the following food groups: fruit, vegetables, fruit and vegetables, seafood, dairy products and eggs. The evidence was uncertain with regard to an association between miscarriage risk and consumption of meat, red meat, white meat, fat and oil, and sugar substitutes

Food group	pooled odds ratio (OR)	95% confidence interval (CI)	I2	Number of studies	Number of participants
Fruit	0.65	0.32 - 1.34	90.5%	4	3331
Vegetables	0.58	0.45 - 0.74	0.0%	4	3066
Fruit and vegetables	0.82	0.43 - 1.54	79.7%	3	8737
Meat	1.30	0.57 - 2.97	86.2%	3	2745
Red meat	0.98	0.81 - 1.18	0.0%	2	7072
White meat	0.80	0.64 - 1.00	0.0%	2	7072
Fish	0.91	0.70 - 1.20	63.6%	5	9568
Dairy products	0.62	0.47 - 0.81	55.7%	6	9827
Eggs	0.78	0.57 - 1.07	77.9%	4	9799
Cereal	0.65	0.51 - 0.87	43.9%	3	1450
Fat and Oil	1.19	0.65 - 2.20	83.0%	3	1326
Soft drinks	1.42	0.37 - 5.43	80.2%	2	656

We did not find evidence of an association between adherence to pre-defined dietary patterns, such as Mediterranean (RR 1.05, 95% CI 0.95 to 1.17) or Fertility diet (RR 0.94, 95% CI 0.86 to 1.03), and miscarriage risk. However, whole diet containing healthy foods as perceived by the trialists, rich in anti-oxidant sources (OR 0.43, 95% CI 0.20 to 0.91) may be

associated with a reduction in miscarriage risk. Conversely, diet high in processed food (OR 1.97, 95% CI 1.36 to 3.34) may be associated with increased miscarriage risk.

Conclusions: Dietary intake that is abundant in food groups such as fruit, vegetables, seafood, dairy, eggs and other food sources rich in anti-oxidants may be

2E.4 Causes of infertility and their effects on pregnancy and neonatal outcomes

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Aim: Latest evidence links infertility to adverse pregnancy outcomes. In this review, we summarise evidence linking polycystic ovary syndrome (PCOS), premature ovarian failure (POI) and decreased ovarian reserve (POR), unexplained infertility, endometriosis as well as adenomyosis with adverse pregnancy and neonatal outcomes.

Methods: An umbrella literature review was conducted covering all current evidence on the above infertility conditions and their associations with obstetrics and neonatal outcomes. The risk of obstetric complications was studied for early pregnancy losses, pregnancy induced hypertension/ pre-eclampsia, gestational diabetes mellitus, placental pathologies, predisposition for antepartum or postpartum haemorrhage, intrauterine growth restriction, adverse outcomes specific to the infertility conditions, predisposed mode of delivery, and other neonatal outcomes at time of delivery.

Results: For each of the above reproductive pathologies the associations were demonstrated as part of an umbrella review analysis. PCOS is described to be associated approximately with a 3-fold increased risk for ectopic pregnancies, pre-eclampsia, gestational diabetes and neonatal mortality. DOR is associated with a 3-fold increased risk of miscarriages and ectopic pregnancies. Unexplained infertility is associated with a 5-times increased risk of pre-eclampsia and preterm births, and a 3-fold increased risk of placental abruption. Endometriosis increases the risk of placental previa 7-times and placental abruption 14-times. Adenomyosis increases the risk of pre-eclampsia 8-times, and is also associated with approximately a 3-times increased risk of pre-term birth and placental abruption.

Conclusion: Reproductive pathologies are associated with increased adverse obstetric and neonatal outcomes. These pregnancies should be triaged as high risk and monitored accordingly throughout gestation, while health care professionals need to be aware of the specific complications and educate patients accordingly.

2E.5 SPINT1: Regulation and function in human placental trophoblasts

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Background: Serine Peptidase Inhibitor Type 1 (SPINT1), expressed by placental trophoblasts, is critical to placental development in mice1,2. In humans, placental and circulating SPINT1 levels are reduced in fetal growth restriction (FGR)3. The aim of this work was to assess the regulation and function of SPINT1 in the developing placenta using a first-trimester human trophoblast stem cell (hTSC) line.

Methods: Placental insufficiency and FGR is associated with placental hypoxia, which reduces SPINT1 in term cytotrophoblasts. We tested whether hypoxia and transcription factors CDX2 and GRHL2 (shown previously to regulate SPINT1 in intestine) regulate SPINT1 expression in hTSCs, and whether its secretion from the cell surface is mediated by matrix metalloproteinases (MMPs) by treating with MMP inhibitor batimastat. We silenced SPINT1 (siRNA) in hTSCs to ascertain downstream effects on trophoblast proliferation and degradative activity of cell surface protease matriptase, using a fluorogenic peptide substrate of matriptase.

Results: Hypoxia (1% Oxygen) significantly reduced SPINT1 mRNA expression by 40% ($p<0.01$) and secretion by 50% ($p<0.01$) relative to normoxia (8% Oxygen). The silencing of the transcription factors did not significantly alter SPINT1 mRNA expression, although cellular and secreted SPINT1 protein levels were reduced by GRHL2 siRNA ($p=0.0079$). SPINT1 secretion into media was also reduced 28% by 10uM batimastat, relative to control ($p=0.012$). A non-significant increase in matriptase activity accompanied loss of SPINT1.

Conclusions: Placental hypoxia and possibly GRHL2 regulate SPINT1 in placenta. While MMPs may contribute to SPINT1 release, there may also be alternate proteases that contribute. We also confirm a likely role for SPINT1 in impairing matriptase activity in human placental cells. Further studies are currently underway to ascertain the molecular regulators of SPINT1 and its potential role in cellular proliferation and differentiation.

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2E.6 Mitochondrial reversal can occur following meiotic spindle transfer, potentially impacting efforts to avoid transmission of mtDNA disorders using this form of mitochondrial replacement therapy

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Background: Mitochondrial DNA (mtDNA) disorders are caused by mutations in the mitochondrial genome, disrupting ATP production. The conditions are characterised by a range of potentially devastating phenotypes and have few treatment options. All mitochondria are derived from the oocyte and consequently mtDNA disorders are maternally inherited. It has been proposed that disease transmission could be avoided if female mtDNA mutation carriers underwent meiotic spindle transfer (MST), removing the chromosomes (held on the spindle) from an affected oocyte and transferring them into the healthy cytoplasm of a donor oocyte. Here we report evaluation of mtDNA in children born following MST.
Methods: Metaphase-II-spindles from patient oocytes were transferred into enucleated donor oocytes, inseminated using ICSI, and transferred to the uterus at the blastocyst stage. The mtDNA was sequenced to identify polymorphisms differing between the patient and oocyte donor. These variations were quantified, revealing the relative amounts of patient and donor mitochondria in the resulting embryos (blastocyst biopsies), during pregnancy (amniotic fluid), in newborns (cord blood, cord tissue and urine), at 3-6 months and at one year (saliva, urine and blood).
Results: Six children born following MST were assessed. The patient's mtDNA represented <1% of the total in blastocysts and remained low in subsequent samples tested from five of the children. However, in one child the very low level of patient mtDNA at the blastocyst stage increased dramatically, coming to represent 30-60% of the total in samples at birth. Levels of donor and patient mtDNA appeared stable after birth.
Conclusion: All the children born following MST were healthy. However, our results clearly demonstrate that a substantial degree of mtDNA 'reversal' is possible. This indicates that mitochondrial replacement therapies might not always be as successful as suggested by embryo analysis. The mechanism by which one mitochondrial type expands at the expense of another remains unknown.

SHORT PAPER PRESENTATIONS FRIDAY

SP3A THE AGEING EGG

3A.1 Aneuploidy does not alter cytoplasmic flow in mammalian oocytes

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Background: Chromosome segregation errors during early development lead to inheritance of incorrect numbers of chromosomes, known as aneuploidy, which causes infertility and birth defects. Brightfield microscopy is an emerging tool in assessing the quality of oocytes and eggs in clinical settings, but whether it can detect aneuploidy is unclear. It is already well established that mammalian metaphase-II eggs undergo stereotyped actin-dependant cytoplasmic movements that can be visualised by non-invasive brightfield timelapse-imaging, termed "cytoplasmic flow". Here, we hypothesised that this cytoplasmic flow might be affected by ploidy status of the egg and therefore be a useful selection tool to select euploid eggs non-invasively.

Methods: To address this, we developed conditions to generate euploid and aneuploid eggs from the same pool of oocytes by treating them with nocodazole during oocyte maturation. We then used DIC live-imaging microscopy, which allowed us to visualise and measure flow without any manipulation to the egg. To further look into cytoplasmic motility, we microinjected fluorescently-labelled beads in the cytoplasm and quantified their movement. Both types of cytoplasmic motility were significantly attenuated by cytochalasin-B, confirming that they are actin-dependent. Importantly, individual eggs were scored for their ploidy status by immunofluorescence, so that cytoplasmic movements could be related to ploidy on an egg-by-egg basis.

Results: There is no difference in cytoplasmic flow between euploid and aneuploid eggs. Furthermore, there was no difference in cytoplasmic flow between oocytes from young females (3mo) and Aged females (15mo). In addition, microinjection of fluorescently labelled dragon beads to analyse deep cytoplasmic movements also did not reveal any difference in the flow between the groups.
Conclusion: Our data demonstrates that ploidy status does not impact biologically-relevant stereotyped cytoplasmic

movements, suggesting that using non-invasive imaging to try to distinguish ploidy status between otherwise healthy oocytes will be challenging.

3A.2 Oocyte and cumulus cell cooperativity and metabolic plasticity under the direction of oocyte paracrine factors

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Background: Mammalian oocytes develop and mature in a mutually dependent relationship with surrounding cumulus cells. The oocyte actively regulates cumulus cell differentiation and function by secreting soluble paracrine oocyte-secreted factors (OSFs). We aimed to characterize the molecular mechanisms by which two model OSFs, cumulin and bone morphogenetics protein 15 (BMP15), regulate oocyte maturation and cumulus-oocyte cooperativity.

Methods & results: Immature mouse cumulus-oocyte complexes (COCs) were treated in vitro with in-house made recombinant pro-cumulin and pro-BMP15, which, as expected, improved oocyte quality as subsequent blastocyst yield was significant increased. Using global proteomic and multispectral autofluorescence analyses, exposure to these OSFs during maturation altered the profiles of both the oocyte and cumulus cells. In oocytes, cumulin significantly upregulated proteins involved in nuclear function. In cumulus cells, both OSFs elicited marked significant upregulation of a variety of metabolic processes (mostly anabolic), including lipid, nucleotide, and carbohydrate metabolism, while mitochondrial metabolic processes were downregulated. The mitochondrial changes were further investigated using functional assays: COC respiration using the Seahorse assay, mitochondrial mitotracker staining, transmission electron microscopy and the adenosine and NAD+ metabolomes were quantified by mass-spec (LC-MS/MS). Cumulin in particular changed mitochondrial function by significantly suppressing COC respiration, and altering mitochondrial morphology and decreasing their numbers in cumulus cells and oocytes. Although ATP homeostasis was maintained, cumulin, and BMP15 to a lesser extent, suppressed NAD(P)H and the REDOX ratio in both cell types.

Conclusions: Collectively, these data demonstrate that the oocyte, via OSFs, instructs cumulus cells to increase metabolic workload on its behalf, thereby subduing oocyte metabolism. Hence the oocyte remodels cumulus cell metabolism during oocyte maturation in preparation for ensuing fertilization and embryonic development.

3A.3 MRNA-miRNA interaction network in the ovarian follicle reveals miRNA target genes crucially involved in the regulation of cell apoptosis

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Background: Tissue specific gene expression profiles provides valuable on the mechanisms controlling reproductive performance in the monovular species including horse and human. In the recent years, many ovarian transcripts have been identified that are correlated with reproduction-related traits. Elucidating interactions between mRNAs and miRNAs has greatly contributed to better understanding the molecular mechanisms controlling ovarian development (1,2). This study aimed to identify mRNAs, miRNAs, and signaling pathways as well as their interaction networks in the equine and human ovarian follicles.

Methods: Available microarray data generated from mare and woman ovarian follicles GSE52109 (3) and GSE107746 (4) were used to identify genes differentially expressed in the granulosa cells during preovulatory follicle development using NCBI GEO2R. In addition, miRNAs predicted to interact with the defined genes were identified using miRTargetLink v2 and miRWalk software as well as their biological processes were studied using DAVID tool.

Results: We obtained a total of 216 common differentially expressed genes for both horse and human granulosa cells from preovulatory follicles using GEO2R. An integrative mRNA-miRNA network was generated containing 52 nodes for mRNA and 122 nodes for miRNA. The two miRNAs with the most connections, regulating the largest numbers of genes were miR-17-5p and miR-22-3p. BDNF, HMGB1, SLC2A1 and JAK1 genes were located in the center of the network as they were regulated by the most miRNAs. Using functional annotation analysis, these genes found to be involved in regulation of the cell apoptosis and to be highly enriched in oocytes and granulosa cells from antral follicles.

Conclusion: A mRNA--miRNA interaction network was generated for equine and human ovarian follicles at preovulatory phase could be used for the future molecular studies which may aid the identification of novel therapeutic candidates to improve reproductive efficiency in the monovular species.

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3A.4 Oxidative phosphorylation genes alteration by insulin resistance or sensitivity in cumulus cells of the PCOS patients

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One of the most prevalent endocrine diseases and the main causes of ovulation is a polycystic ovarian syndrome (PCOS) which leads to sub/infertility (1,2). This multifactorial syndrome is classified as a weak-oxidation state which is associated with decreased levels of serum antioxidants followed by increased production of free radicals such as reactive oxygen species (ROS) (3,4). Previous research investigated that contact between oocytes and the surrounding cumulus cells (CCs) is required for natural folliculogenesis (5,6). This study was performed to evaluate the expression of NCF2, TXNIP, UCP2, ATP5H, NDUFB6, COX7C, NDUFA3, SDHA, and SDHB, as the most relevant OXPHOS genes, in CCs of patients. Therefore, twenty-one women <36 years were included in 3 groups (n=3×7) including PCOS patients with insulin resistance (IR) and insulin sensitivite (IS) as well as control, women with male factor infertility, groups. All cases underwent GnRH antagonist and hCG protocol treatment. After 36 hours, follicle aspiration was performed, from which CCs were collected. The qPCR analysis indicated significant upregulation of NCF2, TXNIP, UCP2, and ATP5H in the IR with P values of <0.001, <0.001, <0.001, and <0.005, respectively. Also, their expression in the IR was higher than that of the IS with P values of <0.01, <0.005, <0.001, and <0.05, in turn. NDUFB6 had higher expression in the IS than the control and the IR with P values <0.05 and <0.01. Moreover, except for COX7C with the upregulation in the IS group, COX7C, NDUFA3, SDHA, and SDHB had a higher expression in the IR, however, none of these differences was statistically significant. Altogether, abnormal expression of genes involved in mitochondrial function was observed in samples from PCOS patients. These results suggest that alteration in OXPHOS gene expression can be considered as a potential molecular factor for the pathophysiology of PCOS.

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SP3B ENVIRONMENTAL INFLUENCES ON REPRODUCTION

3B.1 Reproductive trends in the novel equine model: Declining semen quality and environmental aetiologies revisited

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Introduction: Adverse trends in reproductive health and semen quality are reported in humans (1) and the dog sentinel (2). Poor testicular function is associated with exposure to environmental chemicals (ECs) (3). Assessing equine testicular EC accumulation initiates this species as a sentinel for human reproduction and a bio-monitor for terrestrial ecosystem environmental health. Here we determined trends in semen quality in global and UK-based equine populations and initiated investigations into the aetiological involvement of EC exposure.

Methods: A comprehensive evidence synthesis and meta-regression analysis determined trends (1984-2019) in objectively analysed fresh sperm motility (n=230 articles) from the global equine population. Trends were determined in sperm motility (PMOT; %), concentration (million/ml), output (million) and volume (ml) in a UK-based equine population from a single breeding facility (2001 to 2020; 11,387 samples; 1,036 stallions). A linear mixed model (REML) accounted for predetermined variables. For EC analysis; testes (n=6), feedstuffs (n=6), grass (n=2) and soils (n=2) were analysed for Σ7PCBs, Σ7PBDEs, Σ16PAHs and DEHP (GC-MS). EC concentration statistics incorporated an ANOVA and Tukey's post hoc or an independent t-test.

Results: PMOT declined by 31.89% between 1984 and 2019 irrespective of sensitivity analyses ($p<0.05$). In the UK-based equine population, motility declined by 10.10% (2001 and 2010), whilst concentration, sperm count and volume increased. All chemicals analysed were detected in testicular samples, feedstuffs and pastures.

Conclusions: Adverse trends in two sperm motion characteristics raises concern of the testicular health and fertility of the equine population. This research provides novel data on equine testicular EC accumulation, and suggests ingestion as a key exposure route. The research initiates the use of the novel equine model as a sentinel species for reproductive trends and as a bio-monitor species for terrestrial ecosystems. Further research is required to determine whether EC exposure is associated with declining equine motility trends.

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3B.2 Chronic dietary exposure to a glyphosate-based herbicide in young broiler hen increases ovary weight and modulates ovarian transcriptome

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INRAe

Introduction: Glyphosate-based herbicides (GBHs) are massively used in agriculture worldwide because of their great efficiency. Yet, few studies have considered the effects of GBH on avian species, while there are very likely to be exposed through their food. In this study, we investigated the possible effects of a chronic dietary exposure to a GBH on young broiler hen.

Methods: Female broilers (2 weeks-old, n=75) were exposed to two doses of GBH via their food (47 and 215 mg/day glyphosate equivalent corresponding to about half (GBH-, n=25) and two-fold (GBH+, n=25) the No-Observed-Adverse Effect-Level (NOAEL) as defined by European Food Safety Authority in birds or to GBH-free food (CT, n=25) during 28 days. Plasma glyphosate and AMPA and oxidative stress were assayed by mass spectrometry and TBARS (thiobarbituric acid reactive substances), respectively. An RNAseq analysis in CT, GBH- and GBH+ ovaries was performed to identify differential transcripts.

Results: All animals (CT, GBH- and GBH+) received the same amount of food during the experiment but the more GBH it contained the less they ate. Still, GBH+ animals ingested more glyphosate and AMPA than GBH- animals and consequently plasma glyphosate and AMPA concentration was higher in GBH+ animals. The weight of ovaries based on body weight was higher in GBH+ than CT and GBH- animals. RNAseq analysis on the ovaries revealed that as compared to the control, high GBH doses disturbed sterol and cholesterol metabolic processes as well as steroidogenesis transcripts such as *3B-HSD* and *StAR*, while low GBH doses modified RNA processing functions, including nucleic acid metabolic processes and gene expression.

Conclusion: *In vivo*, chronic dietary exposures to a GBH decreases food intake, increases oxidative stress and modulate ovarian transcriptome profile, resulting potential disorders in ovarian steroidogenesis in young broiler hen.

3B.3 The impact of COVID-19 vaccines on male semen parameters: A retrospective cohort study

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Background and objectives: The emergence of SARS-CoV-2 and the subsequent COVID-19 pandemic necessitated the development of adequate vaccines. Despite prominent vaccines being demonstrated to be safe and effective for preventing severe disease and death, significant levels of hesitancy remain. Reasons include concerns over adverse effects on male fertility, which have not been widely investigated. Therefore, this study aimed to determine the impact of SARS-CoV-2 vaccination on semen parameters in a retrospective cohort study of males undergoing fertility assessment.

Methods: A total of 277 patient records were reviewed to identify adult men who have previously undergone routine semen analysis for fertility assessment at Androcryos Andrology Laboratory (Johannesburg, South Africa) between March 2021 -- March 2022. Included patients also received vaccination within 3 months following this semen analysis, and underwent a second semen analysis any time post COVID-19 vaccination. Out of these patients 46 met the inclusion criteria.

Results: Out of 46 patients included in the study, 29 (63%) received the Pfizer-BioNTech (BNT162b1), 16 (34.8%) the Johnson and Johnson (JNJ-78436735/Ad26.COV2S), and 1 (2.2%) the AstraZeneca (AZD1222) (2.2%) vaccines. Sperm concentration significantly increased post-vaccination (P=0.0001), with no significant changes in semen pH, volume, total sperm count, progressive motility, normal sperm morphology or chromatin condensation. Results were not influenced by age, type of vaccine received and the number of days following vaccination, as depicted by multiple regression analysis.

Conclusion: There is no evidence of a negative impact of COVID-19 vaccination on male semen parameters, which is consistent with the emerging literature on COVID-19 vaccination and male fertility. Hence, COVID-19 vaccinations should not be dismissed based on fear of adverse effects on male fertility parameters.

3B.4 Maternal gut bifidobacterium remotes control placental endocrine function and fetal brain development

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Introduction: Studies have shown that the gut microbiota modulates fetoplacental development in mice. Moreover, we have previously demonstrated that administration of Bifidobacterium to pregnant mice improves fetal growth in association with beneficial changes in the placental transport region and fetal liver growth (Lopez-Tello et al., 2022). Driven by these insights, here we aimed to examine if maternal B. breve administration could also exert changes in placental endocrine function and fetal brain development.

Methods: Germ-free mice were either treated with 100uL of B. breve UCC2003 (concentration of 1010CFU/mL) at gestational days 10, 12 and 14 (BIF group, n=6) or 100uL vehicle control solution (GF group, n=5) by oral gavage. Mice were killed on day 16.5 (term ~day 20), and placental endocrine zones (junctional zone, Jz) and fetal brains collected for molecular analysis.

Results: No differences were found in the size of the Jz. However, increased mRNA levels of Hmgcr and a tendency for elevated Cyp17a1 were found in the BIF compared to the GF group. BIF group also had upregulated mRNA levels of Prl3a1, Prl3b1 and Prl8a8. In addition, increased mRNA levels of specific pregnancy-specific glycoproteins (Psg17, Psg18, Psg19, Psg21) were found in the BIF compared to the GF group. BIF fetuses exhibited reduced brain/fetal weight ratio compared to GF fetuses. Moreover, Foxm1 and the mitotic cycling Cdk1 gene were significantly reduced in the fetal brain of the BIF compared to the GF group. In contrast, the mRNA levels of Slc2a1 and Slc2a3 were significantly elevated in the fetal brain BIF group. Finally, immunoblot analysis revealed an increase in the abundance of phosphorylated AKT (Thr308) and STAT5 levels in the BIF group.

Conclusion: B. breve exerts changes in the placental endocrine function and alters cellular signalling pathways in the fetal brain that are important for cell growth and metabolism.

Lopez-Tello, Jorge, et al. "Maternal gut microbiota Bifidobacterium promotes placental morphogenesis, nutrient transport and fetal growth in mice." Cellular and Molecular Life Sciences 79.7 (2022): 1-16.

SP3C CLINICAL CHALLENGES AND STRATEGIES

3C.1 Intrauterine insemination (IUI): Identifying patient characteristics associated with a successful pregnancy

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Objective: Intrauterine Insemination (IUI) is a useful assisted reproductive technique for couples with certain causes of subfertility. However, there is a lack of current evidence regarding patient characteristics and the likelihood of successful pregnancy. This study aims to identify both male and female patient characteristics that correlate with successful IUI.

Methods: This is a retrospective cohort study of 144 couples undergoing IUI in 2021 at a tertiary fertility unit. Couples were chosen for IUI based on clinical history, suitability and local clinical guidelines. Data including male and female age, body mass index (BMI), diagnosis, parity, cycle number, hysterosalpingogram (HSG) results, laparoscopy and dye test results, treatment medication regime, semen pre and post-wash characteristics and serum estrogen levels were gathered. Treatment medication protocols included either clomiphene citrate (CC), human menopausal gonadotropin (HMG), or both combined (CC+HMG). Primary outcomes included successful pregnancy.

Results: 16% (n=23) of women had a successful pregnancy following IUI. When comparing patient characteristics of successful versus unsuccessful cycles, a statistically significant difference ($p<0.05$) was found between serum estrogen levels prior to IUI and the treatment protocol received. Pregnant women's mean serum estrogen was 2263.19 pmol/L (±1363.72 pmol/L SD) compared with 1613.67 pmol/L (± 1067.69 pmol/L SD) ($p<0.05$) in patients who did not get pregnant. There was a statistically significant difference when comparing treatment protocols in pregnant vs non-pregnant women ($p<0.05$). Women with successful IUI were more likely to have received treatment with CC+HMG ($p=0.04$), whilst CC ($p=0.02$) treatment alone was associated with lower rates of pregnancy. There was no statistically significant difference when comparing pregnancy outcomes and HMG treatment alone ($p=0.14$) or other parameters, including sperm characteristics.

Conclusion: Our study demonstrates that higher serum estrogen levels prior to IUI and treatment protocols consisting of CC+HMG were associated with higher pregnancy.

3C.2 Video consultations in reproductive medicine: Safety, feasibility and patient satisfaction

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Purpose/background/objectives: To study the safety and feasibility of virtual consultations for patients of subfertility from the points of view of patients and clinicians.

Methods: An online survey was offered to all patients attending a video consultation for subfertility, from September 2021 to August 2022. A separate survey was carried out for all clinicians conducting virtual consultations. Consultations were carried out using Attend Anywhere software.

Results: The survey was offered in 4,932 consultations. 577 (11.69%) patients responded and 510 completed the questionnaire (88.3%). The majority of the patients had a positive experience with the video consultation (91.70%) and 48.65% preferred this to an in-person consultation. Most patients agreed that the virtual consultation saved them time and money (average saving £33 per consultation). Most patients (72.68%) felt safer and less exposed to COVID-19. When the risk of COVID-19 subsides, 47.00% would still prefer to have video consultations, while 32.82% would have no preference. Of 39 patients with a disability, 26 (66.7%) agreed that virtual consultations were suitable for patients with a disability. Patients who had a negative experience were statistically more likely to have experienced technical problems in joining the consultation or poor quality of sound and/or video ($p<0.0001$). Twelve out of 15 clinicians preferred video consultations to in-person consultations. Two clinicians raised concerns about safeguarding, medicolegal risk and prescription errors.

Conclusions: This is the largest survey of its kind among fertility patients. It shows that virtual consultations are a safe and feasible alternative to in-person consultations for infertility patients, with a high rate of patient satisfaction. Appropriate patients' selection and patient choice is crucial for successful virtual consultations. Further research is needed to address the ethical and legal challenges of virtual consultations.

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3C.3 Can MPA be a surrogate to GnRH antagonist to prevent premature LH surge during COS in voluntary oocyte donors

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Objective: To determine whether MPA is effective for preventing premature LH (Luteinizing Hormone) surge compared to GnRH antagonist during controlled ovarian hyperstimulation.

Materials and methods: The voluntary oocyte donors were assessed for basal serum LH levels on Day 1 of stimulation (Day 2 of mensuration). The initial dose of Gonadotropin(1) was decided considering Age, BMI, and AFC count(2). The follicular monitoring, dosage adjustment (3) and trigger plan were done as per institutional SOP. The rate of premature LH surge (as defined by rise in serum LH level 2.5 times (150%) from the baseline value(4-6) was compared among two groups as primary outcome measure. The total gonadotrophin required till the trigger day was compared between the two groups. The daily pattern of serum LH from day 6 of stimulation to the day of trigger was analyzed in two groups.

Results: This is a pilot study with a sample size of 72 participants. The baseline characteristics (Age, BMI,AFC and D2 LH) were comparable in both the groups. Premature LH surge observed in 8 cases in antagonist(n=30) and 6 cases in MPA group(n=37) on day 6 of stimulation. However, on D9 of stimulation only one case in antagonist group (n=32) and 3 cases in MPA group(n=35) had LH surge. The average duration of ovarian stimulation in antagonist vs MPA group was 11.38 days vs 11.50 days. The difference in total Gonadotropin used for antagonist group vs MPA group was not significant (2329.41±561.53) vs (2522.37±447.77) (p=0.110). The retrieval rate, FORT, OSI, & M2 rates were comparable among two groups.

Conclusions: MPA is equally efficacious to antagonist in preventing premature LH surge in controlled ovarian hyperstimulation. MPA can be a better & cost effective surrogate to GnRH antagonist for preventing premature LH surge with a better compliance.

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3C.4 Value of intrauterine platelet-rich concentrates in patients with intrauterine adhesions after hysteroscopy: A systematic review and meta-analysis of randomized controlled trials

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Objective: To evaluate the value of intrauterine platelet-rich concentrates among patients with intrauterine adhesions (IUAs) after hysteroscopic adhesiolysis.

Methods: Four different databases (PubMed, Cochrane Library, Scopus, and ISI web of science) were searched for the available studies from inception to November 2021. We selected randomized clinical trials (RCTs) that compared platelet-rich concentrates in the intervention group versus no injection of platelet-rich concentrates in the control group among women with intrauterine adhesions after operative hysteroscopy. Revman software was utilized for performing our meta-analysis. Our primary outcomes were the adhesion score and incidence of recurrence of severe intrauterine adhesions postoperatively. Our secondary outcomes were the clinical pregnancy rate, menstrual flow duration in days, and menstrual flow amount (number of pads). **Results:** Five RCTs met our inclusion criteria with a total number of 329 patients. We found that platelet-rich concentrates were linked to a significant reduction in the postoperative adhesion score (MD = -1.00, 95% CI [-1.68, -0.32], p = 0.004). Moreover, there was a significant reduction in the incidence of severe IUAs recurrence among the platelet-rich concentrates group (7.6%) compared to the control group (23.4%) after hysteroscopy (p = 0.001). The clinical pregnancy rate was significantly increased among the platelet-rich concentrates group (37.1%) in comparison with the control group (20.7%) after hysteroscopic adhesiolysis (p = 0.008). There were significant improvements in the menstrual flow duration and amount among the platelet-rich concentrates group (p < 0.001).

Conclusions: Intrauterine placement of platelet-rich concentrates is an effective method for the treatment of intrauterine adhesions after hysteroscopy.

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SP3D EMBRYO AND FETAL DEVELOPMENT

3D.1 The plastic used for microfluidic device fabrication affects mouse embryo culture outcome

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Background/Objectives: Microfluidic embryo culture has been proposed as a means of improving blastocyst developmental competence. Microfluidic culture devices are frequently fabricated in Polydimethylsiloxane (PDMS), whereas Polystyrene (PS) is widely used for conventional embryo culture. The plastic used may affect oxygen permeability. This study investigated the impact of microfluidic culture in novel devices fabricated in PDMS or PS on markers of murine blastocyst quality.

Methods: Murine zygotes (strain B6C3F1xB6D2F1) were incubated to the blastocyst stage at 37C and 5% CO2. Embryos were cultured in averaged groups of 10 in bespoke microfluidic devices with 400 nl of KSOMaa media (16 PDMS devices, n=172; 17 PS devices, n=182) and 10 control 10l microdrops (n=113) of media under mineral oil (11 replicates). Cell number was quantified in 37 expanded blastocysts (PS=12; PDMS=12; Control=13) using epifluorescence microscopy and ImageJ software following nuclei and actin staining. Blastocyst attachment and outgrowth potential were assessed over 48hrs and 72hrs, respectively, in 102 individual hatched blastocysts (PS=34; PDMS=49; Control=19) by culture in fibronectin-coated, 12-well dishes in KSOMaa overlayered with oil.

Results: Blastocyst production was achieved in PS devices (93.5%2%), but the blastocyst rate was significantly lower ($p<0.05$) following PDMS device culture (92.73%) than in controls (1000%; 11 replicate cultures). The lower blastocyst cell number (37.22.1; n=12) tended towards significance ($p=0.055$) in embryos grown in PDMS vs. PS devices (44.32.5; n=12) and controls (44.32.5; n=13). Embryo attachment significantly increased ($p<0.05$) during outgrowth culture in embryos generated using PDMS (63.37%, n=49) and PS devices (91.23%, n=34) vs. controls (78.910%, n=19) but once attached, outgrowth area was similar ($p>0.05$) across treatment groups.

Conclusions: These results indicate acceptable blastocyst production is possible following culture in bespoke PS microfluidic devices. The differences detected between embryos cultured in PDMS devices vs. microdrops control cultures suggest that PDMS may be leaching toxic compounds into the embryo culture environment.

3D.2 Mouse embryonic stem cell model reveals maternal protein restriction around conception alters embryonic signaling and metabolic phenotype

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Background: Maternal periconceptional malnutrition increases the risk of offspring cardiometabolic disease1. Our mouse model of maternal low protein diet in vivo (over 0.5- 3.5 days to blastocyst Emb-LPD) alters embryo phenotype, and physiology through gestation culminating in postnatal disease2. We used mouse embryonic stem cell (mESC) lines from Emb-LPD and normal diet (NPD) blastocysts to investigate in vitro mechanisms of the origin of adverse programming.

Methods: (i) mESC lines were derived from blastocysts of dams fed isocaloric Emb-LPD (9% casein) or NPD (18% casein); (ii) Male lines were characterized for derivation, pluripotency, proliferation, apoptosis and cell-cycling activity; (iii) Karyotypically normal lines were analysed by metabolomics, enzymatic activity and transcriptomic.

Results: Emb-LPD mESC lines had reduced derivation efficiency ($P<0.01$). While pluripotency expression and cell cycling were unchanged, Emb-LPD lines displayed increased apoptotic cells, and reduced pERK 1/2 survival signaling activity and differential expression ($P <0.001$) of MAPK pathway genes. Global metabolomics identified Emb-LPD alterations in glucose metabolism, fatty acid homeostasis and ascorbate utilization. Emb-LPD lines exhibited increased glucose 6-phosphate and fructose 6-phosphate, reduced downstream metabolites and glycolytic enzyme activity of phosphofructokinase ($P<0.05$) and differential expression of Gpi ($P<0.001$), pyruvate kinase ($P<0.001$) and facilitated fructose transporter that collectively may explain 'log-jam' accumulation of upstream glycolytic metabolites.

Conclusion: mESC models show suitability for analysing periconceptional developmental programming, reducing animal numbers and overcoming limited material in preimplantation embryos. We demonstrate Emb-LPD increased apoptosis, perturbed MAPK and ERK 1/2 signaling, and dysregulated glycolytic pathway. This insight will permit pertinent screening of embryos for origins of disease.

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3D.3 Spatiotemporal control of mitotic exit in the mammalian embryo

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Introduction: During the first mitotic divisions of the mammalian embryo it is essential that chromosome segregation in anaphase and partitioning of the cytoplasm at cytokinesis occur in a tightly controlled sequence during M-phase of the cell cycle to produce euploid daughter cells. In somatic cells these events are under strict temporal control to prevent the gain or losses of chromosomes, termed aneuploidy. Entry and exit into M-phase are coordinated by Cyclin-dependent kinase 1 activity. Chromosome segregation and cell division is triggered by destruction of the CDK1 cofactor cyclin-B, thereby inactivating CDK1. How this series of events is coordinated in mammalian embryos is poorly understood. Understanding this will be essential to understand why chromosome segregation errors that cause aneuploidy are so common in the IVF clinic.

Methods: We used live-cell confocal imaging of cyclinB1:GFP and chromosome marker H2B:RFP to observe the relationship between cyclin B levels, anaphase onset, and cytokinesis during mitosis in the 2-, 4- and 8-cell stage mouse embryo.

Results: We found that the duration of M-phase shortens from the 2-cell, 4-cell and 8-cell divisions. We found that the rate of cyclin-B destruction is relatively similar across developmental stages, and thus fails to explain the changing duration of M-phase. However, cyclin destruction is initiated earlier after entry to mitosis in later developmental stages with shorter M-phases (32.5 +/- 5.24, 27.5 +/- 5.82, 22.5 +/- 3.0 minutes at 2-, 4-, 8-cell mitosis respectively). We also found that chromosome segregation errors have no effect on the rate of cyclin destruction during mitosis ($p=0.8947$), alluding that M-phase duration is not a predictor of aneuploidy.

Conclusions: Our data suggests that M-phase duration is controlled by the timing of cyclin B destruction, and that embryos lack a correction mechanism to slow cyclin destruction and prevent aneuploidy.

3D.4 The effect of chemotherapeutics on the fetal testis

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Introduction: Around one in every 1000 pregnant women is diagnosed with cancer, which can require treatment with chemotherapy. Many chemotherapy agents can cross the placenta but chemotherapy is considered safe to administer to pregnant women after the first trimester. However, to date, studies assessing the side effects of chemotherapy agents on the developing fetal testis have been limited. We have investigated the effect of two chemotherapy agents, cisplatin and doxorubicin, on the fetal mouse testis, both in vivo and in vitro.

Methods: For in vivo experiments, pregnant Bl6 mice were intravenously injected with cisplatin (6 mg/kg), doxorubicin (9 mg/kg), or sodium chloride at embryonic day 13.5 (E13.5), with embryos subsequently collected at E15.5. For in vitro culture, testes from E13.5 CD1 mouse embryos were cultured for 48 hours and exposed to either cisplatin (1 or 3 M), doxorubicin (0.09 or 0.9 M) or saline for the first 24 hours of culture only. Immunohistochemistry was carried out to examine germ- and Sertoli cell numbers, and double-strand breaks, using markers for Ddx4, Sox9 and H2AX respectively.

Results and discussion: In vivo exposure to cisplatin reduced germ cell density by 68.6% ($p<0.05$), but Sertoli cell density was unaffected. In contrast, germ cell density was unchanged following in vitro exposure, whereas Sertoli cell density reduced by 46.2% ($p<0.001$). Doxorubicin administration did not affect germ- or Sertoli cell densities in vivo. However, in vitro exposure lead to a 39.5% decrease of Sertoli cells ($p<0.05$). H2AX expression showed evidence of increased DNA damage in testes exposed to cisplatin in vitro only (380% increase, $p<0.05$). Results suggest that the fetal testis is vulnerable to chemotherapy-induced damage. However, further work is needed to explore the difference between the in vivo and the in vitro systems, and to determine whether similar effects are observed in the human fetal testis.

POSTER PRESENTATIONS

P001 Single-sperm morpho-kinetic association using SiD, a real-time artificial intelligence tool for ICSI sperm selection

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Purpose/Background: Applications of artificial intelligence (AI) in reproductive urology have focused on obtaining semen parameters, spermatozoa viability, DNA integrity and classifying morphology/motility of spermatozoa [1]. The aim of this study is to associate morphological abnormalities with motility patterns in single spermatozoa based on well-documented motility standards[2].

Methods: 2165 single spermatozoa were video recorded for a few seconds from 13 ICSI patient videos (7% PVP, 200X magnification) and their motility patterns were extracted using SiDTM[3] (IVF 2.0 Ltd., UK). Each single-sperm video was assessed by three senior embryologists from different clinics. We excluded videos where the sperm did not swim freely. Videos were labelled as morphologically normal or by type of abnormalities [2] when at least two annotators agreed. Mann-Whitney U test was used with a significance threshold of $p<0.05$.

Results: 612 single-sperm videos were excluded, 257 were labelled as morphologically normal, 1015 with head-defects, 39 with tail-defects, 346 with neck/midpiece-defects, and 42 with an excess of residual cytoplasm (RC). Inter-embryologists concordance of anomalies was 52% for head, 38% for neck and midpiece, 38% for RC, 34% for normal, and 22% for tail. Compared with normal spermatozoa, those with any defect showed a significant reduction of Amplitude of Lateral Displacement (ALH), Mean Angular Displacement (MAD), Wobble (WOB), and Average Path Velocity (VAP). Particularly, statistic evidence suggests that spermatozoa with tail-defects swim slower across a straight line (VSL), a curvilinear path (VCL), and VAP, have decreased WOB and Linearity, and larger MAD; head-defects showed larger ALH and MAD, and lower WOB and VAP; Neck and midpiece-defects showed smaller ALH and MAD, and Excess of RS are slower (VSL, VAP, VCL).

Conclusions: AI-based tools to assist sperm selection for ICSI may reduce subjectivity and bias during procedures. Understanding spermatozoa morpho-kinetic association may improve treatment outcomes.

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P002 Study on sperm parameters and clinical outcome in same sex female couples undergoing intra uterine insemination using frozen thawed donor sperms

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Background: Same-sex female couples who are undergoing fertility treatment will receive either Intrauterine insemination (IUI) or in-vitro fertilization (IVF) as their treatment options. IUI using donor sperm is still widely practised[1]. As fresh donor sperm is less utilized, the therapeutic IUI with donor sperm must utilize cryopreserved sperm[2]. The aim of this study was to determine the relationship between sperm parameters of thawed donor sperms with their pregnancy rates.

Methods: A retrospective analysis was performed in 55 same sex female couples who underwent donor IUI in our tertiary centre, from January 2020 to July 2022.An Infertility database (IDEAS) was used for the data collection. Patient's age, ovulation pathway, donor sperm characteristics and their clinical outcome of pregnancy were included in the analysis. Independent sample T test was used to test the level of significance.

Results: Average age of the patient included in study was 33.8 years (26-42 years). Among 55 couples who underwent IUI, 57 % had natural cycle and 43% had gonadotrophin. In the study group, 29% (n=16) had positive pregnancy using frozen thawed IUI. The couples with positive pregnancy showed a mean sperm concentration of 25.7 x 106/ml (Standard Deviation (SD) 14.7x106/ml) and progressive sperm motility 73.6% (SD 9.6). The couples with negative pregnancy had a mean sperm concentration of 21.1 x 106/ml (SD 15.2) and progressive sperm motility of 70.9% (SD 11.6) Observed mean sperm concentration deference and mean motility deference between two groups are not significant at 95% confidence level (P value=0.032/0.412)

Conclusions: Multiple factors contribute to the success of IUI. The present study did not reveal a significant relationship between the sperm parameters and pregnancy rate. Population based further studies will helpful in demonstrating possible association.

Mohammadi F, et al. Relationship between sperm parameters and clinical outcomes of Intra Uterine Insemination (IUI). Caspian J Intern Med 2021; 12(1): 70-76. Chen L, Zhu L, Cai C, Yan G, Sun H. Clinical and neonatal outcomes of intrauterine insemination with frozen donor sperm. Syst Biol Reprod Med. 2018 Aug;64(4):240-245.

P004 Comparative analysis of the fertility-enhancing potentials of graded doses of newbouldia laevis and zinc in male wistar rats

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Infertility has become a real public health problem because of its increasing prevalence, widespread distribution, and the difficulties inherent in its management. Male factor infertility is thought to be the cause of up to 50% of all infertilities across the world. In some parts of Africa and from time immemorial, a wide variety of plants are of great medicinal importance. Many plant extracts have been used as fertility agents in folklore and traditional medicines to enhance fertility, producing results similar to that of Zinc. According to research. Newbouldia laevis (NL) extract could act as an adjunct that can inhibit or promote hormonal imbalances in males at specific dosages as exemplified in the experimental animal models. The research aimed to determine the comparative effect of Zinc and graded doses of NL on male fertility. The thirty-six male Wistar rats weighing 55 - 125g were used for the research. The rats were randomly assigned into 6 groups of 5 rats each and treated with normal saline, Zinc, NL low dose for a short-term (LS), high dose short-term (HS), low dose long-term (LL), and high dose long-term (HL). Results revealed that at high doses NL impacted negatively on the semen parameters specifically the motility and count of sperm cells irrespective of the duration of treatment, however, the germinal epithelial cell population was unaffected. The testosterone levels were initially impacted but the gonads recovered with long-term treatments. The FSH levels were reduced in all groups treated with NL. This was further appreciated in the number of pubs from each group. Mating with the long-term NL yielded more pubs. The research concludes that moderate use of NL extract for a longer period may have possible beneficial effects on male fertility potential. Keywords: Newbouldia laevis, testes, Zinc, Fertility potential, reproductive hormones

P005 Fibroblast growth factor 21 decreases testosterone production in mouse testis

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Introduction: Lifestyle, environment and excess bodyweight are frequently associated to an increased risk of metabolic disorders, such as type 2 diabetes, but also infertility. Fibroblast Growth Factor 21 (FGF21), a hormone produced mainly by the liver, is closely related to energy status and regulated carbohydrate and lipid mechanisms. Recently, the FGF21 pathway has been shown to be associated with female fertility disorders through a central action, but none or few studies on the role on male fertility have been described. In a previous work, we have described presence of FGF21 in the testis and in human semen. Moreover, a negative correlation between serum FGF21 and testosterone levels was observed. In the present study, we explore the role of FGF21 on steroid function in the testis.

Methods: To demonstrate the presence of FGF21 receptors (FGFR1/3/4) and the cofactor β-Klotho) in Leydig cells, their expressions were detected by RT-qPCR on testis extracts and) proteins were localised by immunohistochemistry on sections of mice testis. To study the role of this factor on steroidogenesis on Leyidg cells, we used the mLTC (Mouse Leydig Tumoral Cells) cell line, and performed treatments with human recombinant FGF21 at physiological concentration (0.01ng/ml; 0.1ng/ml) to pharmacological doses (1ng/ml; 10ng/ml).

Results: In this study, we demonstrated the expression and the presence of FGF21 receptors and cofactor in mouse testis and more specifically in Leydig cells. Leydig cells treated with human recombinant FGF21 showed a decrease in testosterone secretion, associated to an activation of the Akt signalling pathway. rhFGF21 affects both the synthesis of steroid precursors (phospholipids and cholesterol) and the expression of enzymes involved in steroid production (3bHSD and Star). We confirmed these results in vivo after administrations of FGF21 to mice.

Conclusion: Therefore, this work highlights that FGF21 has an inhibiting activity on the testosterone synthesis.

P006 Cimetidine as a fertility protectant against cisplatin-induced damage in mouse spermatogonia

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Background: Cisplatin is a chemotherapeutic agent commonly used to treat childhood cancers, however cisplatin has off target cytotoxic effects. One such effect is the loss of germ cells, including spermatogonia, in the human immature testis (1). Copper transporter 1 (CTR1) has been shown to be involved in cisplatin uptake and cimetidine, a known inhibitor of CTR1, has shown promise as a chemo-protectant against cisplatin in the rat kidney and cochlear organotypic cultures (2, 3, 4). We aimed to investigate whether cimetidine would rescue spermatogonia from cisplatin-induced damage.

Methods: The mouse GC1-spg cell line was used as a model for spermatogonia in the immature testis. RT-PCR and ICC were used to determine CTR1 expression. GC1-spg (n=3) were treated with either cisplatin-only (0.5g/ml-3g/ml) or cotreatment with 1.5g/ml cisplatin and cimetidine (0.01M-10M). To assess the effects of cisplatin and cotreatment with cimetidine, cell confluency and apoptosis were analysed by live imaging using an IncuCyte (Essen BioScience, UK).

Results: GC1-spg cells express CTR1. A dose response in cell confluency and apoptosis was seen in cisplatin-only treated GC1-spg cells. At the final experimental timepoint (45 hours), a significant reduction in cell confluency was seen in all cisplatin doses in comparison to non-treated control cells (98.7% vs 37.3% for 1.5g/ml cisplatin; p<0.0001). A reduction in cell confluency is seen in GC1-spg treated with 1.5g/ml cisplatin in combination with 0.01M-10M cimetidine in comparison to cisplatin-only treated control cells. At the final experimental timepoint (66 hours), analysis showed no significant differences between control and cotreatment groups, suggesting that cimetidine does not protect against cisplatin-induced damage in GC1-spg cells.

Conclusions: Cisplatin treatment prevents cell growth and causes apoptosis in GC1-spg cells. Cotreatment with cimetidine does not appear to protect GC1-spg cells against cisplatin. Additional experiments are ongoing to further analyse apoptosis. Further work is required to establish CTR1 as the receptor for cisplatin in spermatogonia.

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P007 Investigating boar sperm metabolism during capacitation, hyperactivation and the acrosome reaction using 13C-Nuclear magnetic resonance

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Background: The journey sperm make through the female reproductive tract (FRT) has two possible end points: fertilisation or death. During their journey three key events occur: (i) capacitation; (ii) hyperactivation; and (iii) the acrosome reaction. Whilst capacitation is a prerequisite for fertilisation to occur and the acrosome reaction allows sperm to penetrate the egg, the exact role of hyperactivation is debated (1,2). These events likely use ATP generated by glycolysis or oxidative phosphorylation, however it remains unclear what the exact ATP demands are (3). The aim of this research is to investigate live boar sperm energy metabolism during capacitation, hyperactivation, the acrosome reaction and apoptosis.

Methods: Boar sperm aliquots were incubated with 13Cu-glucose at 39C for up to eight-hours and the metabolic pathways utilised by sperm were measured using 13C-NMR (4). Capacitation was stimulated by HSA and bicarbonate, hyperactivation by 4-aminopyridine, the acrosome reaction by progesterone and apoptosis using staurosporine. Capacitation and the acrosome reaction were determined by fluorescent staining; tyrosine phosphorylation and Pisum sativum agglutinin staining respectively. Apoptosis was determined using the TUNEL assay.

Results: Capacitation was significantly increased with 5 mg/mL HSA and 25 mM bicarbonate (p0.0001) and 100 M staurosporine induced DNA fragmentation (p0.05) in boar sperm. However, the results suggest that 10 M progesterone was unable to stimulate an acrosome reaction and 2 mM 4-aminopyridine was unable to induce hyperactivation in boar

sperm. There were no significant differences in normalised lactate (glycolysis) or bicarbonate (oxidative phosphorylation) NMR integrals for hyperactivation, acrosome reaction or apoptosis, however, there was significantly more lactate produced during capacitation (p0.0001).

Conclusions: During capacitation, the significant increase in lactate production suggests a dependence on glycolysis for this process. The bicarbonate production measured during

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P008 Sub-optimal diet influences the gut microbiome and key metabolite levels in male mice

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Background: The mammalian microbiome comprises between 10 and 100 trillion microorganisms in a symbiotic relationship with the host[1]. While our view of our microbes has centred on their role as pathogens, it is now known that our microbiota plays a central role in many developmental, physiological and metabolic aspects of everyday life[2].

Methods: Male C57BL/6 mice were fed one of 5 different diets: a control diet (18% protein, 10% fat, 21% sugar; CD), an isocaloric low protein diet (9% protein, 10% fat, 24% sugar; LPD), an isocaloric methyl donor supplemented LPD (betaine, choline chloride, folic acid, methionine, Vitamin B12; MDL), a high fat, high sugar Western diet (19% protein, 21% fat, 34% sugar; WD), or a WD supplemented with methyl donors (MDWD) for a minimum of 7 weeks. After culling, DNA was isolated from fecal pellets and the hypervariable bacterial V3-V4 region of the 16S gene was sequenced by Illumina HiSeq (Illumina). Liver tissue and serum samples were analysed for levels of cholesterol, free fatty acids, triglycerides, glucose and insulin using commercial assays.

Results: Differences in total body weight at time of sacrifice were only observed between the CD and MDL groups, with MDL males weighing significantly less. However, WD and MDWD males displayed significantly heavier livers and gonadal fat compared to CD males while not having significantly altered total body weights. WD and MDWD males additionally displayed significantly increased levels of liver cholesterol and/or free fatty acids when compared to CD males. Analyses of the mouse microbiota data are currently underway to determine their influence on male metabolic and reproductive health.

Conclusion: These data shed light on how high fat, high sugar diets impact male metabolic and physiological health and suggest that such diets have a larger influence on male physiology and metabolic health than low protein diets, with or without supplementation.

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P009 Testicular morphology and gene expression profiles are modified in a diet-dependent manner in the mouse

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Background: Recent evidence indicates that sub-optimal diet is associated with unfavourable sperm quality, impairing subsequent embryonic development and offspring health[1,2]. Changes in the testicular environment are proposed as a mechanistic cause underlying poor sperm quality and perturbed offspring development.

Methods: C57BL/6 stud male mice were fed either a control diet (18% protein, 10% fat, 21% sugar; CD), an isocaloric low protein diet (9% protein, 10% fat, 24% sugar; LPD), an isocaloric methyl donor supplemented LPD (betaine, choline chloride, folic acid, methionine, Vitamin B12; MDL), a high fat/sugar Western diet (19% protein, 21% fat, 34% sugar; WD), or a WD supplemented with methyl donors (MDWD) for a minimum of 7 weeks. Testes were collected and processed for either histological assessment or genome-wide gene expression analysis using the Clariom S Assay Mouse

GeneChip array (ThermoFisher Scientific) and analysed using the Partek Genomics Suite and the online enrichment analysis tool WebGestalt.

Results: Using a fold change 1.1, FDR cut-off 0.05 (+ *p 0.05*), we observed 910, 2,959, 3,425 and 3,503 genes to be differentially expressed in LPD, MDL, WD and MDWD testes respectively when compared to CD testes. Gene ontology and pathway analysis identified non-coding RNA metabolism and prenatal lethality pathways upregulated in LPD. Both these processes were downregulated in MDL, however, in addition to the downregulation of abnormal cell morphology process. In WD, abnormal mitochondrial physiology and cholesterol metabolism pathways were upregulated. On the other hand, abnormal cell cycle and parathyroid hormone synthesis were upregulated and downregulated, respectively in MDWD. In addition, embryonic organ development was downregulated in both WD and MDWD.

Conclusion: These data shed further light on how sub-optimal diet plays a role in influencing global testicular gene expression patterns. Ongoing studies aim to investigate testicular histology and assess the impacts of these changes on male reproductive fitness and offspring development and well-being.

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P010 The effect of diet extremes on the canine sperm transcriptome

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Objectives: A healthy diet is often prescribed to men to improve semen quality. We have previously shown that extremes of diet have little effect on canine semen quality. Here, from the same experiment, we have explored if extremes of diet influence canine sperm transcriptome in a predictable manner.

Methods: Fresh semen was collected at baseline from foxhounds (n=13) habitually consuming a raw, flesh-based ration and immediately analysed for sperm physiology and morphology, then centrifuged at 13,000g for 10mins and the pellet flash-frozen in liquid N. Foxhounds (n=8) either remained on flesh diet for 9 weeks (i.e. covering a full spermatogenic cycle) or were fed a nutritionally-replete, balanced kibble (n=5). Semen was then repeat sampled and flash-frozen. Sperm total RNA was extracted from all samples for later global transcriptomic analysis (Novogene Ltd) and data analysed

Results: Foxhounds eating a flesh-based diet had poor semen quality at baseline e.g. sperm concentration (425 ± 199; ref = 1022 ± 386, 106/ml), percent normal live sperm (54 [40 -- 70]; ref = 80 [67 -- 85]; %) with a high proportion of sperm abnormalities (e.g. presence of a proximal droplet, 12 (5 -- 19) %. All canines ate the complete diet for nine weeks with no adverse effects. The extreme dietary change had few effects on semen parameters e.g. sperm concentration remained low (337 ± 129, 106/ml). Transcriptomic data at the point of abstract submission was unavailable, but will be presented at the meeting.

Conclusion: Extremes of diet have little effect on key parameters defining quality semen. Potential subtle effects on epigenetic markers on sperm has the potential to influence sperm function, regardless of concentration or motility. This hypothesis is tested here and results will be presented at the meeting.

P011 Nutritional and lifestyle pre-conceptual guidelines for men

Justine Bold; David Swinburne

University of Worcester

A systematic approach was used to undertake a literature review. Findings were used to develop pre-conceptual guidelines for men (a separate abstract to outline methodology and findings has been submitted). Guidelines are for men to be a healthy weight keeping physically active, and to avoid making food an additional stressor, enjoying food and managing their overall well-being. Dietary recommendations are to avoid trans fats whilst eating sufficient healthy polyunsaturated fats (PUFAs) such as the omega 3 oils, whilst avoiding following a low-fat diet (as fats are important for testosterone synthesis). A traditional Mediterranean style diet is recommended including plenty of fruits, vegetables, beans, pulses, fish and nuts. Green vegetables are important as they contain folate. Colourful fruits and vegetables contain antioxidants. Nuts and seeds contain minerals such as zinc which is important for sperm production in addition to PUFAs. If plastics are used for food or drink storage containers made from glass or stainless steel are preferable as plastics are known to be endocrine disrupting. Fruits and vegetables should be washed or peeled to remove pesticide residues. Cola based fizzy drinks and high intake of caffeine in coffee or tea is to be avoided. A moderate intake (e.g. 1-2

cups of coffee a day is better). Moderate to heavy alcohol consumption is best avoided, instead modest consumption within the safe limits is recommended. Professional help should be sought if this is challenging. If a smoker, smoking cessation services should be consulted. Tight underwear and activities that heat up the testes such as saunas should be avoided. A multivitamin and mineral supplement containing folic acid, zinc, and antioxidants maybe useful. Co Q10 may also be useful at a dosage of 200-400mg a day. Nutritional supplementation should however be discussed with a health professional, who should assess any interactions with medication.

P012 A review of nutrition/lifestyle factors and male infertility experience to develop pre-conceptual guidelines for men

Justine Bold; David Swinburne

University of Worcester

Semen parameters can be improved through a healthy diet (1,2) and nutritional supplementation has also been shown to improve semen parameters, clinical pregnancy and live birth rates (3.4,5). Despite this, dietary changes beyond alcohol reduction are rarely recommended. Our aim was to consider the psychosocial impacts of male infertility whilst assessing other interventions that can be used in personalised nutrition-care. A systematic approach was used to undertake a review and findings were used to develop pre-conceptual guidelines for men. Three electronic databases were searched using predetermined Boolean search terms (Academic Search Complete, CINAHL and Medline). Additional hand searches were undertaken, duplicates were removed and predefined inclusion and exclusion criteria were applied. 125 papers were identified and narrative synthesis was used for review and to develop the guidelines. Review data indicates dietary modification or supplementation with antioxidants such as vitamin C, vitamin E, coenzyme Q10, selenium, carnitine and zinc (6) have been shown to improve markers of male fertility and reduce seminal oxidative damage (3,4). Additionally, a Mediterranean diet is also associated with higher quality sperm counts (7,8). Low fat diets can reduce testosterone levels so should be avoided (9). Weight loss, however, is beneficial in terms of normalising endocrine profiles (10) but it is not possible to determine at present if this is the effect of weight loss alone, or the combined effect of losing weight alongside other nutritional improvement. Despite this, infertility is emotionally challenging for men and personalised nutrition and lifestyle therapies have potential to support men trying to conceive. Pre-conceptual nutrition and lifestyle guidelines for men have been developed and the use should be considered as the basis for more tailored nutrition care in practice (particularly in primary care), supporting men in the first stages of their journey. Further research is needed to understand the potential confounding factors.

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P013 Comparison of cost per ongoing pregnancy/live birth for surgical sperm retrieval methods in azoospermia: A pilot study in a mid-sized fertility clinic

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Introduction: Azoospermic males may undergo surgical sperm retrieval (SSR) via PESA, MESA or TESE depending on the type (obstructive or non-obstructive). MESA and TESE require general anaesthesia and more surgical time unlike PESA. There is a higher sperm retrieval rate in PESA. This study aimed to compare the cost effectiveness of SSR procedures and examine the predictive value of male FSH levels on ICSI outcomes.

Methods: Males attending a fertility clinic (400 IVF/ICSI cycles/year) for SSR between 2018-2021, with female partners <40 were included (n=47). Anonymised data on SSR outcome, male FSH, ongoing pregnancy, and live births after first embryo transfer were collated. Primary outcome was cost per ongoing pregnancy/live birth for each procedure. Secondary outcome was impact of male FSH on ongoing pregnancy/live births. Cost effectiveness was calculated as cost per ongoing pregnancy/live birth (number of ongoing pregnancies/live births divided by cost of SSR (PESA=£2,225, MESA/TESE=£3,075)) plus cost of 1 cycle of ICSI (£6,700)). Outcomes were compared using risk ratios (RR) and 95% confidence intervals (CI).

Results: Of those undergoing PESA followed by ICSI, 26% (7/27) achieved a live birth and 26% (7/27) had an ongoing pregnancy. In those with MESA/TESE and ICSI, 10% (1/10) achieved a livebirth alongside 30% (3/10) having an ongoing pregnancy (RR for combined ongoing pregnancy/live birth=1.25, 95%CI 0.66, 2.36). Cost per ongoing pregnancy/live birth was £17,480 for PESA and £28,913 for MESA/TESE. Secondary outcomes indicated that in men with FSH<12, 38% (3/8) achieved a live birth/ongoing pregnancy, while none (0/2) of the men with FSH>12 achieved either (RR=1.6, 95%CI 0.94, 2.74).

Conclusion: MESA/TESE may be less cost effective than PESA, however there is inadequate power to ascertain true cost-effectiveness. Male FSH levels could also be a marker of successful pregnancy following ICSI. A further multi-centre analysis is now underway to explore the results with adequate power.

P014 Scabiosa atropurpurea aqueous extract improves sperm motility and viability in ram

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Background: Scabiosa Atropurpurea sub. Maritima L.,is a plant of the family of Dipsacaceae, genus scabiosa. Due to its high phytochemical content and its antioxidant properties, S.atropurpurea is well known for its pharmaceutical properties including hepato protective and hypoglycemic, diuretic, analgesic and antipyretic activities. In female, it is also able to treat menstrual disorders. However, its effects on the male fertility are unclear.

Objectives: The aim of this study was to evaluate the in vivo effect of S.atropurpurea on ram's sperm parameters. Methods. Eighteen adult rams of the Queue Fine de l'Ouest breed aged between 4 to 5 years were divided in three homogenous groups (n=6 per group): control (CT), G1 and G2 groups. Rams were orally taken 50 ml of fresh water (CT), 50 ml aqueous extract of S. Atropurpurea 1g/l (G1) or 2g/l (G2) every day during ten weeks. For all rams sperm collection was performed using an artificial vagina twice a week. Sperm motility, sperm concentration, live and abnormal spermatozoa were assessed. Furthermore, biochemical and immunocytological analyses were performed to study spermatozoa viability.

Results: The administration of aqueous extract of S. Atropurpurea showed significant effects ($p<0.05$) on: individual motility 78% (CT) vs 91% (G1) and 96%(G2); abnormal spermatozoa rate 20.2% (CT) vs 16.28% (G1) and 14.85%(G2); DNA damage markers 70.55% (CT) vs 36,18 % (G1) and 45.53%(G2); - HSP70 maker 43,71% (CT)vs 81.97 % (G1) and 78.54 %(G2), spermatozoa calcium concentration 5.61 mg/l (CT) vs 7.71 mg/l (G1) and 8.17 mg/l (G2) and ATP (ratio ATP/protein concentration in spermatozoa) 1 vs 2.16 and 3.39 for CT, G1 and G2 groups respectively, at the end of the trial.

Conclusion: S. Atropurpurea aqueous extract improves in vivo sperm quality in ram.

P015 Semen extender for diagnostic testing

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Objective: Routine semen analyses (SA) are considered the gold standard for evaluating male fertility[1]. To minimize sperm deterioration and ensure accurate results for the diagnosis and treatment of patients, tests must be performed within one hour of production, thus specimen collection mostly occurs on site. Enabling the acquisition of samples produced at home through systems prolonging sperm longevity is highly advantageous for both patients and laboratories. One such medium has been validated in this study.

Methods: A total of 56 samples were obtained from men attending for routine diagnostic testing of semen. SA was performed strictly according to WHO 2010 before and after addition of semen extender at different time intervals over a 48-hour period. Samples were stored either between 2-8C or at room temperature (RT).

Results: The results showed a mean decrease of -4.49 1.12 in progressive motility and -2.70% 0.58% in vitality over time in extender, while sperm concentration and morphology were not affected. The rate of change in semen parameters over time was relatively consistent in samples with normal parameters, but not for abnormal samples. Evaluation of trends in parameters for normal samples allowed the calculation of an algorithm to determine the original value from the time of sampling. Storage at RT preserved semen parameters more efficiently compared to refrigerated samples ($p<0.001$).

Conclusions: The use of semen extender may be utilized for diagnostic SA of samples that are more than an hour old with adequate preservation in normal samples of up to 2 days at RT. This may benefit men who are unable to deliver a sample to the laboratory within an hour, and those who find it difficult to produce a sample in a clinical environment. Ease of use with accurate representation of examined parameters is therefore beneficial to both patients and laboratories.

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P016 Understanding the relationship between sperm DNA damage and oxidative stress (ROS and ORP)

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Introduction: Sperm DNA damage is a contributing factor to male infertility, with the leading known cause being oxidative stress. This can be determined by measuring levels of seminal reactive oxygen species (ROS) or the balance between total oxidants and antioxidants (sORP). This study was conducted to determine which assay is more highly correlated with sperm DNA damage.

Method: A total of 175 semen samples were used for this study. Comparison was made between ORP and ROS with DNA fragmentation Index (DFI) and sperm with immature chromatin (HDS). Additionally, DFI and HDS measurements, as assessed using the sperm chromatin structure assay, were compared between 4 different combinations: Group 1 (High ROS and High ORP), Group 2 (High ROS and Low ORP), Group 3 (Low ROS and High ORP) and Group 4 (Low ROS and Low ORP). Oxidative stress was assessed using the chemiluminescence assay for ROS measurement and MiOXSYS System for ORP measurement.

Results: There is a significant increase in DFI and HDS, when levels of seminal ROS are above the threshold value (DFI: 16.8% 11.1% vs 21.1% 10.8%, p=.028, HDS: 9.8% 6.1% vs 14.7% 10.4% p=.011). There is also a significant increase in DFI and HDS (respectively) when levels of seminal ORP are above the threshold value (DFI: 15.9% 10.0%, vs 21.2% 12.3%, p<.003, HDS: 9.1% 5.9% vs 14.9% 8.7%, p<.0001). Both ROS and ORP are positively correlated with DFI (p<.001;p<.001 respectively) and with HDS (p=0.049;p<.001 respectively). There was also a significant difference in HDS between Groups 1 and 4 (p=0.001) and Groups 3 and 4 (p=0.01). **Conclusions:** Both assays for measurement of seminal oxidative stress are correlated with sperm DNA damage. These simple and cost-effective assays may prove useful tools for assessing male infertility.

P017 Panax ginseng supplementation protects against testicular damage induced by electromagnetic radiation from cell phone

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Background: It is well documented that radiofrequency (RF-EMR) from the cell phone contributes to testicular dysfunction with consequent infertility in male individuals. Panax ginseng (P. ginseng) exerts antioxidant, antidiabetic, neuroprotective and anti-inflammatory effects in biological systems. However, its protective role against reproductive dysfunction, including testiculopathy is unclear. This study was designed to investigate the effects of P. ginseng extract on testicular damage induced by RE-EMR from the cell phone in male Wistar rats.

Methods: Twenty adult male Wistar rats weighing 120-150 g were randomly divided into four groups of n=5; Control group received vehicle (0.2 mls of normal saline; po), P. ginseng group received 0.2 mls of P. ginseng extract (po), RF-EMR group was exposed to 900 MHz of radiation and RF-EMR+ P. ginseng group was exposed to900 MHz of radiation and concomitantly treated with 0.2 mls of P. ginseng (po). The treatment was done daily and lasted for 56 days. The animals were sacrificed, and biochemical/endocrine parameters and the histology of testes were evaluated.

Results: There was a significant decrease in spermatogonia, sperm count, sperm motility and sperm morphology with decrease in progressivity in RF-EMR group compared with control. Likewise, a significant decrease was observed in serum gonadotropins (LH), testosterone and glutathione peroxidase with disrupted testicular morphology in RE-EMR

group compared with control. However, administration of P. ginseng attenuated these circulating and testicular alterations.

Conclusions: The results of the present study suggest that supplementation with P. ginseng extract ameliorates testicular dysfunction associated with RF-EMR from the cell phone by antioxidant enhancement.

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P018 Does low-level mycotoxin exposure affect bovine spermatozoa quality?

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Background: Mycotoxins are toxic secondary metabolites produced by fungi which can be detrimental to the health of livestock. Although cattle are quite tolerant to mycotoxins (i.e. clinical disease is present only at high levels of exposure), potential effects of mycotoxin exposure on reproductive cell function at low levels are unknown, which is relevant for non-health attributes such as reproductive performance. This in vitro study aimed to determine the impact of combined, low-level mycotoxin exposure on bovine spermatozoa using the most frequently isolated mycotoxins worldwide; Deoxynivalenol (DON), Deepoxy-Deoxynivalenol (DOM-1) and Zearalenone (ZEA) [1].

Methods: Semen straws from two bulls were processed and spermatozoa (10x106 sperm/ml) were exposed to T1 (DON 10ng/ml, DOM-1 60ng/ml and ZEA 0.1ng/ml), or T2 (DON 20ng/ml, DOM-1 120ng/ml and ZEA 0.5ng/ml) for 8 h, mimicking the time spent by the spermatozoa in the reproductive tract before fertilisation (i.e., following artificial insemination or natural mating in cattle). Mycotoxin concentrations reflect levels detected in clinically healthy cows in either follicular fluid or blood [2-3]. A control solvent (0.5% methanol) was also included. A fluorescence assay [4] was performed to simultaneously assess membrane damage (Hoechst 33342 and DRAQ-7), acrosome integrity (FITC-PSA) and mitochondrial membrane potential (JC-1) via epifluorescence microscopy and Zeiss ZEN software. Data was analysed by Kruskal-Wallis.

Results: No significant difference was observed between phenotypes in previous trials comparing bulls or in the presence or absence of 0.5% methanol. Following 3 replicates, membrane damage (Control=74.5%, T1=78.25% and T2=75.92%), acrosome damage (Control= 90.17%, T1= 90.84%, T2= 92.84%) and low mitochondrial membrane potential (Control= 40%, T1=37.49% and T2=42.07%) were not significantly affected by the treatment groups after 8 h exposure ($p > 0.05$).

Conclusion: Overall, this study suggests low level mycotoxin exposure does not significantly compromise sperm quality during a time period that reflects transport in the female reproductive tract.

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P019 Covid vaccination and booster does not appear to be detrimental to semen analysis

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Background: Concerns about unknown future effects on reproduction and fertility are an important component of COVID-19 vaccine hesitancy, particularly in younger adults. Temporary detrimental effects on sperm concentration following BioNTech, Pfizer vaccine have been reported [1], although other studies show no significant differences in spermatozoa parameters before and after vaccination [2,3]. This study attempted to investigate further whether COVID-19 vaccination has a negative effect on male fertility.

Methods: Healthy volunteer sperm donors (n=20) who regularly donated semen samples for research to Reproductive Medicine Research Group, University of Dundee were included in this study (ethical approval 20/SS/0104 and SMED REC Number 20/45). A minimum of nine samples were collected from each donor longitudinally from November 2019 (pre-pandemic) to July 2022. Semen parameters for each sample included ejaculate volume (mls), sperm concentration (million per ml), total motility (%) and progressive motility (%). Volunteers reported dates of covid vaccination and/or infection. Semen characteristics were analysed for differences pre and post (first and second dose) COVID-19 vaccination as well as pre and post COVID-19 booster.

Results: There was no difference in ejaculate volume before and after COVID-19 vaccination (2.4ml v 2.6ml; p=0.79). A significant reduction in sperm concentration was found following COVID-19 vaccination (83.1 M/ml v 66.8 M/ml; p=0.002) and booster (77.3 M/ml v 61.9 M/ml; p=0.004), although this was unlikely to be clinically relevant. There was no detrimental effect of covid-19 vaccination on total motility (56.6% v 52.7%; p=0.08) or progressive motility (31.2% v 27.1%; p=0.41) nor booster (total motility 57.1% v 53.4%; p=0.18; progressive motility 29.0% v 28.9%; p=0.48).

Conclusions: Results appear to be reassuring regarding COVID-19 vaccination and booster and male fertility, as assessed by semen analysis.

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P020 Evaluating the impact of COVID-19 on healthcare workers in a fertility unit: A qualitative study

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Background: COVID-19 has had a profound impact on fertility services throughout the United Kingdom, leading to significant delays in treatment and increasing distress for patients (1). Consequently, the resources available, workload and waiting times have also been affected, leading to increased pressure on members of staff at fertility units. Studies to-date have focused on patient impact (2, 3). This qualitative study aims to explore the impact of COVID-19 on the wellbeing and working role of workers at a busy tertiary fertility unit.

Methods: All healthcare workers, including clinicians, embryologists, nurses, and administrative staff at a tertiary assisted conception unit were invited to take part in semi-structured interviews. Interviews lasted between 15-45 minutes and explored four domains: the general experience of working during the pandemic; the impact of the change in working roles on mental and physical wellbeing; aspects that went well or could have been improved with regards to their role and patient care; how the pandemic changed their role and functioning of the unit moving forwards. Interviews were transcribed and qualitative thematic analysis was conducted using NVivo.

Results: Thematic analysis of semi-structured interviews (n=8) revealed 'change in role', 'impact on treatment', 'demands of the role' and 'emotional response' as key prominent themes. 100% of participants reported a change in their working role, ranging from assisting other clinical departments to screening hospital visitors for COVID-19 symptoms at the front door. Participants reported feelings of fear, anxiety, and stress to varying levels. Other themes included the rapid digitalisation of healthcare, and the ongoing impact of this on staff and patients.

Conclusion: This is one of the first studies to focus on the impact of COVID-19 on healthcare workers within a fertility unit and helps to identify areas for improvement moving forward.

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P022 Covid vaccination in assisted conception patients

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Background and objectives: By June 2021 COVID-19 vaccination was available to all adults of reproductive age.(1) There has been concern, propagated on social media, about safety of the vaccine and effect on fertility.(2) The British Fertility Society (BFS) and Association of Reproductive and Clinical Scientists (ARCS) recommend that people of reproductive age have the vaccine.(3) Evidence has shown that unvaccinated pregnant women are at increased risk.(4) COVID-19 declaration forms were introduced in our unit in January 2022 to ensure documentation of vaccination status and discussion of risk. The aim was to evaluate COVID-19 vaccination rates and determine the impact of a vaccination declaration form.

Methods: Retrospective analysis of (1) All patients attending the assisted conception unit in September 2021 and (2) All patients starting treatment March 2022 and August 2022. Data was collected from electronic databases. Medical notes were also reviewed for unvaccinated patients. Statistical analysis performed with SPSS.

Results: Of patients attending in September 2021, 82/340 (24.1%) were incompletely vaccinated: 53/340 (15.6%) completely unvaccinated and a further 29/340 (8.5%) only having received a first dose. None were medically exempt. Full vaccination rate (with two doses) in September 2021 for all assisted conception patients was 75.9%. Patients who were vaccinated were on average older than those who were unvaccinated ($p<0.05$), less likely to be from a minority ethnic group ($p<0.05$), less likely to be from an area of high deprivation ($p<0.05$) and less likely to have an unvaccinated partner ($p<0.05$). Vaccination declaration forms were completed for all unvaccinated patients.

Conclusions: This study has shown that vaccination rates in our assisted conception population are higher than national figures for women of reproductive age overall. However, a considerable number remain incompletely vaccinated. Some groups are less likely to be vaccinated, which may inform future strategies to increase vaccine uptake.

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P023 Obstetric outcomes of fertility preservation in women cancer patients: A systematic review and meta-analysis

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Objective: As cancer survivorship increases there is higher uptake of fertility preservation treatments among affected women. However, the evidence remains inconsistent on the longterm use of preserved gametes and pregnancy outcomes in women who underwent fertility preservation (FP) before cancer treatments. We aimed to evaluate the longterm reproductive and pregnancy outcomes following FP.

Design: Systematic review and meta-analysis

Patients: Women who underwent any type of FP treatments (embryo cryopreservation (EC), oocyte cryopreservation (OC) and ovarian tissue cryopreservation (OTC)) before any planned cancer treatment.

Evidence Review: We searched electronic databases (MEDLINE, Embase, Cochrane CENTRAL, and HTA) from inception until May 2021 for all observational studies that met our inclusion criteria. We extracted data on reproductive and pregnancy outcomes in duplicate and assessed the risk of bias in included studies using the ROBINS-I tool. We pooled data using a random-effects model and reported using odds ratios (OR) with 95% confidence intervals (CI). We conducted subgroup analyses to assess the effect across different types of FP treatments.

Main Outcome Measures: Our primary outcome was live birth rate (LBR) in additions to other important reproductive and pregnancy outcomes.

Results: Of 5405 citations, we screened 103 and included 26 observational studies (n= 7061 women). Majority of women underwent FP for gynecological cancers followed by breast and hematological cancers. Twelve studies reported on OTC, eight offered EC, and twelve offered OC. Only 8% of women used their frozen oocytes/embryos post cancer treatment (558/7037, 8.0%), with a LBR of OR 0.38 (95%CI 0.29-0.48, I2 83.7%). The overall cumulative LBR following any FP treatment was 0.046 (95%CI 0.029-0.066). Miscarriages, ongoing pregnancies and biochemical pregnancies were other common obstetric results reported in 14 studies.

Conclusions: FP can be used to protect potential fertility for women cancer patients with good obstetric outcomes.

P024 An audit of outcomes in fertility preservation for oncology patients at Birmingham Women's Hospital from 2018 to 2020

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Background: Fertility preservation in the form of oocyte or embryo freezing is recognised as an important method of preserving fertility for women undergoing cancer treatment. This audit looks at outcomes in fertility preservation in oncology patients from 2018 and 2020.

Methods: All women who had fertility preservation from 2018 until 2020 for oncological reasons were reviewed. Outcomes which were assessed were; reason for fertility preservation and treatment received, investigations prior to treatment, stimulation protocol, oocytes collected, embryos preserved, return to use stored gametes, clinical pregnancy rate and live birth rate.

Results: 51 patients underwent fertility preservation for cancer treatment between 2018 and 2020. The commonest cause for referral was breast cancer. All women underwent oocyte freezing and underwent a random start protocol with stimulation dose based on antral follicle count. 8 women returned to discuss the use of stored gametes. One patient naturally conceived. One patient who returned to use her stored gametes had a successful thaw and achieved one embryo transfer which resulted in a positive pregnancy test. One patient came back to use stored gametes however had a recurrence of cancer so was unable to continue. **Conclusions:** The covid pandemic may have affected the number of patients seen during 2018-2020. The usual advice given to patients is to wait two years before considering a pregnancy due to the risk of recurrence of cancer, the short time frame of this audit may miss women who leave a longer time between treatment and attending to use stored gametes due to a recurrence of cancer. Since 2020, funding opportunities have improved to allow for embryo as well as oocyte freezing which may have an impact on future outcomes. More data is required, over a longer period to assess outcomes of fertility preservation.

The ESHRE Guideline Group on Female Fertility Preservation, Richard A Anderson, Frédéric Amant, Didi Braat, Arianna D'Angelo, Susana M Chuva de Sousa Lopes, Isabelle Demeestere, Sandra Dwek, Lucy Frith, Matteo Lambertini, Caroline Maslin, Mariana Moura-Ramos, Daniela Nogueira, Kenny Rodriguez-Wallberg, Nathalie Vermeulen, ESHRE guideline: female fertility preservation, Human Reproduction Open, Volume 2020, Issue 4, 2020, hoaa052, https://doi.org/10.1093/hropen/hoaa052

P025 Fertility preservation in the context of complex comorbidities: Lessons from a tertiary clinic

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Background: Increasingly, females with an array of complex comorbidities and multi-organ dysfunction present for fertility preservation treatments making the realisation of preserved gametes, ovarian tissue storage or ovarian transposition challenging. Alongside the majority with an oncology diagnosis, females with chronic and serious benign disease constitute around 8-13% of females undertaking fertility preservation (1); where the disease pathology and treatments are detrimental to both fertility reserve and have serious sequelae for systemic health (2).

Methods: We present a case series of 9 female patients who underwent fertility preservation at a tertiary London NHS Trust in 2022 with complex diagnoses. The specific considerations for each case and the learnings are detailed.

Results: All patients achieved successful fertility preservation with egg freezing, embryo freezing or ovarian transposition treatment as planned. For each case, collaboration between the referring team, fertility preservation, anaesthetic and haematology specialities created an MDT to anticipate, communicate and manage the specific potential risks. The significant bleeding and thrombosis risks were minimised through pre operative exchange or platelet transfusions or anticoagulant treatment plans as required. We describe the inherently higher risks within this group requiring this MDT expertise, careful planning and optimal treatment timing to attain safe results within a window of opportunity.

Conclusions: To attempt fertility preservation in the presence of complex diagnoses and comorbidities can be daunting with balance needed between the desire for treatment with the potential for harm. This case series demonstrates an insight into successful fertility preservation treatment despite numerous challenging circumstances and offers optimism and learnings for the pursuit of fertility preservation for females with complex systemic disease.

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P026 Optimising model of care for female oncology patients needing urgent fertility preservation

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Objective: To optimise outcome and treatment experience of female oncology patients undergoing fertility preservation by developing a structured pathway.

Method: A prospective observational study was conducted on patients referred to the Fertility Centre at Chelsea and Westminster Hospital, London, over the period of last one year. A standard operating procedure, information leaflets and forms for referrers and the receiving team, dedicated IVF coordination slots and training were developed for a one-stop fertility preservation service to complete consultations and investigations in one appointment with a view to starting ovarian stimulation within 48-72 hours of referral. Data was collected to assess feasibility and outcome of this structured pathway.

Results: 18 patients with a recent diagnosis of cancer were referred to our fertility unit in the last one year. Two patients declined treatment. The remaining 16 patients received fast-track treatment through the newly introduced one-stop service. The mean age of patients in the study was 31 years. 87.5% patients were nulliparous. The median AMH in this group was 10pmol/L (lowest 0.2pmol/L and highest 44.7pmol/L). The median number of eggs collected after ovarian stimulation was 9, with the lowest being 0 for patient with AMH <0.2pmol/L and highest being 22 with AMH of 36.7pmol/L. The median time between referral to the fertility team and completion of fertility preservation treatment was 17 days. 68.8% patients were seen in the one stop clinic within 24 hours of referral and in 81.3% cases we successfully managed to provide a one-stop service for investigations and consultations at the same appointment.

Conclusion: Potential treatment-related infertility can be a very distressing diagnosis on top of an initial cancer diagnosis. A rapid-access, one-stop fertility preservation service included several distinct elements of success. The quality of experience can be further improved and standardised by digitalisation of the pathway, which is our current direction.

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P027 Male oncofertility audit

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Purpose / Background: Tremendous advances in oncological diagnoses and therapies have resulted in a continual increase in cancer survivors in recent years. This is especially true for cancers that affect younger populations, for example, testicular cancer (1). Infertility or sub-fertility can arise directly from the tumour itself, or from gonadotoxic chemotherapy regimes (2). Hence, many male cancer patients undergo sperm cryopreservation prior to treatment. This project provides an audit of the sperm cryopreservation service for oncology patients at the University Hospital of Wales.

Methods: Databases stored at the Andrology Department at the University Hospital of Wales were analysed to discover the epidemiology of the 545 patients who used the University Hospital service of sperm cryopreservation between January 1st 2008 and December 31st 2021. Digital notes stored on Welsh Clinical Portal and paper hospital notes were also utilised to retrieve information which was transferred to and analysed on Microsoft Excel.

Results: This project resulted in the collation of data and establishment of trends regarding cancer type, age at time of freeze, years of sperm storage, method of storage, number of vessels stored, partner status and average number of patients who stored sperm per year. Results have shown that, overall, there has been a significant increase (more than 400%) in male oncology patients using the service of cryopreservation between 2008 and 2021.

Conclusion: Cancer care should not only be focussed on the survival of the patient but also on quality of life. Sperm cryopreservation plays a huge role in this, especially for aspiring fathers-to-be. This reinforces the importance of an audit of the service provided by the University Hospital.

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P028 Uptake of fertility preservation in BAME women affected by cancer diagnosis

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Introduction: Fertility preservation is a procedure that can be carried out prior to cancer treatment that could cause infertility in future. The different fertility preservation techniques include ovarian tissue embryo and oocyte cryopreservation, Gonadotrophin-releasing hormone (GnRH) suppression and ovarian transplantation. **Aims:** This study provides an insight into the uptake of onco-fertility preservation amongst Black, Asian and minority ethnic (BAME) patients at a University Hospital.

Methods: This was a retrospective audit of data collected at the Fertility Institute from 1st January 2008 to 31st December 2021; data were analysed by Microsoft Excel.

Results: There were 202 patients who underwent fertility preservation between 2008- 2021. 7.92% were BAME patients who underwent onco-fertility preservation at the Fertility Institute. The average age of all the patients was 29.6 years. The most common oncological reason for fertility preservation was Breast cancer. The average number of oocytes collected per patient was 10.7.

Discussion: The findings of this study suggest that the uptake of onco-fertility preservation within BAME patients (7.92%) is similar as to the general population of BAME with a cancer diagnosis (5.7%). A limitation of reaching this conclusion is the small sample size when compared to the number of patients diagnosed with cancer each year. The average number of oocytes collected per patient was10.7, other published literature the mean number of oocytes being 13.8 , this could be explained by different ovarian stimulation protocols being used. Of the 202 patients who underwent onco-fertility preservation 92 patients underwent oocyte cryopreservation, the average number of oocytes cryopreserved was 8.7. The average number of mature oocytes cryopreserved in other published literature was 11.2 per patient. This could be due to different methods within the centers around collection and freezing processes. 92

(45%) patients had embryo cryopreserved, the average number cryopreserved per patient was 4.4. Other published literature discussed the average number of embryos

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P029 Motivations and treatment experiences of women who underwent social egg freezing

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Background: The average age of motherhood has consistently increased over recent decades. Inevitably, this has resulted in more women facing age-related fertility. Some women may undergo social egg freezing (SEF) to preserve their fertility. This study aims to investigate motivations, treatment experiences and potential feelings of regret in patients who underwent SEF. Additionally, it explores the impact of the COVID-19 pandemic on motivations and treatment experiences of women who underwent SEF, which has not been previously studied.

Methods: An online questionnaire was sent to 410 women who underwent SEF at Lister Fertility Clinic between 2011 to 2021. Questionnaire explored motivations, knowledge, treatment experience, impact of COVID-19 pandemic, usage of oocytes and future plans. Statistical analysis was conducted on 165 responses using SPSS.

Results: Concern for age-related fertility decline was the main factor influencing decision to undergo SEF. Only 0.6% of respondents regretted undergoing SEF, however 64.0% wished to have cryopreserved at a younger age. 44.1% of women who underwent SEF during COVID-19 felt the pandemic made them more willing to freeze their eggs. Only 8.2% of women returned to use cryopreserved oocytes.

Discussion: Following completion of SEF, concern regarding number of oocytes cryopreserved decreased significantly indicating a degree of reassurance and satisfaction. Nevertheless, women of an older age may wish to cryopreserve a greater number of oocytes. Women who were not in a relationship at time of SEF were more likely to consider COVID-19 pandemic as a factor influencing decision to undergo SEF. Conclusion Most women who undergo SEF are aged 35 or older, when fecundity decreases at a faster rate. Education of young women by healthcare professionals on age-related fertility decline and fertility options may result in women cryopreserving oocytes at a younger age. Women undergoing SEF may have several concerns, highlighting the importance of emotional support.

P030 The differences in surrogate and intended parent demographics highlight the need for careful management of surrogacy cycles in order to provide appropriate care for all involved

Jemma Walker; Nabiha Chowdhury; Andrew Ahmoye; David Ogutu

Herts and Essex Fertility Centre

Background: For many Intended Parents (IPs) it is difficult to find a suitable surrogate without the help of specialist agencies who facilitate an introduction between surrogate and IPs who otherwise may never have met. The demographics of surrogates and IPs are often vastly different, meaning that the pressures and expectations of these two patient groups can also vary greatly. The modern fertility clinic must understand the needs of both surrogates and IPs in order to provide high quality care to all involved.

Aim: This piece of work aims to demonstrate the many differences and similarities in demographic information from IPs and surrogates treated at one fertility clinic, in order to increase the awareness of these differences by staff and ultimately to improve the care provided.

Methodology: Retrospective comparison of the demographic data of all patients registered at the clinic as either surrogates (77 individuals) or intended parents (144) between 2016 and 2022. Specific parameters included; year of birth, marital status, nationality, ethnicity, parity, location and profession.

Results: Surrogates were generally found to be younger than IPs, although they were more likely to have children already. While IPs were more likely to be in married and working in professional job roles. The nationality, ethnicity and location of surrogates were far more-wide ranging than for IPs.

Discussion: All clinics undoubtably work hard to support surrogates and IPs throughout their care, and are mindful of specific factors in the lives of all patients which may have an impact on their personal requirements. However, the differences in range of demographics between surrogate and IP patient groups serves to highlight the importance of discussing with and accommodating the needs of not only comissioning IPs but also the surrogates with whom they undertake their fertility journey.

P031 NHS-funded IVF treatment criteria excludes majority of those actively trying to conceive

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Background: The Women's Health Strategy has pledged an overhaul of NHS-funded IVF treatment and abolition of the postcode lottery. However, a strategic plan and committed timeline is lacking for the rollout. Meanwhile, the current system and strict eligibility criteria may disadvantage those in need of IVF treatment.

Methods: The most common criteria requirements for funding eligibility was applied to a cohort of actively trying women to assess how fit for purpose the current criteria for NHS-funded IVF treatment is.

Results: The most common criteria for eligibility were: aged 18-42; Body Mass Index 19-30; serum Anti-Mllerian Hormone >5.4 pmol/L; serum follicle-stimulating hormone <8.9 IU/L; non-smoking; and having no living children.
The above criteria were applied to 780 England-based customers of an at-home hormone testing service who were actively trying to conceive (TTC) between September 2020 and August 2022. Of these, 675 had no underlying gynaecological conditions (Group A) and 105 (Group B) had a diagnosis of one or more of the following: endometriosis, pelvic inflammatory disease, Fallopian tube blockage, polycystic ovary syndrome, and premature ovarian insufficiency.
The majority of Group A (65.25%) did not fit the criteria for NHS-funded treatment, irrespective of the time they had been TTC. Additionally, 78.86% of this group had been TTC for <12 months. Similarly for Group B, 74.3% would be ineligible for NHS-funded treatment, and 42.67% had been TTC for <12 months.

Conclusion: The majority of customers would not be eligible for NHS-funded treatment based on biochemical and/or demographic criteria. Furthermore, at the point of analysis, most customers had been TTC for <12 months and therefore would not be referred for further investigations despite not fulfilling the criteria, suggesting the mandatory criteria and 12 month TTC time prior to further investigations is not fit for purpose.

P032 Egg sharing programmes can provide recipients with comparable treatment outcomes to altruistic donation, without jeopardising the treatment outcomes of the egg sharing patient

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Background: Gamete sharing often results in sharers keeping only half of the eggs collected from them, for their own use, and recipients receiving only a proportion of the eggs collected. Potential sharers must be appropriately counselled of the potential impact sharing may have on their care. When counselling patients who are considering using donor eggs, it is also vital that clinics are able to make recipients aware of the potential risks and benefits of accepting eggs from altruistic or egg sharing donors.

Methodology: In order to evaluate the success of one in-house egg sharing scheme, retrospective analysis of the fresh treatment outcomes of over 100 egg sharers were compared with over 100 non-sharing patients. Alongside this, cycle outcomes of over 70 recipients following altruistic donation and 140 following egg sharing were also compared.

Results: Initial interrogation of the data has shown that while egg sharers on average have fewer eggs inseminated than their non-sharing counterparts, there is no significant difference in clinical pregnancy rates following their fresh egg collection and fresh embryo transfer.
Similarly, recipients from egg sharers and altruistic donors also had no significant difference in clinical pregnancy rate when compared to one another.

Discussion: When counselling patients about the risks and benefits of becoming an egg sharer, clinical staff must be aware of the potential impact of egg sharing on their own treatment. Further analysis to understand if there are differences in cumulative pregnancy and time to pregnancy is required. However, the findings of this preliminary service review are encouraging, that sharers are as likely to have a clinical pregnancy following treatment as non-sharers.

For egg recipients it is also encouraging that if they chose to accept oocyte from an egg sharer, they are not likely to be negatively impacting their chances of achieving a pregnancy.

P033 Black women access at-home fertility testing services later in life compared to white women in the United Kingdom

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Background: Black women access fertility care later than women of any other ethnicity in the United Kingdom (UK), which may contribute to lower pregnancy rates. We assessed the age at which women approached an at-home hormone testing service, time spent trying to conceive (TTC), and the role of ethnicity in these timelines.

Method: Data was collected from 61,758 women who completed an online health assessment between September 2020 and July 2022. Users self-reported age, ethnicity, intention to conceive, and length of time spent actively TTC. Average age has been reported as mean SD. Associations of time TTC between ethnicities were assessed using Chi-squared test; p values <0.05 were considered significant.

Results: Of users who were TTC, the majority self-identified as White (83.74%), followed by Asian (6.90%), Black (4.54%), Mixed (3.77%) and 'Other' (1.04%).

A significant relationship between time spent TTC and ethnicity was found, with the strongest association observed between Black and White users (2 (4, n=12,429) = 44.51, p < 0.0001). Comparison of time spent TTC and average age of user showed similar percentages of Black (30.36%) and White (33.87%) users had been TTC for <6 months, and Black users were moderately older (31.5 6.5 vs 29.3 5.9). However, a higher percentage of Black users (15.34%) were TTC for >5 years compared to White users (8.15%), and a larger difference in age was observed (36.8 5.9 vs 32.6 5.1).

Conclusion: This data suggests Black women approach fertility and hormone testing services later than White women, which may contribute to the ethnic disparities in pregnancy outcomes in the UK. Further investigations are needed to identify a causal association and underlying reasons for this.

P034 Fertility coaching - a new way to support patients

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In 2019, coaching was the 2nd fastest growing sector in the world, behind only the IT industry, and according to the ICF and PWC's 2020 report, there are approximately 71,000 people worldwide who identify as a coaching practitioner. Coaching is about supporting someone to find a positive way forward, and to overcome problems that might be holding them back from being at their best. It is a confidential, non-judgemental relationship based on mutual trust and respect. The value of coaching techniques to improve productivity and employer satisfaction within the working environment is increasingly well-recognised by businesses and organisations. The aim of Fertility Coaching is to offer patients a thinking partner to improve confidence and reduce anxiety; this could be using small goal-setting, giving a sense of empowerment by providing high quality listening, and by challenging limiting beliefs. The differences between Fertility Coaching and Fertility Counselling are illustrated in the following table: In September 2022, a pilot study offering Fertility Coaching to patients at Guy's ACU was launched. The coaching was offered before treatment had started, the aim being to understand if and how coaching techniques can support fertility patients. Fertility Coaching will be provided by Eleanor Wharf as part of a level 5 Apprenticeship in Professional Coaching. The pilot study was agreed as a Service Improvement programme by all stakeholders including the departmental Senior Management team. Feedback will be sought during and at the end of every coaching relationship, and qualitative data will be compiled and presented before the end of December 2022 to inform whether Fertility Coaching is an intervention that could be justified as an additional support service to patients alongside (and separate to) counselling.

P036 Diversity, equity and inclusion in fertility and reproductive health societies' leadership

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Background: Reproductive health societies promote understanding and interest in reproductive biology and medicine. They are leading authorities, providing guidelines, opinions, and direction to practitioners, policy makers and the public. Membership on these societies' board is a marker of influence and prestige. Many societies have clear Equality and Diversity Statement on their website, suggesting that they value representation of members from whom they obtain

fees. This study presents a quantification of the diversity in the executive leadership of major fertility and reproductive health societies in Europe, Australia, and North America to evaluate diversity in governance.

Methods: We conducted a review of the websites of ten leading fertility and reproductive health societies in the Europe, Australia and North America to quantity gender and ethnic diversity. Data analysis was conducted on the information obtained in August 2022. We included the executive leadership team /governing board members but excluded subgroup leaders or special interest group leaders.

Results: In total, the number of board level/executive leadership members, responsible for governance in the societies reviewed were 108. Gender diversity was 41% Men, 59% Women, while ethnic diversity was 82% White, 14% Asian and 4% Black.

Conclusion: It is encouraging to see the gender parity in the executive leadership of the organisations review, there remains an important need to improve ethnic diversity in order to better represent the membership and wider community they serve. This has implications for role-modelling, equity, minimising the negative impact of groupthink and giving underrepresented group a voice.

P037 Impact of cannabinoids on fertility: A review

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Background: Cannabis is an increasingly used recreational drug in the western world. Its constituent components are cannabinoids, the main ones of which are tetrahyrdocannabiol (THC), which is the psychoactive molecule and cannabidiol (CBD). CBD is prescribed in the United Kingdom in the treatment of conditions such as multiple sclerosis and epilepsy, and is freely available to purchase in various forumations. However, there is limited data assessing the link between cannabis use and fertility, and less specifically looking at the effect of CBD on fertility. In this review we review the effects of cannabidiol, THC and cannabis usage on fertility.

Methods: A narrative literature search of the major databases (Pubmed, Medline,Google Scholar, Web of Science) was conducted using the words 'cannabidiol, cannabis, marijuana, sperm, tetrahyrdocannabiol, and fertility'. Given the paucity of prospective data available quantitative analysis was not possible, so the literature was critically reviewed to assess the impact on fertility.

Results: Cannabis usage appears to negatively impact ovulation,(1) as well as semen parameters,(2) including sperm count, concentration, morphology, motility, how this relates to conception is less well established with contrasting findings between studies.(3, 4) The data available for late pregnancy and neonatal outcomes is better established with a detrimental effect found with cannabis usage during pregnancy(5).

Conclusions: There is a large gap in data relating to the effects of cannabis and CBD which in the context of declining fertility rates is a subject that warrants further study. In certain countries such as the United States and Canada there has been recent widespread legalisation of cannabis, highlighting the urgent need for such research. In the United Kingdom the increasing use of CBD formulations should prompt similar action as its effect are largely unknown, but appear deleterious.

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P038 Alternative medicine and herbal remedies in the treatment of erectile dysfunction: A systematic review

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Background and objectives: Erectile dysfunction (ED) is defined as the inability to achieve and/or maintain an appropriate penile erection that is sufficient for sexual intercourse. Pharmaceutical treatments for ED may not be accessible for many patients and are associated with adverse effects, within a lucrative international market for alternative treatments. Therefore, the aim of this study is to systematically review the current evidence from

randomised controlled trials (RCTs) that investigated the use of alternative medicines and herbal remedies in the ED management.

Methods: A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-based systematic review using specific keyword combinations was conducted on the PubMed and Scopus databases. RCTs investigating herbal medicine in at least one group and using the International Index of Erectile Function (IIEF) as an outcome in patients primarily diagnosed with ED were included for review.

Results: Following the literature search, screening and eligibility analysis, a total of 42 articles were included. The 42 articles were categorised as single herb extractions (n = 14), combination herbal formula (n = 5), combination of herbal formula and non-herbal nutraceuticals (n = 7), non-herbal nutraceuticals (n = 5), acupuncture and moxibustion (n = 2), diet and nutrition (n = 3), exercise (n = 5), and topical treatments (n = 1). Korean ginseng, Pygnogenol and Prelox, Tribulus terrestris, Lepidium meyenii, L-arginine, acupuncture and lifestyle interventions were the more predominantly investigated treatments interventions for ED. **Conclusions:** Panax ginseng, Pygnogenol, Prelox and Tribulus terrestris have promising evidence as herbal products, alongside L-arginine as a nutritional supplement, for ED based on IIEF outcomes, and warrant further clinical investigation. The mechanisms of action remain unclear, but each of these appears to in part increase nitric oxide synthesis. Importantly, improved diet and exercise should be considered, particularly in patients with obesity or diabetes mellitus.

P039 Fertility treatment: Public attitudes and understanding

Sarah Norcross; Sandy Starr; Jen Willows

Progress Educational Trust

Working in a fertility clinic supporting patients it is easy to find yourself in a closed environment, but what does the rest of the UK population think of your work. Do they support NHS funding? Or even know your work is legal? Understanding public attitudes towards your work can help bring greater understanding regarding the issues your patients may face from families and friends.

Purpose: In anticipation of changes to the Human Fertilisation and Embryology Act and a new Surrogacy bill, a piece of wide-ranging, nationally representative research was commissioned -- to gauge public opinion and understanding of these issues.

Methods: A questionnaire was developed by the authors and their advisers with the input of Ipsos survey research experts. Ipsos interviewed a sample of 2,233 adults aged 16-75 in UK using its online i:omnibus between 24 and 27 March 2022.

Questions topics included:

* NHS funding of fertility treatment, and who should be eligible
* Sex selection
* Willingness to donate gametes, including in relation to identity release
* Posthumous conception
* Legal status of surrogacy and payments
* Awareness of the HFEA

Results included 67% support NHS-funded fertility treatment to people who are infertile and wish to conceive.

Younger (16-24 and 25-34) age bands were more likely to support people being able to choose the biological sex of their child than in older age bands.

60% thought stored gametes from a deceased person should be able to be used by their spouse or partner.

Only 26% thought surrogacy is legal throughout the UK, even though surrogacy has been regulated and permitted by UK law for almost 40 years.

55% were not aware that the HFEA existed, and only 11% knew its name.

Conclusions: Although the findings indicate that there is broad support for fertility treatment there is a clear need for continued public engagement and societal debate to build upon it to improve understanding.

P040 Exploring women's percpectives on managing reproductive health in the workplace

Zoya Ali; Tharni Vasavan; Natalie Getreu; Helen O'Neill

Hertility Health

Background: Women make up 51% of the United Kingdom (UK) population, and 72% of women aged 16-64 are in employment (1). There is limited evidence on the impact of reproductive health-related conditions on workforce participation, productivity and outcomes. Our study investigated women's experiences managing reproductive health issues in the workplace.

Methods: A cross-sectional survey was conducted on 1511 UK-based women aged 20-40 in March 2022. It assessed general demographic information and individual perspectives on managing women's health in the workplace.

Results: Almost all (94.0%) participants believed there is a taboo surrounding discussing women's health issues, with 93.4% agreeing this stigma also exists in the workplace. When asked about issues they would not be comfortable discussing in the workplace, 30.0% selected period pain, 24.0% selected fertility issues, 19.0% selected miscarriage, and 13.0% selected fertility treatments. Most participants (90.4%) reported a lack of support by managers and employers regarding women's health issues, and 26.0% stated they would rather take paid time off than explain requiring leave for women's health issues. Almost half of participants (43.0%) admitted to avoiding discussing a woman's health issue around men to prevent feeling uncomfortable or embarrassed. It was noted that participants aged 20-30 were more likely to report facing embarrassment than those aged 30-40 (90.0% vs 84.2%). Many participants (30.7%) reported that a male colleague has shuddered, made a painful noise, or asked them not to talk about women's health issues on multiple occasions.

Conclusions: Our findings highlight the need for better workplace support for reproductive health and further research to understand women's experiences, impacts of reproductive health awareness and support.

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P041 Closer look at the sources of fertility and reproductive health information: A mixed-methods study

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Background: There has been a concerted effort from policy makers and reproductive health groups to improve reproductive health awareness. Understanding the different sources of information used by the target audience is important for disseminating and improving knowledge. This mixed-methods study therefore aimed to assess the different sources used by individuals when seeking fertility information and the perceived accessibility and reliability of these sources in order to understand what's working, what isn't, and opportunities for improvement.

Methods: A mixed method study was conducted via UK-wide cross-sectional survey and semi-structured interviews. 1082 survey participants were recruited nationwide via online newspaper and social-media adverts. Of those who agreed to follow-up interview, 35 were purposively sampled to reflect the diversity of gender, age-range, ethnic and education. Data analysis was carried out using Tableau for survey, and NVIVO for interviews using framework method.

Results: Sources of information identified included: school education; healthcare-professionals; internet, social media, smartphone-apps, online-forums and blogs; family, friends, and communities; books, magazines, newspapers; fertility-products; workplace, communities and sexual-health clinics/centres, charities, and third-party organisations. School education remains a consistent but often inadequate source. Participants reported varying levels of access, reliability, and trust, in relation to these sources. Interview themes around veracity showed that healthcare-professionals were highly trusted but not easily accessible. The internet was very popular due to accessibility and perceived anonymity but untrusted, and "the plethora of information can be overwhelming." There were recurring themes around discomfort. A respondent recalled that her first discussion of sex with her mother was on her wedding night stating, "...Mum, I'm 28! And you're just discussing this with me now?"

Conclusion: Sources used by participants are not necessarily the most trusted. In addition to online-platforms and products based on robust scientific evidence, opportunities for improvement include using underexploited sources, such as workplace and community settings, and training for providers.

P042 Impact of limited reproductive health awareness on PCOS diagnosis timelines and need for improved patient education

Zoya Ali; Tharni Vasavan; Meeladah Ghani; Eliza Waskett; Natalie Getreu; Helen O'Neill

Hertility Health

Background: Despite affecting up to 20% of women worldwide[1], a Polycystic ovary syndrome (PCOS) diagnosis can take over 2 years and appointments with 3 different healthcare providers[2]. Our study investigated the barriers which contribute to a delay in the diagnosis of PCOS.

Methods: Women aged 18 years old with a self-reported confirmed or suspected PCOS diagnosis were asked to complete an online survey assessing their experiences with receiving a diagnosis, management of their symptoms and impact on quality of life. Of the 341 responses, 184 with a PCOS diagnosis were included in the analysis.

Results: Despite the majority of participants reporting that their symptoms impacted their life, only 7.6% had sought medical help immediately, whilst 57.6% waited for up to 2 years. The most common reason stated for this delay was not realising their symptoms needed attention (59.8%). The majority (36.4%) were diagnosed after consulting only one doctor, 22.8% needed to visit 2 different doctors, 26.0% visited 3 different doctors, 22.8%, and 10.9% visited over 4 different doctors. Anxiety (74.4%), irregular menstrual cycles (65.2%) and weight-related concerns (64.6%) were the most commonly reported symptoms, although this did not translate to what was focussed on during consultations. Following consultation(s), 53.8% of participants were not satisfied with the management information, and 89.1% participants looked for extra information afterwards; the majority of these people (46.7 %) relied on social media to find information. When asked which support pathways would have improved symptom management, 32.0% selected educational resources.

Conclusion: These results suggest that lack of reproductive health awareness contributes to delays in PCOS. Heavy reliance on unregulated channels such as social media to obtain reproductive health information highlights the need for better accessibility to educational resources.

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P043 Quality of life assessment in patients with extremely low ovarian reserve

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Objective: The study evaluates the impact of the diagnosis of extremely low ovarian reserve and poor fertility prognosis on the quality of life of women seeking fertility treatment.

Design: This cross-sectional study is the first of its kind to assess quality of life in women with extremely low ovarian reserve and poor fertility prognosis with in-vitro fertilisation using their own eggs. A tailored questionnaire was designed to assess demographics followed by questions in the core module of FertiQOL questionnaire. This was sent to women with AMH <3.5pmol/L seeking fertility treatment at Chelsea and Westminster Hospital, London, between December 2020 to December 2021.

Method: An online version of the questionnaire was emailed to 30 patients, of which 23 were completed and returned. The total quality of life score was calculated using the online FertiQOL scoring tool. SPSS was used for statistical analysis and comparison between subgroups.

Results: The response rate of patients was 76.6%. The mean core FertQOL score was 63.0. Patients of the Afro-Caribbean race had significantly lower scores compared to those from other ethnic background (60.7 vs. 63.0; p = 0.01). Women who received professional counselling prior to seeking fertility treatment showed higher scores (72.8), albeit not statistically significant, when compared to women who had no counselling (63.0; p = 0.67). There were no statistically significant differences in scores between younger and older women, different education levels, single and married or nulliparous and multiparous women. The relatively small sample size possibly had an impact on the statistical significance of the results.

Conclusion: Undergoing fertility treatment is an emotionally challenging process, which can be aggravated by the knowledge of expected poor prognosis with IVF treatment due to extremely low ovarian reserve. These women should, therefore, be identified as a group that would benefit from extensive counselling and additional support throughout the process.

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P044 Recurrent implantation failure: Understanding patients' experiences after referral for endometrial assessment in a dedicated unit

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London Women's Clinic

Purpose/background/objectives: Repeated IVF failures incur a significant emotional, physical and financial burden for patients. Despite this, few IVF centres provide tailored clinical management and endometrial assessment in these cases

is often overlooked. Following the establishment of a dedicated unit in 2018, we sought to better understand patients' experiences following referral for endometrial assessment and to determine whether patients valued this service.

Methods: A cross-sectional retrospective survey was conducted in November 2021 at a UK-based centre to evaluate patients' experiences at a dedicated unit for endometrial assessment. 320 patients who attended between 2018-2021 were invited to participate via email and responses were anonymised. Continuous variables are reported as mean SD, and categorical variables presented as percentages. Open-answer responses were qualitatively analysed through thematic analysis.

Results: 109 patients responded, with a mean age of 41.8 6 and mean number of embryos transferred of 4.6 1 before referral. 90 respondents had undergone timed endometrial biopsy and 73 already completed their subsequent personalised embryo transfer. Of these, 45 had an ongoing pregnancy, 5 miscarried and 23 had implantation failure. When commencing IVF treatment, 45% believed their chances of becoming pregnant were 50%. Following treatment failure, 78% said their hopes of achieving a pregnancy decreased. 74% felt they received new knowledge regarding treatment at the Implantation Clinic and 62% said their expectations of achieving a pregnancy increased, primarily attributed to change in treatment regime, greater understanding and increased feelings of hope and motivation. Collectively, 91% valued the option of referral and 58% wished they had been referred sooner, irrespective of treatment outcome.

Conclusions: These findings show that a dedicated unit is valued by patients facing repeated implantation failure, who appreciate in particular the greater education, hope, support and personalised care it can offer.

P045 Women's experiences of ovarian hyperstimulation syndrome (OHSS): A qualitative research study

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[1]The University of Sheffield; [2]Jessop Wing, Sheffield Teaching Hospital NHS Foundation Trust

Aims: To highlight the experiences of women who have had OHSS.

Background: OHSS is a recognised side effect of fertility treatment that can significantly affect the health of women. Historically research has focused on the medical aspects of OHSS, such as prevalence, prevention and treatment, with little research to examine the experiences of women who develop OHSS. This qualitative study sought to redress this gap in the research by highlighting the varied experiences of women who have had OHSS.

Methods: Semi-structured interviews were conducted with ten women who have experienced OHSS. Framework analysis was used to identify themes exploring experiences of OHSS.

Results: A wide range, and severity, of OHSS symptoms were described; women were impacted both physically and emotionally. Methods of treatment varied although women were commonly monitored until symptoms became severe. Women suggested that they were left 'in limbo' whilst waiting for symptoms to either improve or worsen. Women described feeling under-informed about both OHSS, and the subsequent impact that it could have on their fertility treatment. This led to them using unofficial sources such as internet searches, forums and social media, which could provide misleading or often distressing information.

Conclusion: There is a dearth of qualitative research exploring the experiences of women who developed OHSS during fertility treatment. This qualitative study highlights the wide reaching effects OHSS can have emotionally and physically, and the need for provision of accurate information.

P046 Fertility intention typology (FIT) and potential applications: A mixed-methods study

Bola Grace[1]; Jill Shawe[2]; Sarah Johnson[3]; Nafisat O. Usman[4]; Judith Stephenson[1]

[1]University College London; [2]University of Plymouth; [3]Qiagen; [4]Kaduna State University

Background: Several studies have highlighted poor fertility knowledge across men and women of reproductive age. As the average age of first-time parents continues to rise, there has been a concerted effort to improve fertility awareness. To ensure that these messages are effective and to deploy the best strategies, it is important to understand reproductive health needs. This study therefore aimed to explore the different fertility intentions to aid tailoring of information to help individuals achieve their family-building desires. **Methods:** We conducted a mixed-method study via a UK-wide cross-sectional survey with 1,082 participants and semi-structured interviews of 20 women and 15 men who agreed to follow-up interviews. Interviews lasted an hour on average, data was transcribed and analysed using the thematic framework method. Ethics approval was obtained from UCL Research Ethics Committee.

Results: We identified six key categories of people, grouped alphabetically, in a user-friendly manner to highlight a spectrum of fertility intentions: Avoiders describe those who have no children and do not want children in future; Betweeners describe those who already have child(ren) and want more in future but are not actively trying to conceive (TTC); Completers describe those who have child(ren) but do not want more; Desirers describe those who are actively

TTC; Expectants describe those pregnant at the time of the study and Flexers describe those who may or may not already have and are unsure about having child(ren) in the future. Survey analysis showed these proportions: Avoiders,4.7%; Betweeners,11.3%; Concluders,13.6%; Desirers,36.9%; Expectants,4.1%; Flexers,28.4% and 2.4% preferring not to answer. A majority of the survey population were pregnant; TTC; or planning to have a child in the future - whether actively, passively or simply open to the idea, with interviews providing deep insights into their decision-making.

Conclusions: We developed a user-friendly, alphabetical FIT, can be used by individuals, healthcare professionals, educators, special interest groups.

P047 Exploring the availability of fertility-friendly policies in Portuguese organisations

Beatriz Trigo; Andreia Trigo; Joana Folgado; Catarina Lucas

Vida Mais Fértil

Companies throughout Europe have been adopting fertility policies to support their employees entering parenthood. However, literature regarding this is scarce in Portugal. This study explores how Portuguese companies support their employees' fertility plans.

Our sample (N=24) is mainly aged over 41 years old (41.7%), working for a large company located (66.7%) in Lisbon and Vale do Tejo (83.3%), and have a leadership position (58.3%). Only 12.5% acknowledged the company they work for does not have any support benefits for fertility preservation or treatment, 20.8% had no knowledge of whether their company offered such support, and 66.7% stated theirs did.

Concerningly, 87.5% of the companies do not have internal policies related to their employees' fertility health and 41.7% do not provide all their employees with healthcare insurance. All respondents attributed moderate to high importance to the existence of such policies (3 to 5 ratings on a scale of 0 to 5). Out of those who do not have such internal policies, 66.7% do not find having them useful. Moreover, 95.8% do not provide employees with any informative resource about reproductive health; of those, 70.8% do not believe it would be useful.

Significant differences were found in terms of providing flexible schedules for consultations and exams (U=16.0, r=-.56, p=.03), allowing time off for mourning (U=16.0, r=-.56, p=.03), and recognizing usefulness in financial contributions to fertility treatment (U=16.0, r=-.50, p=.05) between large (>250 employees) and not large companies (<250 employees). Workers from small companies are more flexible in their schedules and allowed time off, but workers from large companies recognize the usefulness of financial contribution to fertility treatment more. These findings point to the need to educate the policymakers in Portuguese companies in terms of employees' fertility needs and the individual and organizational benefits of supporting them.

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P048 Does size matter? A patient survey about fertility benefits in micro, small, medium and large Portuguese companies

Beatriz Trigo; Andreia Trigo; Joana Folgado; Catarina Lucas

Vida Mais Fértil

European companies have been adopting fertility policies to support their employees' intentions to conceive and become more aware of their reproductive health to prevent low natality. This study aims to explore how supported Portuguese workers feel by their employers.

In our sample (N=107, 95.3% females, Mo=between 35 and 38 years old), 26.2% are trying to conceive naturally, 56.1% are using ART, and 17.8% both. The majority (52.3%) have been trying for more than 24 months. All participants reported their company does not have fertility-supportive internal policies, although 28.0% report being offered some

support benefits. Access to support benefits is considered very important by the majority of participants (69.2% rating it a 5 on a scale of 0 to 5).

The sample was divided into two groups - with benefits(1) and without benefits(2). There are significant differences between the two groups in: frequency of concern/worry (t=-2.31, df=68.15, p=.01), with a medium effect (d=.50), and having considered quitting their job (t=4.94, df=86.63, p<.001), with a large effect size (d=.94).

The sample was divided into two further groups - belonging to a large company(3) and to a non-large company(4). No significant differences between the two groups were found.

These findings show that offering benefits and internal policies for fertility influences some employee-level variables significantly, whereas company size does not. People who are working for a company that offers them fertility benefits are far less likely to consider quitting than those who receive no support. However, people working for a company that offers them support benefits are more worried in their day-to-day lives than those who are offered no benefits. This may be due to support increasing people's awareness about their fertility problems; being open about seeking treatment in the workplace leading to more unwanted comments; and/or receiving benefits putting more pressure on people not to fail.

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P049 Fulfillment, career satisfaction, and professional outlook: Physical and psychological symptoms reported by U.S. embryologists

Anar Murphy[1]; Mark Lapczynski[1]; Glenn Proctor[2]; Elaine Meyer[3]; Timothy Glynn[4]; Alice Domar[5]; Michael Collins[1]

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Background: Fatigue and burnout are commonly reported amongst U.S. [ ] and Spanish [ ] embryologists. This study aimed to explore the impact of job fulfillment, career/workplace satisfaction, and attitude on the emotional and physical well-being of U.S. embryologists, and how organizational changes could lessen these effects.

Methods: In 2022, a cross-sectional web-based survey was emailed to U.S. embryologists, including the validated Perceived Stress Scale (PSS) and the Patient Health Questionnaire (PHQ-15), and a customized occupational questionnaire. Respondents were asked to rank their professional fulfillment, career/workplace satisfaction, and career outlook, and responses were analyzed in conjunction with PSS and PHQ-15 scores. Descriptive statistics and correlations were conducted.

Results: 246/487 (51%) completed the survey--mean age 40, 65% female, 67% worked in private/for-profit, 19% corporate, and 14% academic settings. Of those, 87% reported fulfillment in their positions, 73% were satisfied with their careers, 72% were optimistic about future careers in the ART/IVF field, and 66% were optimistic about their careers compared to other medical fields. However, 65% reported their employers did not understand their occupational challenges. Moreover, (1) 24% would, 48% would maybe, and 28% would not leave their positions to pursue other careers; and (2) 13% would not, 48% would be somewhat likely, and 39% would recommend their position to another person. Most participants self-reported moderate scores on the PSS, other than those with the highest levels of job fulfillment and career/workplace satisfaction.

Conclusions: U.S. embryologists reported fulfillment in their positions, although many were dissatisfied with or pessimistic about their careers, and 65% reported that their occupational challenges were not understood by their employers. Employers could benefit from greater understanding of the unique occupational challenges of embryologists and those challenges that may be mitigated through

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P050 Counseling donor family members: A guide for mental health professionals

Wendy Kramer

Donor Sibling Registry

Families formed and connected via donor gametes are unique in many ways, yet they also share the same joys, disappointments, adventures, concerns, stressors, and love that most families do. It's not uncommon for individuals in donor families to feel a sense of confusion or discomfort about their stories or with their own or their family's boundaries when it comes to using donor gametes or donating them, or to have issues surrounding disclosure or learning about their own donor conception story. It can sometimes be anxiety-provoking to reach out to one's own or their child's new genetic relatives. Grappling with the depth and breadth, and the timing and speed with which they explore their own or their child's origins and expanding families can be challenging, and also deeply profound and rewarding. Clinicians will explore in this presentation the unique issues that can present for egg and sperm donors, parents of donor-conceived children, and donor-conceived people. They will better understand the reasons donor family members may or may not desire to connect with their own or their child's close genetic relatives. They will be better prepared for many of the issues that donor family members might present with regarding their families of origin and with their new donor family relationships. Regardless of the presenting issues for treatment, for these individuals, the challenges of forming and redefining identity and family as they explore their own or their child's new biological connections can seem overwhelming and are therefore very likely to surface as a topic of discussion. Counseling Donor Family Members is intended to be a resource for mental health and medical professionals in any setting, especially for those who are unfamiliar with donor conception. It's a presentation of evolving ideas, recommendations, and talking points that can be used when counseling anyone in the donor family.

Counseling Donor Family Members: A Guide For Mental Health Professionals
https://ethicspress.com/products/counseling-donor-family-members-a-guide-for-mental-health-professionals?fbclid=IwAR0zyBasmU9Bp3Mnc3zognPuur4QmSiASPzLWV-d3miGWJ3nYNTvSIAsUsw

P051 Therapeutic needs and complexities for families after donor conception

Sharon Pettle

Independent Clinician and External Advisor to DCN

Although much of the academic research on families built through donor conception [DC] have been generally positive, there are families and individuals for whom this can be a complicated and challenging pathway. Focusing on the perspective of parents who use DC as a way to build families, this presentation will share information gained over the past two decades working therapeutically in situations across the life span, including cases dealt with in Family Proceedings. It will also refer to the development and delivery of groups for children who are being brought up in families where parents choose to be open. It will highlight the value of support from organisations such as the Donor Conception Network, and the need for therapeutic services addressing the needs of these families, those of donors and their families, and surrogates and their partners and children over the long term. This is particularly salient with the imminent opening of the Register and increased use of over-the-counter DNA tests

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P052 What is the psychological impact of the one at a time embryo transfer policy for women?

Lois Whelan

Hewitt Fertility Centre

The one at a time policy was introduced by the HFEA in 2006 to reduce the high multiple birth rate as a result of IVF. The aim was to reduce the maternal and neonatal risks for the patient with data suggesting the policy was successful at doing this. However, studies across Europe have since suggested that patients still preference a double embryo transfer despite the risks. This presentation considers the reasoning behind this and why patients still preference a double embryo transfer despite the known risks. It will also analyse the education given to patients and if this is sufficient in helping patients to make the decision on if to transfer one or two embryos and suggest how this could be improved going forward. The psychological impact on women of the policy will be analysed and if there are sufficient support mechanisms in practice to support this. Furthermore, it will consider the data regarding multiple births from ovulation induction and intrauterine insemination and why tighter regulations could be suggested for this field to reduce multiple births.

P053 The delivery of AMH testing and interpretation via an app based fertility service - a retrospective review

Anne Howard; Francesca Steyn; Laura Carter-Penman; Emily Yates

Peppy Health

AMH (Anti-mullerian hormone) testing is recognised by NICE 2017(1) as one of the first tests when assessing female fertility. Unfortunately this test may not routinely be freely available to those that require it. By providing AMH testing via an app based fertility service, our aim was to reach out to those who are trying to conceive and begin the process of initial investigation earlier. This enabled our clients to have a clearer understanding of their basic fertility by having access to the test, interpretation and guidance by fertility nurse specialists. Following assessment of the client, against a selection criteria, AMH testing kits were sent to clients in partnership with a UKAS(2) accredited third party provider. The result was then interpreted and provided to the client by competent fertility practitioners and a consultation was offered to explain the result and next steps. Data collected from AMH tests performed between May 2021 and July 2022 showed the following results: A total of 412 tests were completed, 373 tests were analysed with normal results against TDL(3) reference ranges, 39 results (9%) were abnormal (abnormally high or low for age range). Out of the abnormal findings, the highest number of abnormal AMH levels fell in between the age range of 31-35. These results (although small numbers) suggest that those who are at the average age of conception in the UK (Statista 2022)(4), may have unexpectedly abnormal AMH levels. By alerting clients to these results, we have been able to advise and signpost to seek further investigation or intervention sooner than perhaps originally planned. Our recommendations following the review of this preliminary data, has been to continue to offer AMH testing, interpretation and guidance as part of the service to promote greater access to reproductive choices and earlier interventions.

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P054 The challenges, benefits and shared learnings of a UK multicentre scientific training and development focus group

Lynne Nice; Alison Campbell; Zoe Flitter

Care Fertility Group

A review of the UK reproductive science workforce resulted in the establishment of an company based Training and Development Focus Group in 2018 to seek and create solutions to ensure workforce stability, reproductive scientist training and development provision. The national Scientist Training Program (STP) was in place although access to Health Education England (HEE) funded training places had, to date, not proved possible for private service providers. Outcomes: A consortium was formed and training accreditation achieved to enable four company-funded training places on the ACE STP pilot scheme. The rotations were extremely challenging to secure and NHS laboratories were not in a position to host training places for the students. The rotations were completed using a variety of resources relating to the service and tailored to Reproductive Science. These first pilot scheme students all successfully graduated in 2021. A further four graduated in 2022. Having fully engaged in the STP as an organisation and with our focus group leading the initiative, in 2020 and 2021, HEE funding was secured for 4 and 5 students respectively. The consortium currently consists of 13 UK laboratories within the company; all accredited for STP supervision and training. 2022 will see nine

HEE-funded STP students commence training across the company using the new STP embryology curriculum. In addition, company support for STP-equivalence is also being provided. Going forward: Healthcare Science Apprenticeships are being introduced at levels 2 and 4 for 2023 as well as engagement in the Higher Specialist Scientific Training (HSST). Structured in-house developed training and development pathways for Clinical Scientists to professionally develop in one of three core areas; leadership and management, research and academic or technical training has been developed. This dedication to cultivating and developing talent within the UK fertility sector aims to bolster the UK workforce and produce well trained Clinical Scientists.

P055 An investigation into fertility awareness and attitudes towards clinical practice amongst obstetrics & gynaecology and general practice trainees in Ireland

Sarah Petch; Jenny Stokes; Minna Geisler; Moya McMenamin

Cork University Maternity Hospital

Purpose: There is a varying degree of knowledge regarding fertility and assisted reproduction amongst doctors working in Obstetrics & Gynaecology (O&G) and General Practice (GP)[1,2]. Notably, a rotation in O&G is not mandatory for GP trainees in Ireland. The aim of this study is to explore knowledge, attitudes, and approaches to fertility care, Assisted Reproductive Technologies (ART) and Elective Oocyte Cryopreservation (EOC) amongst trainees working in O&G and GP in Ireland.

Methods: This is a cross-sectional study involving an online survey disseminated by email to GP and O&G trainees. The survey was adapted from a questionnaire by Yu et al[1]. Background demographics, questions regarding knowledge of, and attitudes towards, age-related fertility decline and ART and opinions on EOC were included. Ethical approval for the study was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Results: The collection of responses is ongoing. So far, 42 participants have responded, 90% (n=38) of whom are female. Ninety-two percent of respondents (n=39) think that GPs and Gynaecologists should initiate discussions with patients regarding childbearing intentions. Forty-five percent (n=19) reported a woman's ability to conceive markedly decreases over 40 years, and 70% (n=29) stated that couples should have investigations for sub-fertility after trying to conceive for over 1 year. Seventy-six percent (n=32) feel that EOC should not be encouraged for social reasons, but 66% (n=27) would discuss EOC in the case of a cancer diagnosis in a 25 year old patient.

Conclusion: This research is the first to examine knowledge of and attitudes towards fertility and ART amongst trainees in O&G and GP in Ireland. Our findings thus far highlight the need for enhanced training and education. Better knowledge of fertility amongst clinicians would ultimately lead to better fertility counselling and care for patients.

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P056 Virtual consultation (VC) in fertility services: A comparison of different patient pathways and satisfaction

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Background and objectives: The onset of the coronavirus pandemic was the catalyst for many forms of healthcare, including fertility services, to move to virtual methods of appointment delivery. This study aims to assess patient and clinician satisfaction with the use of VC, and clinic attendance rates at one UK fertility institute.

Methods: Analysis of patient and clinician responses to a VC satisfaction survey between August 2020 and March 2022. Analysis of clinic attendance rates between July 2020 and March 2022.

Results: In satisfaction data collected from 82 patients and 161 clinicians, 88% of patients and 63% of clinicians reported their VC quality to be at least "very good". After implementing the Hybrid pathway (of VC and face-to-face appointments) in October 2021, overall attendance reduced to 81%. This was down from 88% during the Covid pathway when only VC appointments took place. Attendance of VC appointments reduced from 88% to 75% after implementing the Hybrid pathway.

Conclusions: Most patients and clinicians were satisfied with their VC experience. VC attendance rates reduced after implementing the Hybrid pathway, which may suggest patient satisfaction with VCs reduced once F2F appointments became available again.

P057 Can EMA, an end-to-end artificial intelligence (AI)-powered platform for fertility clinics, aid in the efficiency of conventional embryo evaluation? An efficiency case study

Achilleas Papatheodorou[1]; Daniella Gilboa[2]; Maya Shapiro[2]; Chara Oraiopoulou[1]; Mary Karagianni[1]; Marianna Papadopoulou[1]; Daniel Seidman[2]

[1]Embryolab Fertility Clinic, IVF Lab; [2]AiVF Ltd.

Purpose and Objectives: The occupational stress often experienced in IVF labs due to high-demand/low-control working conditions can manifest itself in reduced capacity and embryologist burnout. There is a need for more standardized workflows that optimize efficiency, productivity, and capacity. This is even more apparent following the introduction of time-lapse systems (TLS) into the laboratory. Though TLS enables more comprehensive embryo readouts, the need for manual annotations and interpretation increase workload and embryo evaluation variability. In this study, we perform a real-world efficiency and agreement analysis for EMA (AiVF, Israel), an AI-powered platform designed to assist embryologists in evaluating embryo quality. EMA's automated outputs may overcome many of the challenges described above.
Methods: We manually calculated embryo evaluation performance time with and without EMA, which records a continuous score for every embryo. Calculated by: average time spent by 3 senior embryologists to evaluate embryo quality and record decisions (transfer/freeze/discard) per embryo using published consensus guidelines and EMA software. A total of 526 embryos from 49 cycles were included. This analysis was restricted to cycles where 2 good-quality blastocysts were eligible for evaluation. Agreement between embryologist's majority vote and EMA's ranking model was also quantified.
Results: Manual evaluation time without and with EMA: 3.1 minutes versus 30.9 seconds per embryo, respectively. Percent reduction in evaluation time: 83%. Time range (seconds) for embryo evaluation using EMA: 25th /75th percentile - 5.1 and 31.2, respectively. Agreement rate between embryologist majority vote and EMA reached 72%. 85% of embryos deselected by embryologists were similarly scored as low quality by EMA.
Conclusions: Benchmarking and comparing efficiency measurements with and without the use of EMA is used to understand how EMA can standardize and aid in the efficiency of repetitive clinic workflows. Results from this study will further be used to quantify EMA's impact on clinic productivity and operational capacity.

P058 Hype or reality? Is artificial intelligence (AI) solving the real problem in the IVF laboratory

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[1]Ferticentro Fertility Clinic; [2]AiVF Ltd.

Purpose: The impressive performance of AI tools that distinguish between top/good/poor embryos for implantation prediction has been shown in numerous published reports. We hypothesize that this does not reflect the AI's "real-world" efficacy; characterizing AI performance on a dataset using top/poor embryos that would otherwise be manually selected/deselected skews its presentation of efficacy and benefit. To reflect true clinical utility, the performance of AI tools should be assessed using a cohort of homogeneous B-grade embryos -- i.e., the subset of embryos most relevant for AI decision-support. In this context, we compare the discriminative performance of EMA (AiVF, Israel), an AI platform for embryo evaluation, and another commercially available embryo scoring tool.
Methods: We used a multicentric annotated dataset of 647 blastocysts with known implantation data (KID). EMA and the commercially available tool were used to output numeric scores for each embryo. We examined the distribution of scores, through their averages and quartiles, for each category: top, good, fair (A, B, C-grade embryos, respectively). We then focused on the subset of embryos that were not top/poor quality (i.e., B-grade embryos).
Results: Manually graded A/B embryos from the dataset had comparable implantation rates (67% and 65%, respectively, no difference); likewise, mean EMA scores for the two categories (A/B) were comparable ($p=0.24$; no difference), accurately reflecting the KID. A statistical difference in scores was observed for the commercially available tool ($p<0.01$), inaccurately reflecting the KID. When examining the distribution of scores for B-grade embryos only, EMA showed statistically higher scores for the B-grade implanted group, compared to the B-grade nonimplanted group ($p=0.03$). This difference was not observed for the commercially available tool ($p=0.33$).
Conclusions: When focusing on the subset of embryos most relevant for AI analysis, EMA more accurately differentiated between implanted/nonimplanted subgroups and reflected the KID. EMA offers clinical benefit to enable objective, accurate embryo evaluation decision-support.

P059 Using artificial intelligence to automatically annotate time-lapse videos: Saving precious embryology time

Rebecca Matthews[1]; Andrew Thompson[1]; Sally Dodge[1]; Suzanne Cawood[2]; Mina Vasilic[2]; Samantha Knight[3]; Raj Joshi[3]; Anastasia Mania[4]; Ippokratis Sarris[4]; Alexa Zepeda[5]; Noam Bergelson[5]; Adriana Brualla[5]; Cristina Hickman[5]

[1]CRGW Plymouth; [2]CRGH; [3]Harley Street Fertility Clinic; [4]Kings Fertility; [5]Fairtility

Introduction: The aim of this study was to compare the manual annotation by embryologists with the automated annotation by an AI-based decision support tool (CHLOE-EQ, Fairtility).

Methods: 8368 embryos from ICSI/IVF cycles cultured in Embryoscope incubators from 2021 to 2022 at 4 clinics (n=362, 5591, 653, 1762) were annotated as per routine clinical practice. The same videos were blindly assessed retrospectively using CHLOE-EQ (Fairtility). Lin's concordance correlation coefficient (CCC) were calculated between CHLOE-EQ and embryologist annotation times for each of the morphokinetic parameters assessed using two-way model for agreement. Five categories of agreement were determined based on CCC score; very weak (0-0.20), weak (0.21-0.40), moderate (0.41-0.60), strong (0.61-0.80) and very strong (0.81-1.00). The level of agreement was quantified separately for each clinic and presented as (clinic 1, clinic 2, clinic 3, clinic 4).

Results: All the CCC for all the morphokinetics across all 4 clinics were at least strong level of agreement between CHLOE-EQ and human embryologists: tPNf (0.97, 0.63, 0.95, 0.66), t2 (0.84, 0.87, 0.92, 0.74), t3 (0.8, 0.81, 0.84, 0.84),t4 (0.89, 0.87, 0.74, 0.76), t5 (0.76, 0.89, 0.78, 0.73), t6 (0.77, 0.68, 0.74, 0.69), t7 (0.72, 0.76, 0.80, 0.80), t8 (0.73, 0.79, 0.72, 0.83), tsB (0.75, 0.89, 0.9, 0.92), tB (0.74, 0.92, 0.92, 0.95).

Conclusion: Manual annotations are time-consuming and subjective, prone to inter and intra operator variation. CHLOE-EQ automatic annotation is equivalent to the annotation by experienced embryologists. This equivalence has been demonstrated across different clinics with different types of patients and following different protocols. Automatic annotations help save precious embryology time.

P060 Comparative performance of EMA, an artificial intelligence (AI) platform for embryo evaluation decision support, and a commercially available embryo scoring tool

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[1]Ferticentro Fertility Clinic; [2]AiVF Ltd.

Purpose: Several semi-automatic tools for scoring embryos claim to decrease inter- and intra-user variability while increasing reproductive outcomes in the IVF lab. However, the clinical utility of these tools in a real-world setting is debated since they rely on manual annotations and movement of data. In this analysis, we externally validated the accuracy of EMA (AiVF, Israel), which uses a convolutional neural network (CNN) with ResNet50 backbone to assess embryo quality and developmental competence, and a commercially available semi-automatic tool that processes annotated morphokinetic/morphological parameters to rank embryos.
Methods: A retrospective analysis on 643 embryos with known implantation data (KID) from a single European fertility center that employs both models in the lab. The performance of both models for predicting implantation was compared using the area under curve (AUC) of the receiver operating characteristic curve. Student's t-tests were used for statistical analyses.
Results: AUC values for the semi-automatic tool and EMA were 0.58 and 0.62, respectively. EMA outperformed the tool in sorting embryos by implantation outcome across all possible thresholds, as a relation of false positive rate and true positive rate. AUC for the semi-automatic tool was lower than the value recorded in previously published reports, highlighting the need for clinical validation and generalizability during model testing. Both models showed statistical differences between mean scores for implanted/nonimplanted embryos ($p<0.01$). Both models showed statistical differences between mean scores for grade-A/grade-B embryos ($p<0.01$), demonstrating robust ability to distinguish between morphology classes.
Conclusions: Both models' learned features are rooted in biologically meaningful parameters of the embryo that relate to its quality and implantation outcome. EMA's improved performance may indicate that it applies its learned features more robustly and objectively than the semi-automatic tool, which relies on manual annotations as input parameters. EMA's automated framework increases standardization without compromising on performance.

P061 Is an AI embryo selection tool more predictive for pregnancy with fewer embryos compared to patients with a higher number of embryos?

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Manchester Fertility

Purpose/background/objectives: Selecting the best quality embryo to transfer is a key part of IVF and typically relies on subjective human morphological assessment (1). Advancing artificial intelligence (AI) has the potential to innovate the process. Using deep learning of time-lapse data with clinical outcomes, Vitrolife have created AI tools (KIDScore and iDAScore) to aid embryo selection (2). As there is currently limited research on iDAScore this study aims to assess if: iDAScore is a significant tool to aid embryo selection for patients with fewer embryos compared to patients with a higher number, using iDAScore improves clinical pregnancy rate (CPR), the iDAScore value increases chance of CPR.

Methods: A retrospective study of 96 patient cycles, (maternal age up to 37, 2+ utilised, using own eggs and donor sperm) extracted from our clinic database. The data was then cross-matched with iDAScore and analysed.

Results: 77 patients had the embryo with the highest iDAScore from their cohort of embryos transferred. The group was categorised by the number of embryos utilised and there were no significant difference in CPR between all groups, 2 vs 3-4(p=>0.9999), 2 vs 5-11(p=0.5532), 3-4 vs 5-11(p=0.5532). 19 patients had the second highest iDAScore embryo transferred. The difference in CPR between the highest and second highest iDAScore was not significant (55.8% and 47.4% respectively, p=0.29213). No significant difference was seen in iDAScore value vs CPR, p=0.9207.

Conclusions: The use of iDAScore is equally predictive for CPR for a small cohort of embryos compared to large, indicating that iDAScore could be helpful in selecting embryos regardless of morphology. Trends observed lacked significance, potentially due to the patient numbers. Based on inclusion criteria the patient cohort were a good prognosis subgroup, likely affecting the results. For 80% of patients iDAScore agreed with the embryologist, validating the tool for use in our clinic.

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P062 Barriers and drivers to AI adoption in clinical care and the views of 144 fertility professionals on AI tools in clinical practice

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[1]Apricity; [2]Cardiff University; [3]Centre Hospitalier Universitaire de Nantes

Introduction: The uptake of AI in fertility is slow despite its promised benefits. The objective was to understand the views, needs and barriers towards the use of AI in clinical practice.

Methods: A questionnaire was developed with 4 sections investigating demographics, knowledge, experience and current use of AI. 1419 fertility professionals were invited to respond through emails and LinkedIn messages. Response rate was 10%, with 144 respondents (occupation: 97 embryologists, 45 doctors, 2 academics; source of funding: 70% private; average 15 years experience) from 37 countries. Perceptions were graded (1:Strongly Against, 2:Against, 3:Neutral, 4:For, 5:Strongly For).

Results: Respondents showed a more positive view towards AI compared to the use of decision support tools (average score for AI vs DST: 3.9 vs 3.7, p=0.003). Age, source of funding and occupation did not affect perceptions. Respondents were split on whether AI tools help reduce the burden of clinical decisions (1:7%,2:13%,3:37%,4:40%,5:13%). Barriers included inexperience (14/29 that disagreed had never experienced the use of AI tools in clinical practice) and lack of knowledge (11% indicated discomfort in using AI tools in clinical practice, 79% of whom self-described as having poor knowledge of AI). Needs included evidence of improved live birth rate (120/144 considered critical or important), necessity for the tool (106/144), cost (105/144) and training (100/144). The preferred manner of implementation was directly to the equipment used in clinical practice (50%), directly into EMR (18%), standalone software (17%), web-based (13%).

Conclusion: Overall, fertility professionals hold a positive view of AI. The low implementation of AI to date can be explained by the barriers: insufficient experience, knowledge, validation. Unmet needs identified included the need for increased validation with respect to live birth outcomes, training programmes, and direct integration into clinics' digital ecosystems.

P063 The use of machine learning to enable predictive algorithms for reproductive and gynaecological conditions through digital diagnostics and at-home hormone testing

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Problem: A healthy reproductive cycle, leading to a successful pregnancy requires a complex calculus of ovulatory, menstrual, lifestyle factors and hormone levels. While there are some known associated symptoms with the diagnosis of common gynaecological pathologies and reproductive conditions, their biological relevance and pathophysiology are not well understood. The majority of symptoms are highly pervasive yet varied among individuals. The resulting difficulty in interpreting relevant combinations of symptoms and menstrual patterns can lead to delays in diagnosis and referrals for secondary care or fertility treatment.

Methods: A virtual health assessment containing 1500 variables and risk factors for reproductive pathologies was created to assess relevant biometrics regarding health, such as age, BMI, medical history, symptoms, menstrual cycle

patterns, exercise frequency and ethnic background. Internally embedded algorithms enabled weighted signposting for potential diagnoses based on current clinical diagnostic criteria and international guidelines for 9 reproductive pathologies. Tailored endocrinology panels were created for a given suspected diagnosis to enable further confirmation. Upon completion of capillary blood tests analysing a varied selection of endocrine markers, including AMH, FSH, LH, Oestradiol, Prolactin, TSH, FT4, Testosterone, SHBG, PRG, Anti-TPO, TG, FT3, 54,000 variables of outcomes were created.

A supervised machine learning approach was used on a database of 4311 women who completed both a health assessment and blood test. This algorithm was trained selecting for data where pathology of interest was selected a priori.

Results: The combination of algorithms applied to data regarding the test can detect (at present) 9 of the most common benign gynaecological pathologies.

Conclusion: The combination of four disciplines: endocrinology, reproductive science, clinical gynaecology and machine learning can leverage data to create digital biomarkers. Disruptive technologies in consumer health landscapes have the potential to harness diverse, stratified and population-wide data points that can inform the prediction of diagnoses and fertility outcomes.

P064 An analysis of PGT-M cases from 2014-2021 from a single testing laboratory organisation. Are we still seeing disorders new to PGT-M?

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[1]University of Kent / CooperSurgical; [2]CooperSurgical; [3]University of Kent

Objective: The number of monogenic disorders potentially considered suitable for Preimplantation genetic testing for monogenic disorders (PGT-M) is steadily growing as genetic screening becomes more available[1]. Here we determine whether this increase in patient testing has impacted PGT-M through analysis of a retrospective case series.

Methods: Workup of cases with known monogenic disease status for PGT-M included individual genetic counselling upon enrolment and was performed by three CooperSurgical laboratories between January 2014 and June 2021. Confirmation of genetic status of patients and relatives through external mutation reports was an inclusion criterium. Cases were processed using Karyomapping, a haplotyping test, with concurrent direct mutation analysis by Sanger sequencing. All prepared PGT-M cases were reviewed, and trends were established via annual growth analysis.

Results: A total of 8,400 cases were prepared for PGT-M, with an average annual increase of 14.07% for cases enrolled between 2014 and 2019, followed by a decrease in 2020 and 2021. Altogether, 915 individual monogenic disorders were identified and the most frequently occurring disorder was cystic fibrosis (n=767) with a growth rate (GR) of 7.15%. This was followed by Fragile X Syndrome (n=645, GR=8.61%), then sickle cell anaemia (n=425, GR=20.95%), hereditary breast-ovarian cancer-1 (n=384, GR=21.11%) and Huntington disease (n=372, GR=9.24%). On average, 109 unique disorders were added to this list annually, although this trend is showing a decline.

Conclusions: Karyomapping is a highly versatile technology facilitating PGT-M and enabling increased disorder screening. Sickle cell anaemia and hereditary breast and ovarian cancer-1 appear to show the greatest increases in testing frequency although cystic fibrosis remains the most frequently tested disorder. Despite the increased uptake of PGT-M and the concomitant annual rise in testing for novel unique disorders from 2014-2019, a decline in cases was observed between 2020 and 2021, perhaps due to limited genetic testing or IVF treatment access during the pandemic.

[1] Besser, AG; McCulloh, DH; Caroline McCaffrey, C; Grifo, JA. (2021). Trends in Preimplantation genetic testing for monogenic disorders (PGT-M). Fertility and Sterility Volume 116, Issue 3, E35, September 01, 2021 DOI:10.1016/j.fertnstert.2021.07.104

P065 A novel approach for UK fragile X preimplantation genetic testing for monogenic disorders (PGT-M) cases, with the inclusion of direct mutation testing for assessing triplet expansion size

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Aims: Current Whole Genome Amplification (WGA) methods approved in the UK for PGT-M are not compatible for downstream direct mutation testing for triplet repeat disorders, such as Fragile X. This study aimed to validate a Multiple Displacement Amplification (MDA) approach to WGA and its applications in downstream PGT-M via Karyomapping and direct mutation testing of FMR1 CGG triplet repeat size, in the UK.

Methods: Cells, which mimic Trophectoderm biopsies, were selected for the trial. Samples (n=92) were amplified by MDA WGA, alongside negative controls containing mastermix only (n=4). 10 successfully amplified samples were subsequently run on Karyomapping. Direct mutation testing was performed on 14 successfully amplified samples, by PCR and capillary electrophoresis, to establish FMR1 CGG repeat size. Pass criteria for amplification and downstream Karyomapping was 100% concordance. Direct mutation testing required peaks in all positive controls for validation.

Results: WGA by MDA passed acceptance criteria for its performance, use in downstream Karyomapping and use in downstream direct mutation testing. 100% of positive controls successfully amplified by WGA via MDA. Samples submitted to downstream Karyomapping produced a 0.5 call rate, with 100% concordance. Importantly, 100% of samples produced peaks following downstream direct mutation testing.

Conclusions: WGA via MDA for Karyomapping provides a novel approach to direct mutation testing for UK Fragile X patients. With MDA successfully used for both Karyomapping and direct mutation analysis in New Jersey since 2014, this study demonstrates the applications of WGA via MDA, including repeat size detection. This will increase the accuracy of PGT-M, reducing reliance on Haplotype analysis alone. Further advantages include the ability to prioritise transfer of embryos with smaller premutation repeat sizes, hence potentially reduce the risk of affected individuals in further generations. With additional verification studies, this method is planned to be introduced in the UK next year.

P066 A comparison of HFEA approved PGT-M conditions to a series of 8,424 international cases and 915 disorders internally approved from a single genetic testing organisation

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Aims: Analysis of the largest reported PGT-M case/disorder series from a single testing laboratory. How does the spectrum of outside UK, internally approved, disorders compare to HFEA approved conditions?

Methods: Retrospective analysis of clinical cases approved for PGT-M via Karyomapping between January 2014 and August 2021. Cases were obtained from CooperSurgical London, New Jersey, and Michigan. Cases outside the UK, hence not applicable for HFEA licensing, were submitted to an internal review, where the condition was assessed against criteria largely analogous to the HFEA. For case technical acceptance; patient, partner, and reference mutation status was confirmed by external testing, and where possible, in-house Sanger sequencing. For UK cases, only monogenic diseases approved by the HFEA could be accepted. Disorders accepted for PGT-M were tested via Karyomapping, a SNP array based haplotyping method, and compared to the HFEA's 1,454 approved disorders.

Results: 8,424 cases were approved and tested for PGT-M via Karyomapping between 2014 and 2021, consisting of 3,571 UK and 4,673 US cases. 915 unique disorders were approved for PGT-M, with 197 cases requiring PGT-M for more than one monogenic disorder. 350 disorders within the case series were not yet approved by the HFEA. Surprisingly, disorders not yet approved included some of the top 40 most common conditions observed in this study. This included Gaucher disease-1 and Familial Mediterranean fever, with 43 and 35 cases, respectively. Although, some of the 350 disorders tested in this series may have been rejected for approval by the HFEA.

Conclusions: Karyomapping is a highly versatile technology with applications to monogenic disorders both within and beyond HFEA approved conditions. This study and comparison to HFEA approved conditions indicates over 1,700 disorders may be appropriate for PGT-M. This is the largest analysis of the total number of potential and performed PGT-M conditions yet reported.

P067 Exploring the patient population that presents for PGT-M testing for BRCA1/2

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Background: *BRCA1/2* variants correlate with an increased lifetime risk for several cancers, and have relevant implications on family planning options, including potential preimplantation genetic testing for monogenic defects (PGT-M) (1). We sought to describe characteristics of families opting to pursue PGT-M for *BRCA1/2*, including personal and/or family history of clinical disease.

Methods: A retrospective case series of *BRCA1/2* families referred for PGT-M between 2017-2021.

Results: A total of 305 families were referred to our laboratory by 129 in vitro fertilization (IVF) clinics for PGT-M for *BRCA1/2*. 61.0% of referred cases were for *BRCA1* and 39.0% were for *BRCA2*.

95.1% of cases also pursued PGT-A testing. The sperm contributor (SC) carried the variant in 23.9% of cases (*BRCA1*, 22.6%; *BRCA2*, 26.1%). The average age of the egg contributor (EC) at egg retrieval was 33.2 years when the EC carried the variant, compared to 35.0 years when the SC carried the variant.

The most common variants were *BRCA1* c.68_69del (62/305) and *BRCA2* c.5964del (36/305). For these variants, the SC was a more frequent genetic carrier, representing 33.9% and 27.8% of cases, respectively.

40.3% of referred families carried one of three Ashkenazi Jewish (AJ) founder mutations. Most of these families reported AJ ancestry (102/123).

When detailed family history was discussed, both EC and SC frequently reported a cancer diagnosis in a 1st degree relative (EC, 65.5%; SC, 73.0%) or 2nd degree relative (EC, 77.4%; SC, 73.0%). BRCA1/2 variants were typically maternally inherited (EC, 64.5%; SC, 70.0%).

Conclusion: *BRCA1/2* represents a frequent cause of referral for PGT-M for both EC and SC. Family and IVF cycle characteristics were similar for both EC and SC carriers. Close family history of cancer and/or identified Ashkenazi Jewish ancestry remain common in families pursuing PGT-M for *BRCA1/2*, and AJ founder mutations remain the most frequent PGT-M targets.

1. Vukovic P, Peccatori FA, Massarotti C, Miralles MS, Beketic-Oreskovic L, Lambertini M. Preimplantation genetic testing for carriers of BRCA1/2 pathogenic variants. Vol. 157, Critical Reviews in Oncology/Hematology. Elsevier Ireland Ltd; 2021.

P068 Development and validation of a preimplantation genomic test for monogenic disorders using high-read depth targeted next-generation sequencing (PGTMxNGS)

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Background: Investigation of the inheritance of affected alleles from monogenic disorders in embryos prior to IVF is performed using PGT-M, which assesses single nucleotide variant (SNV) data to determine via haplotyping whether embryos risk passing on the disorder. Previous PGT studies have found NGS-based technologies were more sensitive than microarrays in detecting mosaic and non-mosaic aneuploidy with higher resolution, accuracy, and reliability (1). This study compared contemporary microarray methodologies for PGT-M and investigated whether NGS techniques could confidently resolve challenging sample types, gene targets, and reference relationships.

Methods: Genomic DNA obtained from parental buccal swabs, a familial reference, and amplified trophectoderm biopsies formed the 40 PGT-M cases (100 embryos) used in the method comparison. The data is representative of clinical observations i.e., disorder gene targets and inheritance patterns. Samples were processed in parallel using array-based techniques and high-read depth targeted NGS. SNV data was analysed using bioinformatic techniques to resolve the embryo haplotype status and hence risk of passing on disease.

Results: The mutational status, gene targets, and reference relationships were 100% concordant between the array-based and NGS-based methods. SNV density was also analogous in the methods (~10 SNV/Mb) with a high confidence measure (~.98 out of 1.0). Through the genome ~96% was phased concordantly, where discordant regions may be the result of an advanced phasing algorithm and NGS data improving resolution in recombinant and repetitive regions. These regions were investigated further assessing their content and the cause of discordance.

Conclusions: Finding 100% concordance between the methods suggests PGTMxNGS is practicable. Across platforms, the SNV data was of similar quality, consistency and distribution, showing the high accuracy of PGTMxNGS. Further, where cases provided greater complications (i.e., de novo mutations and - repetitive/recombinant targets) PGTMxNGS provided accurate, highly resolved data. PGTMxNGS represents a streamlined platform capable of efficiently generating and resolving large SNV datasets.

1. Tong J, Niu Y, Wan A, Zhang T. Next-Generation Sequencing (NGS)-Based Preimplantation Genetic Testing for Aneuploidy (PGT-A) of Trophectoderm Biopsy for Recurrent Implantation Failure (RIF) Patients: a Retrospective Study. Reprod Sci [Internet]. 2021 Jul 1 [cited 2022 Aug 30];28(7):1923-9. Available from: https://pubmed.ncbi.nlm.nih.gov/33709375/

P069 Failed thaw cycles (FThC) as a key performance indicator (KPI): Accounting for patient diagnosis and preimplantation genetic testing (PGT)

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Objective: PGT is associated with improved FThC (percentage of thaw procedures that failed to produce any embryos suitable for transfer) and live birth rate (LBR). We examined whether the presence of specific infertility diagnoses (Dx) could further impact the extent of improvement related to PGT on FThC and LBR.

Methods: Real-world data analyses using data from the Society for Assisted Reproductive Technology (SART) United States Registry (2014--2019) were performed, including a descriptive review, focused on diminished ovarian reserve (DOR), endometriosis (E), ovulatory dysfunction (OD), male factor (MF), and unknown factor (UF). These Dx were chosen based on the high prevalence of thaw cycles and/or historical difficulty to treat. Thaw cycles (N=9393) were analysed for first transfer 12 months after retrieval, second transfer, or later transfers. Fisher's Chi Square was used for PGT use over time and FThC stratification. Linear regression was used for LBR trend.

Results: From 2014 to 2019, FThC improved for all Dx, except for OD. PGT use increased significantly for all Dx ($p<0.0001$) with the greatest increases observed in DOR, UF, and MF. Only DOR showed significant difference in the proportion of FThC with PGT (4.37% [2014] to 0.39% [2019]; $p<0.0001$), versus without PGT (1.77% to 1.60%; $p=0.8043$). The average proportion of FThC was highest in patients with DOR (all years: PGT, 0.98%; non-PGT, 1.69%), and lowest in those with UF, followed by E and MF, all with PGT, and significantly decreased over time without PGT ($p<0.0001$). LBRs for all Dx improved over time ($p<0.0001$), with higher LBR observed with versus without PGT. OD showed no significant differences in FThC (regardless of PGT) and LBR.

Conclusions: This is the first study evaluating the impact of specific Dx and PGT on FThC. Infertility Dx and PGT should be taken into consideration when developing and/or evaluating FThC KPIs.

P070 Non-invasive PGT is associated with low accuracy rates, calling into question its clinical utility

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Background: The analysis of embryonic DNA in spent culture media (SCM) has been proposed as a non-invasive strategy for preimplantation genetic testing (niPGT). However, concordance rates when comparing niPGT to standard (invasive) PGT-A have varied greatly between studies, likely due to differences in the degree of embryo manipulation before media collection. We aimed to evaluate the accuracy of a non-invasive PGT protocol, where embryo manipulation is minimised prior to SCM collection.
Methods: Three clinics collected 128 SCM samples from embryos cultured to day-5 or day-6. Embryos were produced using ICSI, had not previously been cryopreserved and underwent minimal manipulation prior to SCM collection. Media samples were subjected to whole genome amplification and next-generation sequencing using PG-Seq Rapid Non-Invasive PGT (Perkin Elmer). Results were compared to those subsequently obtained following PGT-A of trophectoderm biopsies.
Results: Contamination with female (likely cumulus cell) DNA was frequently observed, affecting 23% to 44% of samples depending on the clinic. Higher concentrations of libraries and superior sequencing quality scores were observed in SCM collected on day-6 compared to day-5 ($p=0.011$ and $p=0.002$). Considering only samples without evidence of DNA contamination, 50% of SCM samples had identical karyotypes to corresponding trophectoderm biopsies. 80% of 'abnormal' embryos (as defined by standard PGT-A) received the same classification from SCM analysis (without necessarily having identical aneuploidies), while 62% of PGT-A 'normal' embryos were also euploid according to SCM.
Conclusions: Most reported niPGT methods cannot be considered truly non-invasive, as they include embryo manipulations that increase the likelihood of cell death and DNA release. Our results show that it is challenging to obtain reliable niPGT results without deviating significantly from optimal embryological protocols. Maternal DNA contamination is particularly problematic, and difficult to eliminate even when applying stringent methods. Currently, the accuracy of niPGT seems insufficient to justify routine clinical use.

P071 Is it time to reconsider how we perform fertilisation assessment through the use of genetic pronuclear testing? A case series

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Background: Normal fertilisation is determined by the presence of two pronuclei (2PN) and two polar bodies (2PB). Oocytes with 1PN and 3PN are routinely classified as abnormally fertilised and often discarded because of their potential haploid or triploid genetic status.

Methods: Case report series including three patient treatments where abnormally fertilised oocytes developed into blastocysts. Trophectoderm biopsy was performed on day 5/6 of development for four blastocysts derived from

abnormally fertilised oocytes. Artificial intelligence (AI), copy number variation (CNV), single nucleotide polymorphism (SNP) and parent of origin analysis (PGT-A Complete, Cooper Surgical, USA) were used to perform genetic PN testing. PGT-A Complete indicates parental origin for meiotic whole chromosome aneuploidies and meiotic segmental aneuploidies 10Mb.

Case 1; patient aged 44 (egg provider) and partner aged 38 (sperm provider) undergoing their first IVF cycle, with two previous miscarriages. Two blastocysts derived from abnormally fertilised oocytes (3PN and 1PN) were biopsied.

Case 2; patient aged 39 (egg provider) and partner aged 40 (sperm provider) undergoing their second IVF cycle. One blastocyst derived from an abnormally fertilised oocyte (3PN) was biopsied.

Case 3; patient aged 40 (egg provider) and partner aged 48 (sperm provider) undergoing their fifth ICSI cycle with PGT-SR and PGT-A. One blastocyst derived from an abnormally fertilised (3PN) oocyte was biopsied.

Results: In both IVF cycles the PGT-A Complete results showed that the blastocysts derived from the abnormally fertilised oocytes were biparental diploid with additional aneuploidies. The blastocyst derived from the abnormally fertilised oocyte using ICSI was triploid.

Conclusion: Abnormally fertilised oocytes classed as 1PN/3PN can develop into biparental diploid blastocysts. Although in the cases described here all blastocysts were aneuploid, genetic PN testing could potentially increase the number of embryos available for transfer.

P072 Birth of a healthy child to a mother with fibrodysplasia ossificans progressiva using karyomapping for successful preimplantation genetic diagnosis

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Background: Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant condition that leads to significant disability and morbidity, characterised by the formation of heterotopic hard tissues within connective tissues.(1) The aim of this study is to report the world's first case of a healthy infant born following in vitro fertilisation (IVF) and preimplantation genetic diagnosis (PGD) using karyomapping for FOP.

Material and methods: A 30-year-old female with FOP presented to the Centre for Reproductive and Genetic Health, London with her partner seeking fertility treatment to ensure she achieved pregnancy with an embryo unaffected by FOP. The couple underwent IVF and PGD with Karyomapping. The outcome measure is a live birth of a healthy child, not a carrier of the gain of function mutation on chromosome 2 typical of FOP cases.

Results: A multi-disciplinary team approach was utilised in the planning of this case, considering the additional risks of oocyte retrieval, pregnancy and childbirth in women with FOP. The oocyte retrieval was covered with a 5-day course of prednisolone to reduce the risk of a localised inflammatory reaction which could result in subsequent heterotopic ossification. This was subsequently weaned down with reducing doses every two days. The patient underwent uncomplicated oocyte retrieval, yielding 12 mature oocytes. Following ICSI, ten 2 PN zygotes were cultured, and six underwent trophoectoderm biopsy and vitrification 5-6 days after retrieval. PGD via Karyomapping revealed 4 out of 6 (66.7%) of blastocysts were not carriers of the FOP-allele. In total the patient had three separate embryo transfers. She successfully achieved pregnancy following the third embryo transfer, which went to 37 weeks' gestation, and delivered by Caesarean section. The baby was born in excellent condition and is unaffected by FOP.

Conclusion: IVF/ICSI and PGD using karyomapping can successfully be used to identify embryos carrying the FOP allele.

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P073 Improved clinical outcomes for cycles using preimplantation genetic testing for aneuploidy (PGT-A) may be associated with a change in genetic service provider

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Background: An increasing number of IVF cycles include PGT-A to assist in the selection of euploid embryos for transfer to the uterus. Modern PGT laboratories utilise next generation sequencing (NGS) to predict the copy number of each chromosome. Given this technical convergence, it has often been supposed that the choice of PGT-A provider need only depend on factors such as the quality of the user experience, convenience and price. Here we consider whether the choice of provider might have more profound affects, potentially impacting clinical results.
Methods: A large network of IVF clinics switched from PGT-A provider 'A' to provider 'B'. The final 6 months of clinical

data using A was compared to 6 months of data after the switch to B. No significant changes in any aspect of patient population, treatment or embryological practice occurred between the two time periods evaluated.
Results: Within the time periods considered, 9,091 embryos underwent PGT-A using A and 9,550 using B. The average female age was 39.0 and 39.3, respectively. Differences in important clinical outcomes were observed, which were particularly apparent for female patients <38 years of age. For that group, A versus B results were: 52.5% of embryos classified euploid vs. 57.8% (p<0.0001); 45% ongoing pregnancy after the first embryo transfer vs. 53% (p=0.04); 18% miscarriage rate vs. 11% (p=0.048).
Conclusions: PGT-A and NGS are umbrella terms encompassing different methods with widely varying levels of validation and accuracy. The results of this study suggest that the choice of PGT-A provider has implications for clinical results. One possible interpretation of the data is that higher rates of euploidy and pregnancy seen with B might be a consequence of provider A wrongly classifying some chromosomally normal embryos 'aneuploid', while incorrect labelling of abnormal embryos as 'euploid' could explain the relatively higher miscarriage rates when using A.

P074 Modelling the cost-effectiveness of pre-implantation genetic testing for aneuploidy (PGT-A) in the UK using national registry data - the patient perspective

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Background: Pre-implantation genetic testing for aneuploidy (PGT-A) is available as a treatment add-on for patients undergoing in vitro fertilisation (IVF) despite a lack of randomised control trials demonstrating its effectiveness in improving live birth rates. In the UK, the cost of PGT-A is borne entirely by patients, even in NHS funded cycles. There are no studies investigating the cost-effectiveness of PGT-A in the UK but this information might help patient decision-making and provide more data to inform the ongoing PGT-A debate.

Methods: A decision tree model was developed to estimate the cost-effectiveness of PGT-A versus no PGT-A for frozen embryo transfers from a patient perspective. Data from 48,047 cycles in the 2017-2018 Human Fertility and Embryology Authority (HFEA) anonymised data register were used to calculate the probabilities of births in these two cohorts. Average costs of ten randomly sampled UK fertility clinics were used. Theoretical cohorts of 100 patients were assigned to each treatment arm (PGT-A or no PGT-A) in the model to estimate the cost-effectiveness. The primary outcome was the incremental cost per additional birth per woman. Subgroup analyses (age, male factor infertility, unexplained infertility) and sensitivity analyses were also conducted.

Results: PGT-A led to more births and higher costs when compared to no PGT-A in the overall and subgroup analyses. The overall incremental cost per additional birth per woman with PGT-A was 569. In the subgroup analyses, the incremental costs per additional birth per woman with PGT-A were 156 (aged 18-34), 196 (aged 35-37), 146 (aged 38-39), 555 (aged 40-42), 1,265 (aged 43-44), 4,529 (aged 45-50), 1,511 (male factor infertility) and 347 (unexplained infertility).

Conclusions: This simulated model suggests that PGT-A results in more births but with higher costs. Our data suggest that PGT-A is most cost-effective when aged 38-39 and when the cause of infertility is unexplained.

P075 Genetic laboratories report significantly different aneuploidy rates for identical patient populations, a finding with important implications for clinics using PGT-A

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Background: Our clinic recently began working with a new PGT-A company. After a few months, a review was undertaken to examine whether there had been any change in the proportion of blastocyst stage embryos classified as 'euploid' after PGT-A.
Methods: Our previous PGT-A company issued results for 1,384 blastocyst biopsy specimens over ~15 months. Subsequently, the second company tested 374 biopsy samples in 4 months. Patient populations during the two periods were essentially the same, although the average age of female patients was slightly older in the second period (37.7 years versus 39.0).
Results: The first PGT-A company classified 52.0% of blastocysts aneuploid, 3.5% low-level mosaic, 44.5% euploid. In contrast, the second company reported more embryos euploid (55.6%; P<0.0001). Even if low-level mosaics are considered for transfer, the second company is still associated with a relative increase greater than one-sixth in the number of potentially transferable embryos.
Discussion: It is concerning that aneuploidy rates for the same patient population and clinic can vary depending on the

company used for PGT-A. If the second company's results are correct, it implies that potentially viable euploid embryos may have previously been miscategorised as aneuploid. A larger number of embryos categorised 'euploid' means fewer cycles without a transfer, and more opportunities to combine other embryo selection methods with PGT-A, both of which should lead to improved pregnancy rates. However, if the first company is correct, it would suggest some aneuploidies may be missed by the second company, risking the inadvertent transfer of aneuploid embryos, lower pregnancy rates and a higher incidence of miscarriage. 299 transfers following PGT-A using the first company produced 209 pregnancies (69.9% per transfer). Thus far, only 16 single embryo transfers have taken place after PGT-A with the second company, resulting in 14 pregnancies (87.5% per transfer). More transfers are scheduled.

P076 Does the time of spermatozoa incubation prior to insemination of oocytes affect ART outcomes?

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Background: Capacitation of spermatozoa is temperature and time dependent and incubating spermatozoa at 37°C enables capacitation. A capacitated spermatozoon has a lifespan of 1-4 hours. There is limited research on the incubation period of spermatozoa and the effect on ART outcomes, while professional bodies such as ARCS and ESHRE provide no best practice guidelines. The aim of this study is to determine whether there is an optimal incubation period for spermatozoa prior to inseminating oocytes.

Methods: A retrospective study of IVF/ICSI data between July 2017 and December 2019 (n=636), were categorised into five groups based on the time of incubation prior to insemination (0-59 (n=56), 60-119 (n=103), 120-179 (n=208), 180-239 (n=198) and ≥240 (n=71) minutes). Maternal age and AMH were compared by a Kruskal-Wallis test and a post hoc Mann-Whitney for maternal age. A Kruskal-Wallis test compared the fertilisation and utilisation rates, and a Chi-Squared test with a Bonferroni correction was used for the biochemical (BPR), clinical (CPR) and live birth rates (LBR).

Results: There was no statistically significant difference in AMH between the groups , however the maternal age was statistically different for ICSI patients within the 180-239m group compared to <59m (35.32 vs 32.79, p=.017) and 120-179m (35.32 vs 33.40, p=.020) groups. Less embryos were utilised for ICSI patients when spermatozoa were incubated for 180-239m compared to <59m (39.64 vs 48.69%, p=.048) and 60-119m (39.64 vs 52.93%, p=.003) group. There was no difference between the incubation groups for fertilisation rate, BPR, CPR, and LBR.

Conclusion: Significantly less embryos were utilised for ICSI patients when spermatozoa were incubated for 180-239m when compared to those incubated <59m however this is likely due to maternal age as a confounder. No other differences were observed, suggesting the time spermatozoa are incubated prior to the insemination of oocytes does not need to be specific.

P077 Analytical investigation of the profile of human chorionic gonadotropin in highly purified human menopausal gonadotrophin preparations

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Objective: Highly-purified human menopausal gonadotropin (HP-hMG [Menopur, Ferring Pharmaceuticals, Switzerland]) contains 1:1 follicle-stimulating hormone (FSH) and luteinizing hormone (LH) activity. Its *in-vivo* LH-receptor-mediated bioactivity is attributed to naturally occurring pituitary human chorionic gonadotropin (phCG) in postmenopausal urine adjusted in bioactivity with placental hCG for standardization. Sulfated phCG has a shorter half-life than placental hCG, which allows pulsatile phCG secretion. We analyzed HP-hMG samples for the presence of sulfated glycans, diagnostic for phCG, and for presence/abundance of gonadotropins compared to a reference.

Methods: Two HP-hMG samples were analyzed using reverse-phase ultra-performance liquid chromatography to estimate gonadotropin abundance. The reference was a mixture of r-hFSH/follitropin-alfa (GONAL-f), r-hLH/lutropin alfa [Luveris] and r-hCG (Ovidrel) (Merck KGaA, Darmstadt, Germany) constituted based on their reported activity (in IU) in the HP-hMG product label. Glycopeptide mapping using liquid chromatography-tandem mass spectrometry was applied using high-resolution Orbitrap mass spectrometer to identify N-glycan species-specific signals. Presence of sulfated glycans was assessed to differentiate between pituitary and placental hCG, using a commercial standard containing sulfated moieties as positive control.

Results: FSH, LH and hCG are all present in HP-hMG samples. The ultraviolet signal intensity of uhCG relative to uLH in HP-hMG samples was higher than the UV signal intensity of r-hCG relative to r-hLH in the reference. In addition, signals

related to impurities were detected in HP-HMG, probably associated with non-gonadotropin molecules and not observed in the reference. In HP-hMG samples, sulfated glycans were not detected by glycopeptide mapping, indicating potential absence of phCG and suggesting that placental hCG is likely the main source.

Conclusions: Advanced molecular analysis of HP-hMG showed that the relative presence of hCG compared to LH may be higher than in the reference, and revealed absence of sulfated glycans, suggesting a mainly placental origin of hCG, with possible impact on receptor affinity/activation and clinical effect.

P078 Fresh versus cryopreserved microscopic epididymal sperm aspiration (MESA) in cases of obstructive azoospermia (OA) undergoing intracytoplasmic sperm injection (ICSI): Retrospective study

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Introduction: Azoospermia is the absence of spermatozoa in the ejaculate. Obstructive azoospermia (OA) is the consequence of an obstruction or bilateral absent vas deferens. Microscopic epididymal sperm aspiration (MESA) can treat infertile male patients with OA to obtain sperm sufficient for the Intracytoplasmic sperm injection (ICSI) procedure.

Aim of the study: To compare the effect of using fresh and cryopreserved epididymal sperms obtained by MESA in cases of OA on live birth rate (primary outcome), fertilisation and clinical pregnancy rates (secondary outcomes).

Study design: This is a retrospective study of 122 men with normal spermatogenesis who were diagnosed with obstructive azoospermia and underwent ICSI between 2016 and 2020. The first group included 90 ICSI procedures using fresh MSA, while the second group included 32 ICSI procedures using frozen MESA. MESA procedures were undertaken at 25x magnification micro-puncture. Primary and secondary outcomes were compared in both groups.

Results: The average age of men is 34.87 ± 8.73. In both groups of the 90 Fresh MESA and 32 cryopreserved MESA ICSI cycles, the live birth rates per transfer were (35/90) 38.9% and (11/32) 34.4%, respectively. The fertilisation rates were 70% and 61%, respectively. The clinical pregnancy rates were (42/90) 46.7% and (14/32) 43.8%, respectively.

Conclusion: This study showed that using fresh MESA in cases of OA gives high fertilisation, clinical pregnancy and live birth rates compared with using cryopreserved MESA before the ICSI procedure.

Limitations and reasons for caution: 1) Larger (n) number of patients needed for stronger statistical evidence. 2) This is a retrospective study. 3) Results could be affected by other cofactors such as female age and ovarian reserve.

Keywords: MESA; ICSI; assisted reproduction; infertility; obstructive azoospermia (OA).

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P079 Transferable embryo to oocyte ratio in IVF/ICSI correlates positively with livebirth rate but negatively with oocyte number: Analysis of 14,156 fresh cycles

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Background: Static fresh-cycle live birth rate (LBR) with high oocyte yield, other than impaired endometrial receptivity, could be due to a relative shortfall of competent oocytes. Objective of this study was to investigate the correlation of the proportion of oocytes that produce transferable embryos with the oocyte yield as well as LBR.

Methods: Retrospective analysis of a national database published by HFEA, UK. Population included couples at their first IVF treatment with single (or had no available) embryo for transfer, due to tubal or unexplained infertility among women aged <40 years.

Results: 14 156 fresh IVF/ ICSI cycles met the inclusion criteria. The number oocytes that produce a transferable embryo had a moderate correlation (r= 0.569972, p <0.0001), however, the oocytes that did not produce a transferable embryo had a much stronger positive correlation with the oocyte yield (r= 0.916676, p <0.0001). Consequently, there was an inverse correlated between the transferable embryo to oocyte ratio (TEOR) (r = - 0.331431, p <0.0001) and the oocyte yield. Embryo utilisation rate (EUR) (r= -0.250047, p <0.0001) also had an inverse relation. Both TEOR (P< 0.0001) and EUR (p= 0.01) correlated positively with LBR.

Both TEOR and EUR declined steadily with the increasing number of oocytes until it reached a nadir, at a cohort of 8-9 oocytes when the LBR in a fresh cycle reached its peak. The TEOR, EUR and LBR- all plateaued with higher yield.

Conclusions: The transferable embryo to oocyte ratio and embryo utilization rate correlate negatively with the number of oocytes collected but positively with LBRs. The LBRs in fresh IVF/ICSI cycles plateau at a cohort of oocytes when TEOR and EUR reaches a nadir. The focus needs to be on generating more competent oocytes and finding more efficient embryo selection method to improve fresh.

P080 Investigating the effectiveness of standard operating procedures in minimising volatile organic compounds in an IVF laboratory

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Background: Volatile organic compounds (VOCs) are carbon-containing air pollutants (1). In an in vitro fertilisation (IVF) laboratory, they are present in, for example, plastic-ware packages (2). VOCs above 400 parts per billion can damage embryonic development (2). Few clinics routinely measure VOCs, instead, they adopt standard operating procedures (SOPs) to improve air quality. Whether these minimise VOCs remains unexplored. The study aimed to investigate whether the SOPs implemented in an IVF laboratory are effective in minimising VOCs. Such SOPs include perfume-free zone, using Oosafe (SparMed, Denmark) for cleaning, restricting personnel entry and opening plasticware packaging to off-gas before use.

Methods: A photo-ionization detector with VOC probe was used to measure the total volatile organic compounds (TVOCs) during the different procedures and rooms within the laboratory (Graywolf DirectSense, Ireland). TVOCs were also measured throughout the week in the embryology laboratory. TVOCs before and after a semen sample production in the men's room were measured. Calibration was done weekly with isobutylene span gas.

Results: TVOCs were measured for 47 days. The different procedures did not differ in TVOCs ($P>0.05$). No difference in TVOCs was found throughout the week in the embryology laboratory, however, a trend suggested that TVOCs were higher on Mondays. Embryology and micromanipulation laboratories emitted less TVOCs compared to the office ($P=0.001$ and $P=0.038$ respectively). Footfall affects TVOCs as a difference was found when two embryologists were present versus one and four ($P=0.003$ and $P=0.04$ respectively). In the men's room, there was an association between high TVOCs concentration and the presence of aftershave ($P=0.011$). Oosafe emitted less TVOCs compared to ethanol ($P<0.0001$).

Conclusion: The laboratory's SOPs are effective in minimising VOCs. No change in practice is required. As this is a pilot study, we encourage IVF clinics to measure their VOCs whilst completing embryology duties to determine whether their laboratory maintains a safe level

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P081 In-vitro fertilisation in a 55-year-old woman resulting in severe life-threatening pre-eclampsia and acute fatty liver disease of pregnancy; ethical reflection and learning points

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Background: The age at which women are choosing to have children continues to rise worldwide. The number of in-vitro fertilisation (IVF) cycles per year in the UK has increased more than ten-fold over the past three decades, and the percentage of cycles in women over 40 has doubled[1]. Adverse pregnancy outcomes associated with advanced maternal age include miscarriage, preterm birth, foetal growth restriction, stillbirth, hypertensive disorders, diabetes, and Caesarean delivery[2]. In the UK, there is an upper age limit of 42 for IVF on the NHS[3], but there is no legal age limit in the private sector.

Case summary: A 55-year-old primigravida presented at 33 weeks' gestation following IVF in Sri Lanka. She described a five-day history of constipation, nausea and vomiting, and feeling generally unwell. Her blood pressure was 170/122mmHg and urine protein-creatinine ratio 95mg/mmol. She was diagnosed with severe pre-eclampsia, complicated by renal and hepatic dysfunction (creatinine 221mol/L, ALT 157U/L, AST 169U/L, bilirubin 69mol/L), and suspected foetal compromise as computerised CTG did not meet criteria with short-term variation <4.0ms. She was treated with antihypertensives and intravenous magnesium sulphate, and delivered by emergency Caesarean section. The neonate required admission to the neonatal unit for four weeks. The woman developed renal failure and hepatic encephalopathy secondary to acute fatty liver disease of pregnancy necessitating intubation and subsequent

tracheostomy, renal replacement therapy, and a five-month hospital admission with four weeks in a liver intensive care unit.

Discussion: This case highlights the ongoing need to examine the ethical considerations and legal and regulatory standards for provision of assisted reproduction treatment (ART) in very advanced maternal age (45 years or older) globally. This case exemplifies that ART in women over the age of 50 requires due deliberation of the significant risk of adverse pregnancy outcomes, associated healthcare costs, and consideration of the welfare of the unborn offspring.

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P082 Benefit of endometrial receptivity array in a highly selected patient population

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Background: Endometrial receptivity array (ERA) is used to optimise the timing of embryo transfer in frozen embryo transfer (FET) cycles. 25% of women with recurrent implantation failure (RIF) may have a displaced implantation window (1, 2). However, the clinical applicability of the ERA is debated (3,4,5).

Since 2020, our clinic has recommended ERA testing in patients who have had ≥2 consecutive failed FET cycles with good or top quality embryos. An initial audit indicated improved pregnancy outcomes following transfer time adjustment based on the ERA results.

The aim of this study was to compare FET pregnancy outcomes of our ERA cohort with those of a matched cohort who had not had an ERA test.

Methods: Retrospective audit of all women who had an ERA test performed, between 2020 and 2022. Pregnancy outcomes on the subsequent cycle were compared with those of a matched group who had also had two or more consecutive failed FET cycles without ERA testing.

Results: Study groups comprised 19 patients with ERA and 20 controls. Livebirth rates in the ERA group were markedly higher than in the non-ERA group [53% (n=10) vs 25% (n=5)]. Unsuccessful outcomes in the ERA group vs the non-ERA group were as follows: biochemical miscarriage 5% (1) vs 40% (8), negative pregnancy test 32% (6) vs 30% (6), and miscarriage 10% (2) vs 5% (1). There was no difference in age between cohorts but more of the control group had a previous livebirth (16/20 vs 7/19).

Conclusions: We have shown improved outcomes following ERA testing in a group of patients with RIF. Limitations here include the small cohort, the retrospective approach and the lack of PGT embryo testing. After 2+ failed FET cycles with good embryos, most couples seek answers and, in our hands, performance of an ERA seems to be of benefit.

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P083 Donor vs partner sperm: How the sperm source affects IVF pregnancy rates

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Background: There is an 8% year-to-year increase of IVF cycles using donor sperm (1) and a growing number of same sex and single females going through fertility treatment (2). Advising patients on general pregnancy rates without considering the impact that donor sperm makes might therefore no longer be accurate.
Objectives: To compare pregnancy rates using donor and partner sperm in IVF and ICSI treatments carried out with the patients' oocytes.
Methods: A retrospective study of IVF/ICSI data from April 2021-March 2022. Pregnancy rates were compared between patients using partner and donor sperm. Data was categorized into age groups of <35, 35-37, 38-39, 40-42 and >42. Comparisons were made using the Fisher's Exact Test.
Results: The use of donor sperm increased biochemical pregnancy rates per cycle in the <35, 38-39 and 40-42 age groups by 19% (p=0.0240), 39% (p=0.0016) and 11% (p=0.4447) respectively. Clinical pregnancy rates increased in in all groups except >42 (by 8% (p=0.3954) for <35, 1% (p>0.9999) for 35-37, 31% (p=0.0107) for 38-39 and 10% (p=0.4295) for 40-42).
Conclusions: The use of donor sperm increased pregnancy rates for most age groups but the difference was only statistically significant for biochemical pregnancy in the <35 age group and both biochemical and clinical pregnancy in the 38-39 age group. Further analysis is required to ensure this is a consistent trend, whether the differences are similar on live birth rates and if there is any more variation between specific patient groups. Personalizing fertility treatment to the specific patient's needs is common practice and with more information about outcomes in different patient groups, the way they are informed about the potential outcomes could be personalized as well.

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P084 The impact of BMI on fertility in an otherwise healthy population: A systematic review and meta-analysis

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Obesity is a growing problem globally. In 2016 more than 1.9 billion adults were overweight, and over 650 million were obese. Studies have demonstrated an association between excess female weight, decreased fecundability and adverse pregnancy outcomes. With increasing rates of obesity recorded and the NHS currently spending approximately £68 million per year on in vitro fertilisation treatments, it is necessary to explore and address modifiable fertility risk factors and fully understand the effects of excess weight on fertility when no other co-morbidities are present to be addressed. This study aims, for the first time, to consolidate published data on the association between increased BMI and fertility in patients with no other diagnosed medical co-morbidities. Methods Systematic review was conducted following PRISMA guidelines and prospectively registered with PROSPERO (CRD42022293631). Cochrane CENTRAL library, EMBASE and Medline were searched for eligible studies. All primary studies reporting the effects of raised BMI on fertility in women with no other medical co-morbidities were selected. Modified Newcastle Ottawa scale was used to assess risk of bias. Results This systematic search identified nine eligible studies; only one explored natural conception, leaving eight ART studies. A total of 4,108 cycles from 3,770 women were included in the meta-analyses. Women with a BMI ≥ 25 kg/m2 are significantly less likely to have a clinical pregnancy when compared to women with a healthy BMI (OR:0.76, p=0.007). In addition, women with a raised BMI required a longer duration of stimulation (Standard Mean difference= 0.20, 95% CIs 0.07-0.34, p=0.002) and had reduced oocyte harvest (Standard Mean Difference= -0.11, 95% CIs -0.18- -0.04, p=0.002). Conclusions Being overweight/ obese has significant adverse effects on conceiving through ART even when no other co-morbidities are present. Women need to be made aware of the facts, counselled appropriately and assisted with optimisation of their weight to ensure best pregnancy outcomes.

P085 Establishing the sex-specific impact of assisted reproductive technologies on offspring health

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Background: Assisted Reproductive Technologies (ART) are widely used as successful infertility treatments. There is still debate over potential long-term impacts on the health and wellbeing of offspring conceived using ART and whether these have sex differential effects. Therefore, we conducted a scoping review of the existing human and animal models literature on the effects of ART on offspring.

Method: We searched three databases (Embase, PubMed, and Medline) for studies published between 2016 to 2021 to answer the question: Does ART affect embryo quality or offspring sex ratio compared with natural conception among various species? Therefore, we included ART studies that reported a comparison between male and female offspring. We excluded studies using donation gamete, non-standard ART practices (e.g.nuclear transfer), or those focused on medication, mental health, biopsy effects, or external environmental factors (e.g. pollution).

Results: We identified a total of 56 studies, 40 in humans and 16 in animals (cattle, mice, dogs, and horses). Of these studies, three observed male offspring were negatively affected by ART, while 6 found females more sensitive than males. However, 13 studies found a negative impact on both genders, while 6 reported no sex-specific effect. Additionally, we observed 37 studies that included sex ratio in their results, 29 human and 8 animal studies, with 17 studies observing an increase in sex ratio from male to female while 15 studies found no difference in sex ratio.

Conclusion: Our results indicate that offspring respond to routine ART practices in a sex-specific manner. Although the effects are still inconclusive, female offspring seem to be more vulnerable. Animals can be adequate models to study ART differential effects due to the large number of cycles carried out regularly.

P086 Assisted reproduction requirements following trachelectomy for stage 1B cervical cancer - a 10 year single centre retrospective study

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Introduction/Background: - Radical trachelectomy is a fertility preserving operation for women with stage 1B cervical cancer. -Pregnancy following radical trachelectomy for cervical cancer is feasible however patients often have more difficulty in becoming pregnant. -A retrospective cohort study was carried out to investigate assisted reproduction requirements in these women following treatment for their cervical cancer **Methodology:** -10 years data of patients who had radical trachelectomy was collected retrospectively between 2012 and 2022- total 20 women. -Data was collected from their electronic care record (ECR) and Northern Ireland Regional Maternity System (NIMATS) -Data collected included - age, BMI, previous pregnancy, future pregnancy, spontaneous or assisted conception, live birth rate, pregnancy complications. -Limitations -- early miscarriages not recorded, short follow up window for some patients not giving time to allow for fertility follow up.

Outcomes: Total number = 20 Age range 25-40 years (Mean 32.5 years). BMI range 18-40 (Mean 26) 90% (n=18) patients were primiparous prior to trachelectomy 50% (n=10) patients were referred to the regional fertility centre for assisted reproduction techniques 10% (n=2) patients required cervical dilation for cervical stenosis. 0% (n=0) of these patients have had a successful pregnancy. 35% (n=7) had at least one IVF treatment. 10% (n=2) are awaiting assessment. 20% (n=4) patients had future pregnancies. Of these : 100% (n=4) required IVF. 25% (n=1) had a miscarriage. 25% (n=1) had an ectopic pregnancy. 25% (n=1) had a term pregnancy delivered by caesarean section. 25% (n=1) was complicated by severe OHSS requiring hospital admission. This pregnancy is ongoing.

Conclusion: 100% of the pregnancies following trachelectomy required assisted reproduction, highlighting the difficulty in becoming pregnant following this surgery. Limiting factors of the study included short follow up time for some patients, as well as early pregnancy records not being accessible. This is a small study however larger numbers could enable more thorough assessment of outcomes.

P087 An umbrella review of meta-analyses regarding the incidence of female-specific cancers following fertility treatment

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Background: Since 1991, 1.3 million IVF cycles have been performed(1). The association between fertility treatment (FT) and female-specific cancers remains contentious, largely due to the conflicting outcomes published in various meta-analyses. We aimed to evaluate the validity of the association between FT and the risk of female-specific cancers (ovarian, breast, endometrial and cervical).

Methods: A thorough literature search (Cochrane Database of Systematic Review, EMBASE, Google Scholar and Pubmed) was done to identify relevant systematic reviews and meta-analyses up until April 2022. The inclusion criteria included original studies stating the incidence of cancer in FT and control groups (non-FT). The principal outcome of interest was the incidence of cancer (breast, endometrial, cervical, and ovarian) in FT and non-FT groups. The effect estimates, Hazard ratios and Odds Ratios (OR) were extracted or calculated de novo. The strength of evidence and extent of potential biases were summarised.

Results: In total, 3129 publications were identified, and 11 meta-analytical reviews were included. This umbrella review synthesised 324 meta-analyses, including data from over 20 million patients. The incidence of ovarian cancer (OR 1.21;95%CI 1.00-1.45) and borderline ovarian tumours (BOTs) (OR 1.87;95%CI 1.18-2.97) was higher in the FT compared to non-FT group. The meta-analyses related to ovarian cancer was statistically significant (p<0.05), using a random-effects model, but this association was not supported by highly suggestive evidence. There was no significant association demonstrated between FT and the incidence of cervical, breast or endometrial cancer.

Conclusions: This umbrella review has synthesised data from over 20 million patients, to evaluate the association between FT and female-specific cancers. We have demonstrated that FT exposure does significantly increase the risk of ovarian cancer and BOTs, albeit a weak association. Understanding the potential long-term sequelae of FT is fundamental to guide counselling and enable individuals to make informed decisions about their reproductive future.

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P088 Warm, biopsy and re-vitrification (WBR) cycles: Blastocyst suitability for biopsy based on the stage and quality prior to vitrification and clinical outcomes

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Background: Patients that have blastocysts in storage may consider pre-implantation genetic testing for aneuploidy (PGT-A) before planning a frozen embryo transfer. However, as the embryo quality has to meet a certain threshold for biopsy, not all the blastocysts may be suitable for biopsy post-warm.

Methods: Retrospective study of WBR cycles performed at a single clinic between January 2021 and May 2022. Blastocysts were scored according to a modified Gardner's grading system. Blastocysts graded A or B for either the inner cell mass (ICM) or the trophectoderm (T) were considered good quality, while those graded between B and C (i.e., B-) for either ICM or T were considered borderline quality. Blastocysts were warmed in the late afternoon, laser-assisted hatched and cultured overnight. Suitability for biopsy was assessed the next morning. Statistical analysis was performed by Chi-square test.

Results: A total of 197 blastocysts were warmed from 41 patients, of which 152 were suitable for biopsy (77%). Suitability for biopsy was higher for day 5 than for day 6 blastocysts (133/163, 81% vs 19/34, 56%, p=0.002). Out of the 179 blastocysts graded as good quality prior to vitrification, 38 were not suitable for biopsy; out of the 18 blastocysts graded as borderline quality, six were not suitable for biopsy (21% vs 33%, p=0.4). 38 patients had at least one embryo suitable for treatment (euploid/mosaic). To date 31 euploid blastocysts have been warmed, 30 survived (97%) and 15 resulted in a clinical pregnancy (50%). Clinical pregnancy rates are comparable to fresh PGT-A cycles performed during the same period (87/168, 52%, p=0.9).

Conclusions: WBR may be a viable option for patients with blastocysts vitrified on day 5. Blastocysts grade did not seem to impact the suitability for biopsy. More data is required to confirm these findings.

P089 Effectiveness of two consecutive cycles of single blastocyst transfer compared with one cycle of double blastocyst transfer in advanced maternal age

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Background: Women with advanced maternal age are considered poorer prognosis patients with lower live birth rates compared to their younger counterparts. They are more likely to have a double embryo transfer with the associated risk of multiple pregnancy. We aimed to compare - Live birth (LB) and multiple pregnancy (MP) rates following double versus single blastocyst transfer - Cumulative LB and MP rates following two consecutive single blastocyst transfers (SBT) versus one cycle of double blastocyst transfer (DBT)

Methods: Retrospective analysis of 511 IVF/ICSI cycles between January 2010 and 2020. Data was collected on women aged 40 with elective SBT (Group 1, n=81) or DBT (Group 2, n=430), excluding those with >3 previous IVF attempts.

Results: Women in group 1 were marginally younger (40.2±0.6 v 40.8±1.2, p<0.005), had more eggs collected (13.6±7.3 v 11.3±5.5, p=0.009) and more blastocysts frozen (3.4±2.6 v 1.1±1.7, p<0005). Controlling for maternal age, number of previous IVF cycles and blastocyst quality, Group 1 had a lower likelihood of LB (aOR 0.550, 95%CI 0.306-0.988) and MP (0% v 24%, p=0.024) in the fresh cycle. Importantly, for those undergoing their first IVF cycle (n=359), there was no

difference in LB (aOR 0.617, 95%CI 0.329-1.156) after controlling for age and blastocyst quality. Twenty-five women from Group 1 underwent a subsequent SBT frozen cycle. The additional frozen cycle resulted in a cumulative pregnancy rate of 28% for Group 1. This was comparable to Group 2 (28% v 28%, p= 0.997) but importantly with a lower MP rate (0% v 24%, p= 0.009).

Conclusion: Women should be advised that double blastocyst transfer results in a higher multiple pregnancy rate but with no difference in overall live birth rate when compared to those undergoing two consecutive SBT cycles. Single embryo transfer should therefore be encouraged where a blastocyst is available.

P090 Catch before they hatch! Recommendation to improve survival and implantation of vitrified blastocysts

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Purpose/background/objectives: During routine key performance indicator (KPI) analysis, embryo survival rate is assessed. Data from January to March 2022 indicated a reduced survival rate for frozen embryos following warming (94%, target 98%).

Methods: Data from January 2021 to March 2022 were analysed, from six sister clinics (Nottingham/Birmingham/Bath/Tunbridge Wells/London/Woking) following the same laboratory protocol for vitrification and warming, to investigate a possible cause for embryo survival reduction in one laboratory. Clinical outcome data were compared for 2623 non-hatching blastocysts (NHB), 834 hatching blastocysts (HB) and 338 induced hatching blastocysts (IHB), due to laser-facilitated breaching for PGT. The total number warmed and transferred embryos was used to calculate the percentage survival rate.

Results: A higher proportion of blastocysts failed to survive warming (6%) in the HB group, compared with NHB and IHB (4% and 2%, respectively). There were 35 cycles where a further HB embryo was thawed due to no survival of the first thawed HB. Implantation rates per transferred blastocyst were 36%, 48% and 50% for NHB, HB and IHB, respectively.

Conclusions: Non-PGT blastocysts, should ideally be vitrified on the morning of day 5, and prior to initiation of hatching to increase their chance of survival. Where fresh embryo transfer is planned, best practice is to select the embryo for transfer on the morning of day 5, and to proceed with vitrification of the remaining suitable early stage to expanded blastocysts as early on day 5 as possible. Limitations and further studies Several confounders may impact these findings such as patient or practitioner variables. Further studies are required to further optimise practice and understand the difference between IHB and HB, which is causing a decreased warming survival rate - fragility due to increased blastocoel and thin zona pellucida.

P091 Investigating the use of zygote number to identify good prognosis patients for blastocyst transfer

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Background: Good prognosis patients benefit from blastocyst culture for embryo transfer, whereas the benefits for an unselected population are less clear (1). Our clinic favours a policy to identify good prognosis patients for day 5 transfer over blastocyst culture for all patients. Patients aged 38 are booked for a preliminary day 3 appointment which is rescheduled to day 5 if they have 2 good quality embryos on day 3. However, this results in uncertainty for clinicians and patients, and an increased workload. We investigated if there is an optimum number of zygotes which could act as a cut-off to identify patients aged 38 who could be booked for a blastocyst transfer at fertilisation check.

Methods: Logistic regression and ROC curve analysis were carried out on retrospective data from patients 38 years undergoing fresh IVF/ICSI treatment cycles between January and December 2019. Blastocyst transfers were carried out for patients with 2 good quality embryos on day 3. Data from patients 37 years, for which a zygote policy is already in place, was used to define an acceptable percentage of false positives.

Results: Results revealed a positive correlation between zygote number and day of transfer. ROC curve analysis and area under the curve showed it is acceptable to predict day 3 or 5 transfer based on zygote number. False positives resulting in transfer appointments being rescheduled from day 5 to 3, with very little notice, are the least desirable outcome. If 6 zygotes is selected as a cut-off, the rate of false positives is acceptable.

Conclusion: Results support use of zygote number to identify patients, aged 38, in our population who are likely to meet the blastocyst criteria. Embryologists can book patients for day 5 transfer, with no preliminary day 3 appointment, which should reduce disruption for both patients and staff.

1. Kolibianakis EM, Zikopoulos K, Verpoest W, Camus M, Joris H, Van Steirteghem AC, et al. Should we advise patients undergoing IVF to start a cycle leading to a day 3 or a day 5 transfer? Human Reproduction. 2004;19(11):2550-4.

P092 A single-unit approach to tackle the new storage regulations implemented July 2022

Morven Dean; Rachel Murphy; Sarah Martins Da Silva; George Hughes

NHS Tayside

Introduction: On the 1st of July 2022, legislation changed to allow the storage limits of gametes and embryos belonging to patients to be extended up to 55 years. In response, the HFEA introduced a 2-year transition period to offer patients who currently have gametes and embryos stored under the old legislation, the opportunity to extend beyond 10 years. Whilst the uptake of patients who decide to opt for extended storage is currently unknown, the anticipated increased storage capacity, patient contact time, counselling, and financial implications associated with these changes, creates concern for clinics across the UK. This study details the methodological approach one centre has created to ensure an efficient transition for patients with gametes or embryos currently in storage.

Methods and results: Phase 1 involved accumulating patient details with gametes and embryos in storage and recording their eligibility for NHS funded storage. Of the 1375 patients with embryos stored, 716 patients with sperm and 43 patients with oocytes, approximately 17%, 70% and 88% of patients in each cohort were eligible for NHS funded storage, respectively. Each cohort was subsequently categorised into batches (1-10) according to duration of storage.

Phase 2 involved notifying patients, beginning with those approaching storage expiry and sequentially working through each batch. Using IDEAs software, letters were generated to be sent in alignment with specified timelines, ensuring all patients are notified before May 2024, allowing appropriate response time. Plans for phase 3 involve ensuring patient requests are implemented in a scheduled, systematic manner.

Conclusion: The transition to new storage regulations has presented a complex challenge for fertility centres within the UK. Here, we present a systematic approach that one centre has adopted to address these changes within the allocated transition period. It is of interest to explore how other centres are responding to accommodate these changes.

P093 Review of HFEA reported live birth rates across UK centres

NOTE: still awaiting confirmation of results and will submit before publication or presentation.

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Introduction: The demand for ART continues to rise globally and with this, advancements in technology and practice is developing exponentially. Centres require HFEA licensing to provide ART in the UK, which offers an opportunity to access national and individual success rates. This study explored the inter-centre variability of success rates with fresh and frozen embryo transfers, as reported by the HFEA.

Methods: An anonymised list of success rates for live birth per embryo transferred between 2016-2019 was retrieved for each licensed centre in the UK. Descriptive and comparative statistics were performed and the variability in success rates was assessed using GraphPad prism.

Results: Success rates from 80 centres were isolated and reviewed. The mean live birth rate per embryo transfer was 27% for combined fresh and frozen cycles (N=78), 26.1% for isolated fresh cycles (N=80), and 29% for isolated frozen samples (N=78). The highest performing centres achieved success rates of 34.66%, 36.22% and 39.06%, respectively. The lowest performing centres achieved success rates of 14.21%, 14.17% and 13.33%, respectively. There was no significant difference identified between success rates of fresh and frozen embryo transfer cycles ($p>0.05$).

Conclusion: The extensive inter-centre variability of success rates creates concern in the rapidly evolving field of ART in the UK. Factors including funding availability, patient demographic, geographic region, variation in practices and use of add-ons could be contributing to these results findings. It is of great interest to improve success rates in all centres and standardise treatment for patient across the UK.

P094 In-depth glycosylation profiling of r-hFSH-alfa and r-hFSH-delta in comparison with human pituitary follicle stimulating hormone (p-hFSH)

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Objective: The glycosylation profile of recombinant human FSH (r-hFSH) is an important attribute of this molecule, defining its net biological effect, safety and efficacy. The aim of the current study was to compare the glycosylation profiles of r-hFSH-alfa (GONAL-f, Merck KGaA) and r-hFSH-delta (Rekovelle, Ferring Pharmaceuticals) with endogenous pituitary-derived human FSH (p-hFSH).

Materials and methods: The glycosylation profiles of r-hFSH-alfa, r-hFSH-delta and p-hFSH were characterized and compared using a highly efficient glycopeptide mapping liquid chromatography--mass spectrometry-based method. This method utilized a very low sample volume and was developed and used alongside a robust screening workflow for data processing with a database of more than 200 N-glycan combinations, allowing for site-specific profiling of N-glycans. The distribution of sialylation, antennarity and fucosylation were obtained for each of the four N-glycosylation sites (Asn52, Asn78, Asn7 and Asn24), as well as information on sialic acid linkage (2,3 vs. 2,6).

Results: Overall, the three preparations had distinct glycosylation profiles, and substantial differences were observed in the site-specific distribution of N-glycans, in terms of sialylation, fucosylation and antennarity, across all N-glycosylation sites. As expected, both the N-acetylglucosamine (GluNAc)--N-Acetylgalactosamine (GalNAc)--N-Acetylneuraminic acid (NANA) arm and the 2,6 NANA linkage were absent in r-hFSH-alfa, but were present in r-hFSH-delta and p-hFSH. However, compared with the beta subunit of p-hFSH, the beta subunit of r-hFSH-delta had substantially lower 2,6 NANA linkage distribution and a shift towards higher tri- and tetra-sialylated glycans.

Conclusions: Glycosylation profiles, antennarity, sialylation and fucosylation levels were different among p-hFSH, r-hFSH-alfa and r-hFSH-delta, with differences observed at N-glycosylation sites on both the alpha subunit (directly involved in receptor interaction and activation) and the beta subunit (important for circulatory half-life). Despite being produced in a human cell line, r-hFSH-delta showed lower 2,6 NANA linkage distribution in the beta subunit than p-hFSH.

P095 Abnormal HSG - what happens next? Fertility outcomes following abnormal HSG findings - A retrospective single unit cohort study over a 1 year period 2016-2017

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Introduction: -Hysterosalpingogram is a stalwart method of investigating infertility in females. It assists assessment of anatomy of the uterus and patency of fallopian tubes. -We collected data from HSGs performed in a district general hospital to investigate the rates of successful imaging and abnormal outcomes. We used this data to discover future fertility outcomes following an abnormal findings.

Methods: - 1 year of patient data was collected from a single units Radiology department records. A retrospective list of all women who had an HSG from April 2016 to April 2017 amounted to 124 women - Data was collected from their electronic care record (ECR) and Northern Ireland Regional Maternity System (NIMATS) - Data collected-- age, BMI, previous obstetric history, adequate images, findings, subsequent treatments, pregnancies and spontaneous or assisted conception.

Results: N=124 * Age range : 21 -- 45 (mean 31.1) * BMI range : 17 -- 55 (mean 26) * 63% (n=79) were primiparous * 37% (n=46) were parous * 90% (n=112) achieved an adequate radiological view. * Of the adequate views, o 72% (n=81) normal. o 27% (n=30) abnormal. * Of the 30 that where abnormal o 23% (n=7) bilateral tubal blockage o 43% (n=13) unilateral tubal blockage only o 13% (n=4) previous salpingectomy with normal contralateral tube o 17% (n=5) Mullerian duct abnormalities (eg. Septate/bicornate uterus) o 3% (n=1) an intramural fibroid * Of the 30 women who had abnormal HSG findings o 83% (n=25) referred on for further fertility treatment o 43% (n=13) had a future pregnancy o 40% (n=12) had a live birth o 23% (n=7) were spontaneous conception o 23% (n=7) required reproductive assistance ♣ 43% (n=3) IVF ♣ 29% (n=2) Clomid ♣ 14% (n=1) ICSI

Conclusion: HSG is a useful first line investigation for female infertility. This study shows that a significant number of women have abnormalities detected that can help guide their future treatment and management.

P096 Ultrasonography for the assessment of fallopian tube patency: A comparison between HyFoSy and HyCoSy

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Background: Assessment of fallopian tube patency is key in the subfertility diagnostic work-up and is becoming a more and more necessary procedure. Despite that, the available gold standard, hysterosalpingogram is old, necessitates the involvement of large diagnostic centers and radiologists and entails radiation exposure. For these reasons, ultrasonographic alternatives have been sought, so that they can be carried out by a trained clinician in an outpatient

clinic, without the need for radiation exposure. In this systematic review, these methods, namely HyCoSy and the newer HyFoSy, will be compared on their diagnostic efficacy in tubal patency assessment.

Methods: Studies were sought systematically using the PICOS format in the databases Scopus, PubMed and Web of Science, with stringent inclusion criteria enforced according to the PRISMA 2020 guidelines. Resulting eligible studies were further evaluated for risk of bias using the QUADAS-2 tool.

Results: Data on HyFoSy, especially comparative data, was very limited, with only 6 available studies being ultimately included. However, a consistent trend among these studies is that HyFoSy is a very promising diagnostic modality and according to some studies is superior to HyCoSy, with some noting its remarkable positive predictive value.

Conclusions: Ultrasonographic assessment of tubal patency is on the rise, as it offers considerable advantages. From the limited data that is available for now, HyFoSy is a very promising diagnostic test, which could be a valuable asset in subfertility assessment, however further research is required to confirm these preliminary findings.

P097 In-vivo pressure build-up within the reproductive tract during hysterosalpingography: A pilot feasibility study

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Background: Meta-analyses have shown enhanced fertility after hysterosalpingography (HSG) with oil-based contrast compared to water-based contrast (Wang, 2020). It is hypothesized that infusion of contrast media may lead to a hydraulic pressure build-up proximal to a "debris plug", dislodging it and improving cilia function. Currently, data on pressure build-up patterns during HSGs are lacking. The primary objective of this study was to determine the feasibility of recording the pressure build-up within the reproductive tract during HSG.

Methods: A prospective, single-center study in 10 subfertile women. HSGs were performed under oral pain medication (paracetamol 1000mg and Naproxen 500mg). The oil-based contrast (Lipiodol Ultra Fluid, Guerbet) was aspirated into a disposable fluid dispensing syringe with an integral pressure transducer (DiamondTOUCHT, Merit Medical) and infused under fluoroscopic guidance. Pain scores were evaluated on the Visual Analogue Scale (VAS, 0-10 cm, no pain-unbearable pain).

Results: The minimum in-vivo pressure was 0.3bar during all HSGs. The maximum pressure was <1bar in 10%, 1-2bar in 70%, and >2bar in 20%, with an average of 1.71±0.58bar. In four women the HSG showed non-fully patent tubes (maximum pressure 1.6, 1.8, 1.8 and 2.3bar). In one of these women the HSG was halted, because intravasation occurred (maximum pressure 1.8bar). The highest maximum pressure (2.9bar) was reported during an HSG with bilateral patent Fallopian tubes, however, this woman reported the highest pain score (VAS 10). For all HSGs a pressure behavior graph was created.

Conclusions: This study shows that recording the pressure build-up within the reproductive tract is feasible during HSG. We advise additional studies on the pressure build-up pattern in relation to pain scores and fertility outcomes. Furthermore, studies on the characteristics of the different oil-based and water-based contrast media are required to define which contrast properties are related to effective dislodgement of debris from the Fallopian tube.

Wang R, Watson A, Johnson N, Cheung K, Fitzgerald C, Mol BWJ, et al. Tubal fushing for subfertility. Cochrane Database Syst Rev. 2020; 10:CD003718.

P098 Macroscopically and pathological studies of genitalia of ewes slaughtered in Duhok abattoir

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Background: Reproductive disorders (congenital and acquired) which cannot be seen by routine clinical examination regarded as major causes infertility and loss of sheep production in ewes.

Aim: The present study was aimed to determine the common genital tracts pathological disorders of ewes in Duhok.

Methods: A total 270 genital tracts of non-pregnant ewes collected immediately following slaughtering was examined macroscopically. Samples were collected from September 2019-March 2020.

Results: The results of the current study found that overall prevalence of abnormal reproductive tracts in ewes was 24.1% (65/270) and 75.9% (205/270) were normal. Of 65 abnormal genital tracts, 48 tracts (17.8%) had different pathological lesion related to the ovaries, 9 tracts (3.3%) had related to the oviducts and 8 tracts (3.0%) had related to the uterus. The main pathological lesions were uterine infection and inflammation (2.2%), hydrometra (0.4%), mummifies fetus (0.4%), ovarobursal adhesion (14.8%), adhesion between two ovaries (0.4%), ovarian cysts (2.2%), ovarian atrophy (0.4%), uterine tubes obstruction (2.6%) and hydrosalpinx (0.7%).

Conclusion: This study has concluded that pathological lesions, such as uterine inflammation, ovarian cysts and hydrosalpinx in ewes might be the main reason of infertility or sterility in ewes which leading to economic losses to farmers.

P099 Determining the effects of follicle stimulating hormone (FSH) biosimilars on immunogenic and angiogenic responses of human granulosa lutein cells

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Background: The glycoprotein hormone, FSH, is essential for reproduction, with its receptor a key target of IVF. There has been an emergence of recombinant FSH (r-hFSH) biosimilar preparations utilised in IVF that differ in glycosylation, with implications for distinct in vitro and in vivo bioactivities. Vascular endothelial growth factor (VEGF) and cytokines are important modulators of ovarian function and hypothesised mediators of OHSS. Clinical trial data suggests that ovarian stimulation with different r-hFSHpreparations may affect OHSS rates, yet how/if different r-hFSH; preparations modulate cytokines and VEGFs in granulosa cells remains unknown.

Aim: To compare the effects of r-hFSH; preparations on cytokine and VEGF expression and secretion in human granulosa cells (hGLs).

Methods: hGLs were isolated from follicular aspirates obtained by informed patient consent from women undergoing routine IVF. Inclusion criteria: 31 years old, no endocrine pathologies/endometriosis, BMI25 and r-hFSH ovarian stimulation. hGLs were cultured for 5 days before stimulation -/+ 10ng/ml Gonal F (originator), Bemfola or Ovaleap (biosimilars) for 0, 8, 24,48-hours. Cytokine and VEGF synthesis and secretion were analysed via cytokine array, ELISA and qRT-PCR. n=4-6

Results: Cytokine arrays revealed PAI-1, MIF, IL-6 and IL-8 were the predominant cytokines secreted in control and 24-hour r-hFSH- treated hGLs. ELISA confirmed r-hFSHpreparations enhanced IL-8 secretion at 8 hours, with equal potency, plateauing at 24 and 48-hour timepoints, likely due to an observed ~70% reduction in IL-8 mRNA expression at 8, and 24-hour timepoints. All r-hFSH preparations increased PAI-1 secretion, with little effect on PAI-1 mRNA expression. Interestingly, r-hFSH preparations enhanced VEGF secretion, with a trend for 24-hour-Bemfola treatment more potently enhancing VEGF secretion and VEGF-A mRNA expression by ~25%.

Conclusions: r-hFSH preparations modulate the expression and secretion of cytokines and VEGF in hGLs, with potential distinct regulation of VEGF. This has implications for r-hFSHpreparation-specific regulation of granulosa cell functions.

P100 Impact of r-hFSH alfa forced degraded variants on in-vitro and in-vivo biological activity

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Objective: The rat in-vivo bioassay (EU Pharmacopoeia 2285) is routinely used to assess r-hFSH-alfa (GONAL-f [Merck KGaA], follitropin-alfa) potency by measuring ovarian weight increase. We evaluated if an in-vitro bioassay has the same ability as the rat in-vivo bioassay to detect changes in biological activity caused by r-hFSH-alfa structural modifications.

Methods: Three r-hFSH-alfa batches were force-degraded to produce r-hFSH-alfa variants by chromatographic separation (acidic/basic enrichment), chemical/physical stress (acidic/basic pH incubation, thermal/oxidative stress) and enzymatic treatments (de-galactosylation and de-sialylation). r-hFSH-alfa potency was measured via a rat in-vivo bioassay, assessing ovarian weight increase, and an in-vitro bioassay (cell line expressing the transmembrane hFSH-R), assessing cell-specific metabolic cascade. Force-degraded and untreated samples were compared via analysis of variance.

Results: r-hFSH-alfa forced degradation increased the presence of r-hFSH-alfa oxidized forms and/or free subunits up to 50% above product specification and generated significantly more hyposialylated forms when compared with the

untreated control samples. The increase in r-hFSH-alfa free subunits significantly reduced biological activity vs untreated samples, in both the in-vivo and in-vitro assays (p <0.001 for both). The increase in oxidized forms significantly reduced biological activity in-vitro (p <0.001) and, to a lesser extent, in-vivo (p <0.006). A gradual decrease in r-hFSH-alfa sialylation decreased r-hFSH-alfa biological activity in-vivo, indicating a second-order polynomial correlation, and increased r-hFSH-alfa biological activity in-vitro, indicating a negative linear correlation (slope significance p<0.001, R2 =0.996). De-sialylation reduces r-hFSH-alfa steric hindrance during the interaction of r-hFSH-alfa and the FSH-R, increasing both r-hFSH-alfa--FSH-R affinity and r-hFSH-alfa biological activity in-vitro, while increasing the rate of r-hFSH-alfa metabolism, resulting in decreased r-hFSH-alfa biological activity in-vivo.

Conclusions: The in-vitro bioassay showed similar ability to the in-vivo bioassay for estimating the impact of r-hFSH-alfa variants (sialylation, oxidation, free-subunits), resulting from process-related modifications, on biological activity.

P101 Dose accuracy of the follitropin-alfa, follitropin-alfa/lutropin-alfa and choriogonadotropin alfa pen injectors used for fertility treatment

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Objective: This study aimed to confirm if the incremental dose/clicks system was accurate for equivalence of volume (dose) delivered at standard atmospheric conditions for the complete Merck family of fertility pen injectors (follitropin-alfa, follitropin-alfa/lutropin-alfa, choriogonadotropin-alfa) in compliance with ISO 11608-1:2000/2012/2014, which specifies essential performance requirements for pen injectors.

Methods: Laboratory-based dose accuracy testing was carried out under standard atmospheric conditions, between 2015 and 2020 at two manufacturing sites. Set doses (Vset) for three dose dial settings (minimum [Vmin], midpoint [Vmid] and maximum [Vmax] [follitropin-alfa, follitropin-alfa/lutropin-alfa and choriogonadotropin-alfa]) or a single Vset (choriogonadotropin-alfa) were assessed, as appropriate. The last dose administered by the multi-dose device and cartridge and total extractable dose were assessed on the 900 IU and 300 IU follitropin-alfa and the 900 IU/450IU and 300/150 IU follitropin-alfa/lutropin-alfa.

Results: Dose accuracy tests for Vmin, Vmid and Vmax (12.5 IU, 87.5 IU and 150 IU, respectively, for 150 IU pen [n=2,226, one site]; 12.5 IU, 162.5 IU and 300 IU, respectively, for 300 IU pen [n=742/site]; and 12.5 IU, 237.5 IU and 450 IU, respectively, for 450 IU [n=180/site] and 900 IU pens [n=410/site]) for the follitropin-alfa and the follitropin-alfa/lutropin-alfa pen injectors were within the acceptable limits. Last dose and total extracted volume for each presentation were also within acceptable limits. Dose accuracy tests for the single use/single dose device classification (D1) of the choriogonadotropin-alfa pen injector (n=210 [one site]) showed that Vset (6,500 IU) was within the acceptable limits. Dose accuracy tests for the single use/variable dose device classification (D2) of the choriogonadotropin-alfa pen injector (n=180 [one site]) showed that Vmin (260 IU), Vmid (3,380 IU) and Vmax (6,500 IU) values were also within acceptable limits.

Conclusions: Each presentation of the family of fertility pen injectors performed as per the specifications for dose accuracy in ISO 11608-1:2000/2012/2014 under atmospheric conditions.

P102 Investigating intrauterine fetal growth after IVF-assisted conception

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Background: IVF-conceived offspring are at increased risk of low birthweight which may increase adulthood non-communicable disease risk. There is a research gap concerning how IVF-conceived fetuses grow in utero. We aimed to compare intrauterine growth and birthweight z-score by mode of conception (MOC).

Methods: A prospective study of IVF-conceived fetuses (START clinic: N=15 fresh embryo transfer [ET], N=21 frozen ET [FET]) was compared to a spontaneously conceived (SC) cohort (NUPS: N=103). Ultrasound measured fetal biometry (biparietal diameter, head circumference, abdominal circumference [AC], femur length, humeral length, and fractional arm and thigh volumes [AVol/TVol]) and 2D/3D EFW trajectories were compared between MOC by mixed models. Birthweight z-scores were compared between MOC by linear regression.

Results: Birthweight z-score was heavier in FET-conceived compared to SC infants (0.5-z-score; 95% CI 0.1, 0.9). Skeletal growth (represented by growth of biparietal diameter, head circumference, femur length, and humeral length) did not differ by ET type (p>0.1). Fresh ET-conceived fetuses had larger AC than SC peers from ~10 to 30 weeks (p=0.003). FET-

conceived fetuses had larger AVol than fresh ET-conceived peers from ~23 weeks onwards (p=0.001). FET-conceived fetuses had larger EFW (2D and 3D) than SC peers from mid-pregnancy onwards (p<0.001).

Conclusion: FET-conceived infants are born larger than SC or fresh ET-conceived counterparts even when gestational age differences are considered. Potential differences in intrauterine growth are observed after IVF-assisted conception, and differ by ET type, but are likely subtle in magnitude. Prospective investigation in appropriately powered studies is required to determine the nature and magnitude of any differences and to understand potential consequent long-term health of IVF-born individuals.

P103 Predicting the number of oocytes retrieved from controlled ovarian hyperstimulation with machine learning using a secured third-party data access solution

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Purpose: To build, train and test machine learning models aiming at predicting the number of oocytes retrieved from controlled ovarian hyperstimulation in IVF/ICSI treatments using a secured third-party data access solution.

Methods: A dataset containing 14,415 stimulation cycles related to IVF/ICSI treatments performed at a single external clinic in France between 2009 and 2020 was used. The dataset logged the values of 96 covariates pertaining to patient characteristics, treatment details and outcome.
LightGradientBoosting models were fitted on this dataset. No data transfer took place and data remained hidden to us. Instead, a secure data collaboration framework was used to send the models to the external clinic, fit them and return the final models.

Results: Two approaches have been successfully implemented, resulting in different levels of pre-processing. Overall, three different predictive models have been built, trained and tested thanks to the third-party solution. One that directly predicted the number of oocytes retrieved, deviated from the ground truth by 3.80 oocytes. Two other models predict which of a set of bins provided by two clinicians [A&B] the number of oocytes retrieved fell into. They respectively deviated from 0.73 bins and 0.63 bins.
For all models, performance was better within the first and third quartiles of the target variable, with models underpredicting extreme values of the target variable (no oocytes and large numbers of oocytes retrieved). Nevertheless, the erroneous predictions made for these extreme cases were still within the vicinity of the true value.

Conclusion: Three machine learning models were successfully developed using data without the need for an inter-clinic data transfer and guaranteeing total security. In this context, the performance of each model is really encouraging. It demonstrates the promise of using a secure third-party allowing external researchers to provide and use clinically-relevant insights on sensitive fertility data in a secure, trustworthy manner.

P104 Assessment of telomere length in human cumulus cells as a potential biomarker in assisted reproduction outcomes

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Background: Cumulus cells (CCs) are essential for producing competent oocytes, transducing external signals to the oocyte, and providing resources via gap junctions. Given their key role in oocyte development, the biology of CCs is of clinical relevance. In this study, we measured the relative telomere length (RTL) in CC samples and examined potential associations with patient and embryo characteristics. Telomeres are specialised structures at the ends of chromosomes, composed of repetitive DNA sequences and associated proteins. Telomeres shorten with each mitotic division, as well as due to oxidative damage, eventually reaching a critical threshold at which point cellular senescence occurs.

Methods: Quantitative PCR (qPCR) was used to measure RTL in 182 human CCs samples, collected from 52 IVF patients. qPCR data, obtained using PCR primers specific for the telomere repeat, was normalised with respect to a single-copy gene, adjusting for variation in cell number between samples. Associations were assessed between RTL in CCs and patient age, cause of infertility, BMI, as well as rates of fertilisation, and embryo morphology and suitability for transfer/cryopreservation.

Results: The amount of CC telomeric DNA tended to be greater for patients with higher BMI (P=0.002). Additionally, average RTLs were longer for CCs associated with embryos considered non-viable (discarded) compared with those transferred or cryopreserved (P=0.019). No other significant associations were detected.

Conclusions: Currently, a method of evaluating the competence of oocytes prior to use in IVF is lacking. If such a method could be developed, it might add to existing embryo selection strategies based upon morphology and chromosomal analysis. However, its greatest value could be for evaluation of oocytes vitrified for fertility preservation, or stored in donor banks. In this context, the identification of lower RTLs in CCs associated with embryos considered suitable for transfer is a potentially important observation that warrants further investigation.

P106 Increasing the amount of evidence provided by systematic reviews of infertility treatments by combining clinical pregnancy and live birth data in meta-analysis

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Background: Previous meta-epidemiological research has demonstrated that the results of meta-analyses looking at the effect of fertility treatments on livebirth are generally too imprecise to be useful. This is partially attributable to the fact that many RCTs do not report the outcome live birth, but instead report a surrogate outcome, clinical pregnancy. We are interested in exploring whether a method called bivariate meta-analysis could lead to clearer answers about the effects of treatments on live birth, by incorporating information about the effects on clinical pregnancy and the relationship between clinical pregnancy and live birth.

Methods: In an attempt to avoid the research waste, we employed the use of bivariate meta-analysis to analyse the effect of fertility treatments on clinical pregnancy and live birth so as to improve precision of the results. Data was extracted from systematic reviews with interventions intended to improve live birth in Cochrane Gynaecology and Fertility. The data was re-analysed using bivariate meta-analysis. Then the bivariate meta-analysis results were compared to the ones obtained using separate meta-analysis.

Results: We extracted 17 Cochrane Gynaecology and Fertility Reviews with 52 meta-analyses from the last 2 years and applied bivariate meta-analysis and separate meta-analysis, so as to compare performance. Sixty three percent of the meta-analysis included live births. In most studies, the higher the within-study correlation the more precise the bivariate analysis results became.

Conclusions: It may be useful to take into account the correlation between outcomes in a trial, as the bivariate meta-analysis led to more precise estimates than the separate analyses.

P108 In search of lost time: Assessing assisted reproductive therapy referral practice to Birmingham Women's Hospital Fertility Centre

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Background: Assisted reproductive therapy (ART) is a time-critical intervention. While success rates of ART decrease with age[1], increasing maternal age correlates to reduced live birth rates in ART pregnancies, particularly from the age of 35[2]. Due to ART's age-related decline in efficacy, delays to intervention may negatively affect treatment outcomes. Long waiting times to access ART services only heighten the importance of eliminating avoidable delays. Referral practice to Birmingham Women's Hospital Fertility Centre (BWHFC) was assessed to evaluate whether preventable delays were being introduced by referral practices.

Methods: The information required for an assisted reproduction appointment at BWHFC was identified as menstrual status, ovulatory status, BMI, hormone profile (LH, FSH, day-21 progesterone, oestrogen) transvaginal ultrasound scan (TVUSS) and seminal fluid analysis (SFA). Referrals to BWHFC between 12/21 - 06/22 were randomly selected and assessed for the inclusion of this clinical information. Patient demographics and referral origin was recorded.

Results: 173 referrals to BWHFC between 03/12/2022 - 24/06/2022 were assessed. 84 referrals included menstrual status, 14 included ovulatory status and 65 included BMI. 112 included hormone profile, 73 included TVUSS, and 78 included SFA results (where applicable). 21% of referrals did not include laboratory or imaging investigations, 58% lacked at least one of these investigations. The modal age group was 30-34 and 39% of patients were aged 35 or over. 158 referrals originated from primary care, the remainder from secondary care. 112 were for NHS funded treatment, 67 for self-funded treatment.

Conclusion: Poor referral practice is introducing avoidable delays to treatment. Over half of referrals lack at least one key laboratory or imaging results (TVUSS, SFA, hormone profile), leaving patients waiting for time-consuming investigations to be completed before treatment can begin. Delays to treatment may be negatively impacting outcomes, especially in the over 35 population, a notable proportion of the patient population.

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P109 Improving ICSI success rates following root cause analysis and use of system behaviour charts

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Background: A fertility clinic observed a reduction in its fresh ICSI implantation rate key performance indicator (KPI) below benchmark threshold which was further monitored but did not improve. The clinic had been performing ICSI successfully for >16 years with good ICSI implantation rates meeting benchmark level. A root cause analysis was conducted, including the input from an external observer, reviewing all systems and processes. A bundle of recommended changes was implemented as part of an improvement cycle with the aim to increase fresh ICSI implantation rates back to benchmark.
Methods: Quality improvement (QI) methodology was applied to identify improvement interventions and to test them. Many QI tools were used including Statistical-Process-Control charts (BaseLine© SAASoft). Measurements included standard clinical outcome data.
Results: KPI's were tracked following defined and controlled clinical and laboratory changes. Fresh ICSI implantation rates improved significantly (p = 0.013, ChiSq). The improvement work was limited by its design of a plan-do-study-act (PDSA) cycle 'intervention bundle' as opposed to small PDSA cycles of single changes. Therefore, the improvement could not be attributed to any singular intervention within the bundle.
Conclusions: The QI project highlights the difficulty for clinics with low cycle volumes to sensitively monitor KPI's in a timely and responsive way. The need to accumulate sufficient data to be confident of any trends/concerns, means small clinics could be less responsive to any problems or too reactive to false positives. It is important to disseminate the learning from this improvement work because there is currently no agreed standardised optimal protocol for ICSI[1], resulting in clinics using slightly different approaches, and there are limited published reports where embryology KPI's are tracked following defined and controlled laboratory or clinical changes[2]. This project provides useful knowledge about ICSI improvement interventions.

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P110 Detailed sub-group analysis for development of individualized treatment strategies for women over 40 to optimize pregnancy outcome

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Background: The number of women 40 and above seeking fertility treatment is increasing despite the poor prognosis. POSEIDON criteria recommended the usage of ovarian predictive markers and female age to predict "low prognosis" in IVF/ICSI. However, even this classification may not be detailed enough to lead to specific recommendations for individual management in older women, as there is rapid decline of oocyte-quality and quantity. Here, we assess predictors of pregnancy outcome after stratification by ovarian reserve in women 40 and above.

Methods: We conducted a retrospective cohort study of 382 fresh IVF/ICSI cycles for 253 women 40 and above from January 2019 to December 2021 in a tertiary fertility centre. The participants were divided into Group A, "normal ovarian reserve" (AMH 2.5-29.9; 271 cycles), Group B, extremely poor ovarian reserve (AMH <2.5 pmol/l; 85 cycles), and Group C with high ovarian reserve (AMH 30 pmol/l; 26 cycles). Chi-square, Mann-Whitney U and multivariate logistic regression tests were conducted where appropriate.

Results: In Group A, multivariate logistic regression revealed female age, male age, number of previous IVF cycles, and number of embryos as independent factors contributing to pregnancy.
In Group B, predictors of pregnancy included female age, gravida, previous successful IVF and number of eggs fertilized ($p<0.05$). Other factors influencing positive pregnancy outcome included pre-treatment with testogel, addition of Lubion and combination of FSH and LH versus FSH alone.
In Group C, lower AMH level positively influences pregnancy rate ($p<0.05$). Other factors associated with positive outcome included antagonist cycles, and usage of FSH and LH versus FSH alone.

Conclusion: This detailed analysis will allow us to better understand the prognostic factors in women 40 and above to individualize their care. In the era of personalized medicine, this approach in preference to blanket cover treatment may be more acceptable to women in this age group.

P111 Protecting our in vitro future - a clinic's call for sustainable change in ART

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Background: The 2022 Health and Care Act issued new legislative law for the NHS to consider their environmental footprint, yet non-NHS facilities were not included [1]. Numerous systematic reviews have reported associations between increased pregnancy risks and adverse birth outcomes with factors related to climate change: higher temperatures and air pollution [2][3]. In a field protected by the Hippocratic Oath, we should try combating one of the greatest ethical tragedies of our lifetime to protect our patients and future generations.
Aims/Methods: To design a model for a small-medium sized private fertility clinic to operate following the Health and Care Act 2022. Based on the NHS Sustainable Development Unit Survey [4], all staff undertook a formal assessment to ascertain current attitudes towards the environment and discussed in a formal presentation how best to effectively tackle issues within the clinic.
Results: 100% of staff agreed the health and care system should work in a way that supports the environment, whilst only 18.8% strongly and 43.8% somewhat agreed the clinic actively supported the environment. Staff suggested including sustainability in job descriptions, clinic campaigns and induction/training would be most effective. Actions taken include switching to recycled paper alongside increased digitisation, ceasing the purchase of single-use cups and forming a green committee. The committee agreed to meet quarterly with updates from representatives of each department and publish a clinic 'Green Plan'.
Conclusion: The clinic identified key areas for environmental change, set up a review management system and actioned sustainable improvements - a small review and strategy model, if successful, other clinics can adopt. The reproductive field needs stronger sustainable action taken by regulatory bodies and societies; the BFS and HFEA may look at our data and other published literature to provide guidelines on how clinics and research facilities can reduce their carbon footprint.

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P112 The use of cryokinetic variables to ensure correct functioning of liquid nitrogen dewars

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Background and aims: Ensuring proper liquid nitrogen (LN2) dewar integrity is vital in any IVF Clinic. This has been historically assessed by internal measurements of LN2 temperature or LN2 level. In recent years, there has been growing evidence on the advantages of using weight-based systems to assess dewars' health. However, the potential of other cryokinetic variables, such as dewar surface temperature and surface temperature difference remains to be explored.

Methods: In this study, four dewars were individually placed on top of a monitoring device[1] for 360 hours (15 days) uninterrupted and undisturbed. In order to recreate a critical dewar failure, one dewar was then monitored without the lid. Additionally, and to valuate the effect of dewar usage over time, dewars with different manufacturing dates were used (2005, 2008 and 2x 2020). The device collected data on four cryokinetic variables: weight, evaporation rate, surface temperature and surface temperature difference. A conventional temperature probe was placed inside each dewar, throughout the experiment, to measure its internal temperature. ANOVA and Pearson correlation tests were used to analyse differences and correlations amongst the variables.

Results: After normalising evaporation rates to the neck diameter no differences were found between the four dewars in terms of daily weight loss and evaporation rates. When comparing all dewars with the failure one, statistical significance ($p<0.001$) was obtained for daily weight loss and evaporation rate normalised to neck diameter. As for

performance, differences were noted for each dewar between the observed evaporation rate and the one provided by the manufacturer. The oldest dewar had an increase of 22.5% over 16 years, whereas the increase difference was less accentuated for the remaining dewars (6.9-12.2%).

Conclusion: Our study suggests that the assessment of the cryokinetic variables is a powerful tool to monitor and validate dewars internally, even allowing the operator to predict dewar's lifespan.

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P113 Doing today's work today: Real-time data recording and rolling audit in an IVF clinic

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The Assisted Conception Unit (ACU) at Sheffield Teaching Hospital NHS Foundation Trust provides in vitro fertilisation (IVF) treatment. A team of seven embryologists provide a routine clinical laboratory service, involving culture and storage of embryos. This requires a series of management and statutory data-administration and communication tasks. We were aware that these were often done many days after clinical tasks, resulting in delays sending patient correspondence and unavailability of clinical notes for multidisciplinary team (MDT) cycle-review meetings. Embryologists also complained that transcribing data was time-consuming and duplicated across our IDEAS software, spreadsheets and paper. We process mapped our processes and gathered staff views on problems and potential solutions. The baseline average Total Cycle Time (TCT) for completion of all administrative steps was around 17 days; data administration time (DAT, data 'touch time') was around 30 minutes per patient. We embarked on this QI project to reduce waste in TCT and DAT, and have data available for patient communication and MDT deadlines. Exploration of IDEAS' capabilities led to progressive realisation of how much could be transferred to this single data system, removing a lot of off-putting redundancy. Through this we developed a 'to-be' vision of all data entry being real-time, as part of the clinical 'jobs'. We conducted five PDSA cycles plus two more to test performance and sustainability as processes bedded-in and an external constraint disappeared. We have cut TCT to 0 or 1 days and DAT to around 18 minutes. All project metrics are reliably within our targets, and data are now always available for timely patient letters and the MDT. Other benefits include easy access for all staff to patient records and removal of paper and spreadsheets. A further, unanticipated, benefit was a switch from a tedious two-yearly storage tank audit to a more-agreeable and safer rolling audit.

P114 A service evaluation reviewing datix incident reports from 2013 - 2022 across the fertility institute (FI), and comparing this to the human fertilisation & embryology authority (HFEA) national audit

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Introduction The Fertility Institute (FI) is an NHS facility licensed by the HFEA. Who, monitors and inspects fertility clinics across the UK, and collects data from these to ensure the best quality care is being provided. Datix is the electronic incident reporting system in which you report an incident. Methods Data was collected from 612 Datix reports. Each incident was categorised and graded to enable easy comparison to the HFEA National Audit data. Results Grade of incidents reported 93% of the incidents were graded as 'No Harm (1)', followed by 4.4% incidents graded as 'Low (2)' and 2.1% incidents graded as 'Moderate (3)'. The results from the 3360 incidents reported to the HFEA in the same time frame, show the majority were 'Grade C' (50 % of incidents), followed by 'Grade B' (41.6%), 'Near Miss' (8.3%) and 'Grade A' (0.1%). Categories of incidents reported The most common category of incident reported by the FI was 'Administration' (157 incidents), closely followed by 'Clinical' (132 incidents) and 'Communication' (58 incidents). The least common categories included 'Lab operator' (1 incident) and 'Consent' (12 incidents). In order to compare results between HFEA and FI incident data, the results from the 1496 incidents reported to the HFEA show the most common category of incident was 'Clinical' (44% of all incidents), followed by 'Administration' (22.3%) and 'Laboratory Processes' (9%). The least common categories were 'Clinical equipment and 'Security' (0.54%) and 'Resources/organisational' (0.27%). Conclusions This project has revealed that the most common type of incident reported both to the FI and the HFEA as a whole are either Administrative or Clinical incidents.

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P116 Do urine measurements of luteinising hormone (LH) and follicle stimulating hormone (FSH) provide a good enough proxy for serum levels in polycystic ovary syndrome (PCOS)?

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Background: Polycystic Ovary Syndrome (PCOS) is a common endocrine pathology characterised by polycystic ovaries, ovulatory irregularities & hyperandrogenism (1,2). Current research tends to focus on serum LH and FSH levels in PCOS and non-PCOS populations (3-6). Acquiring serum samples can be laborious and time consuming, therefore, if urine measurements could be used as a proxy for serum levels, this could potentially facilitate diagnosis/monitoring of PCOS.

Aims: To explore the potential associations between urine and serum LH and FSH measurements in PCOS women. To investigate associations between BMI, vitamin D levels and status, waist:hip ratio, homeostatic model assessment 2 (HOMA2), insulin resistance status and free androgen index (FAI) with urine and serum LH and FSH in the same population.

Methods: Data were collected between 2018 to 2020. Investigations from 56 women with Rotterdam Criteria diagnosed PCOS included fasting glucose and insulin, and a range of hormones - testosterone, sex hormone binding globulin (SHBG), LH, FSH and, vitamin D. Urine samples measured LH, FSH and, creatinine.

Results and conclusions: All correlations and associations between urinary (both corrected and non-corrected) and serum LH and FSH were statistically significant (correlations $p<0.05$, associations $p<0.001$) with correlations ranging from a moderate to very strong correlation. There was a significant univariate association between corrected urinary LH against both BMI ($p=<0.001$) and HOMA2 ($p=0.003$), urinary LH against BMI ($p=0.011$) and urinary FSH against vitamin D levels ($p=0.013$) and status ($p=0.047$). The only significant variable in a multivariable regression model was corrected urinary LH against BMI when adjusted for all other covariates ($p=0.005$). These findings show that corrected urinary LH and FSH can be used as a proxy for serum measurements in women with PCOS; which may have future implications to improve monitoring of PCOS in settings such as general practice.

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P117 The use of urine hormonal data to assess the severity of vitamin D deficiency and insulin resistance in women with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine dysfunctions in women,[1] with a prevalence between 5.6% and 35%[2,3]. Insulin resistance and vitamin D deficiency heighten PCOS severity, increasing cardiovascular manifestations, subfertility and poor pregnancy outcomes[4]. The use of hormone metabolites from urine to measure PCOS severity would be a more convenient and less invasive marker for diagnosing and monitoring PCOS compared to the more common use of serum equivalents, however, there is a lack of research supporting this.

Aims: To investigate potential associations between urinary hormone profiles in women with PCOS and the severity of their presentation of vitamin D deficiency and insulin resistance.
To explore associations between levels of E3G, P3G, LH and FSH measured in urine of women with PCOS to their Vitamin D status, BMI, waist-hip ratio, AMH, FAI, total testosterone and insulin resistance status.

Methods: Cross-sectional cohort study, data collected from sixty-five women with PCOS. Blood and urine samples were taken and assayed. Characteristics assessed were used to analyse univariable and multivariable regression for each urinary hormone with the markers or potential explanatory variables.

Results: A significant positive association was seen between urinary E3G and the vitamin D deficient/replete groups (p=0.01). Negative associations between urinary P3G with BMI (p=0.02), HOMA2 (p=0.003) and the insulin resistance/sensitive groups (p=0.02). Additionally, urinary LH had a negative association with BMI (p=<0.001) and HOMA2 (p=0.01) and a positive association with AMH (p=0.01). No significant associations were seen with urinary FSH. Further correlations between metabolic and anthropometric measures were also explored.

Conclusion: These findings support the use of urinary hormonal profiles to measure severity of insulin resistance, but limited evidence for its use measuring vitamin D severity. Secondly, this study supports the use of other serum endocrine and metabolic markers to measure PCOS severity.

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P118 FSHR trafficking is modulated by differential FSH glycosylation variants

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The heterodimeric glycoprotein hormone, follicle-stimulating hormone (FSH), and its target G protein-coupled receptor (FSHR) are essential for reproduction, and key targets of assisted conception. Post-translational modification of FSH gives rise to two predominant glycosylation variants, which are modulated with ageing. Partially glycosylated FSH (FSH21/18), predominates in women's reproductive prime (20's), demonstrates faster binding kinetics to the FSHR, and

more potent at activating cAMP-dependent signalling. This is in contrast with fully glycosylated FSH (FSH24) which predominates in peri-menopausal women (50's) and is less bioactive. Although recent studies have suggested a link between receptor trafficking and signalling, how FSH glycoforms modulate FSHR trafficking remains unclear. Therefore, we aimed to determine how FSH glycoforms modulate FSHR trafficking and impact on signal activation. HEK293 cells transiently expressing the FSHR were pre-treated with 50µM of a dynamin inhibitor (Dyngo-4a®), to inhibit FSHR internalisation, before treatment with either FSH21/18 or FSH24, and cAMP signalling analysed. Pre-treatment with Dyngo-4a® significantly reduced both FSH21/18- and FSH24-dependent FSHR cAMP signalling and cre-luciferase activity. Confocal microscopy analysis of FSHR endosomal routing revealed temporal FSH-glycoform-dependent differences in the routing of FSHR to EEA1-positve endosomes, indicating that the differences in FSH glycosylation status may mediate differential FSHR trafficking. Interestingly, knockdown of the early endosomal adapter protein (APPL1), linked to cAMP production and receptor recycling, had no effect on FSH21/18 dependent cAMP-signalling, however, it enhanced FSH24-dependent cre-luciferase activity. Together, these data suggest that differential FSH glycosylation may distinctly modulate the endosomal routing of FSHR to fine-tune cAMP production. This may have implications for altered FSH/FSHR actions in the ageing ovary, highlighting potential novel targeting mechanisms for enhancing assisted conception.

P119 Tool development for the in vivo analysis of the physiological role of FSHR oligomerisation

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G protein-coupled receptors are the largest family of mammalian receptors, with key roles in controlling most physiological processes. Ovarian function is no exception, with a key ovarian GPCR, follicle stimulation hormone receptor (FSHR), and its endogenous ligand, FSH, critical for pre-antral-antral follicle growth and survival. An increasingly important way that GPCRs have been shown to regulate ligand specificity and signal amplitude is via association and formation of dimers/oligomers. Although FSHR has been demonstrated to self-associate and homomerize, the physiological regulation and significance of this remains unknown. This study therefore aimed to determine the modulation and functional consequences of FSHR oligomerisation in native ovarian granulosa cells. To do this, an N-terminally FLAG-tagged knock in FSHR mouse was generated. Phenotypic characterisation revealed that ovarian and uterine weights were the same between wild type (WT), FSHR FLAG-/+ and FSHRFLAG+/+suggesting FSH-mediated oestrogen production was maintained. Gross morphological analysis of the reproductive tract and ovaries revealed no differences between these three genotypes. Histological analysis of ovaries showed the presence of follicles at all stages of follicular development in WT, FSHRFLAG-/+ and FSHRFLAG+/+ mice. Additionally, corpora lutea were present in all models supporting intact ovulation. Breeding strategies confirmed fertility of FSHRFLAG-/+ and FSHRFLAG+/+. Isolated of granulosa cells and super resolution analysis of FSHR monomers, dimers and oligomer populations showed ~40% of FSHR were basally associated, comparable to previously published work in HEK293 cells expressing FSHR. Additionally, analysis of the types of FSHR oligomers suggested a predominance of lower order oligomeric complexes. These data support the utilisation of this mouse model for monitoring endogenous, native FSHR oligomerisation, and provide an exciting tool to unravel the physiological roles of these receptor complexes in ovarian function and ageing.

P120 Investigating the potential role of iron in the endometrium during the menstrual cycle

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Background: Menstruation is a repeated physiological injury/repair cycle of the endometrium, a mucosal surface of the reproductive tract (1). Heavy menstrual bleeding (HMB) has a debilitating impact on quality of life, affects 1 in 3 women of reproductive age, and is a leading cause of iron deficiency (ID) and iron deficiency anaemia (IDA) in menstruating women (2,3). HMB can occur due to impaired repair of the endometrium (4). Iron is implicated in injury/repair at other body sites, e.g., the gastrointestinal tract (5). The role of iron in endometrial repair has not to our knowledge been explored.

Hypothesis: Iron plays a role in endometrial injury/repair, thus implying a role in the mechanisms underpinning HMB.

Methods: Standard qRT-PCR was used to detect the presence of markers of iron metabolism: Ferroportin, Hepcidin and Transferrin-Receptor1; subsequent Immunohistochemistry (IHC) was used to detect the presence of two markers of iron metabolism: Ferroportin and Hepcidin; in the endometrium, across the menstrual cycle, in women with normal menstrual blood loss (NMB; n =21) and HMB (measured menstrual blood loss >80mls (2); n=25); Ethical approval (20/ES/0119).

Results: qRT-PCR confirmed Hepcidin (Human HAMP), Ferroportin (Human SLC40A1) and Transferrin-Receptor-1 (Human TRFC) are expressed in the endometrium of women with normal and heavy menstrual bleeding. Expression was not significantly different ($p>0.05$) between women with NMB and HMB across the cycle. IHC confirmed Hepcidin and Ferroportin protein localisation in the endometrium. Both Hepcidin and Ferroprotin were localised in the cytoplasm of endometrial stromal cells, from women with NMB and HMB, across the menstrual cycle

Summary of findings: This pilot study has demonstrated the presence of markers of iron metabolism in the endometrium. There was no significant difference in levels of protein observed in samples from women with NMB and HMB. A role for iron metabolism throughout the menstrual cycle warrants further investigation.

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P121 The impact of body mass index and lifestyle factors on serum concentrations of reproductive and thyroid hormones in premenopausal women

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Hertility Health

Background: The impact of BMI, exercise, smoking, drug and alcohol use on reproductive and thyroid hormones has little or conflicting evidence. We therefore aimed to investigate the association between these factors and serum hormone concentrations.

Methods: Capillary blood samples were taken on menstrual cycle day 3 from 932 eumenorrheic users of a UK-based at-home hormone test between Sept 2020 and June 2022. Serum concentrations of Anti-Müllerian hormone, Estradiol (E2), Luteinising Hormone (LH), Follicle-Stimulating Hormone, free Thyroxine, Thyroid-Stimulating Hormone (TSH) and Prolactin (Prl) were measured via chemiluminescence immunoassay. Women self-reported their height, weight, exercise frequency, alcohol consumption, recreational drug use and smoking status. Following stratification into two age groups (18-30 and 31-40), data was log-transformed and the Pearson correlation coefficient (r) between pairs of variables was calculated; p values < 0.05 were considered statistically significant.

Results: In 18-30 year olds, BMI negatively correlated with E2 ($r=-0.13$, $p=0.02$, $n=308$) and LH ($r=-0.24$, $p<0.01$, $n=306$) and positively correlated with TSH ($r=0.12$, $p=0.04$, $n=292$). Exercise frequency positively correlated with LH ($r=0.16$, $p=0.02$, $n=201$) and drug use negatively correlated with Prl ($r=-0.2$, $p=0.02$, $n=151$). In 31-40 year olds, BMI negatively correlated with E2 ($r=-0.11$, $p<0.01$, $n=548$), LH ($r=-0.20$, $p<0.01$, $n=545$) and Prl ($r=-0.12$, $p=0.03$, $n=352$).

Conclusion: These data suggest BMI has a weak but significant negative association with reproductive hormones and a weak positive association with TSH. Exercise frequency and drug use have a small positive association with reproductive hormones, whilst alcohol consumption and smoking status did not appear to have a significant impact. Further investigation into these associations is required.

P122 Reduced accuracy of gold top blood collection tubes for reproductive hormone profiling in capillary blood samples

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Hertility Health

Background: While evidence of concordance between reproductive hormone measurements in venipuncture and capillary serum exists, variation between blood collection tubes has not been investigated. We compared the performance of two capillary and venipuncture blood collection tubes (red top and gold top) and assessed variation between two leading tube manufacturers.

Methods: To compare tube types, two venipuncture and two finger prick capillary samples were concurrently collected from 11 premenopausal women into red top and gold top tubes. In all four sample types, serum concentrations of Anti-Müllerian Hormone (AMH), Estradiol (E2), Follicle-Stimulating Hormone (FSH) and Luteinising Hormone (LH) were

measured via chemiluminescence immunoassay. To compare capillary tube manufacturers, the same hormones were measured in a second cohort of 8 premenopausal women using Greiner Bio-One (Germany) and Becton Dickinson (BD) (U.S.A) red top tubes. Data was log-transformed prior to statistical analysis via paired t test and subsequent Cohen's d (d) to calculate effect size; p values=<0.05 were considered significant.

Results: Gold top capillary tubes produced higher AMH (p<0.001, d=0.12), FSH (p<0.001, d=0.30) and LH (p<0.001, d=0.29) measurements compared to gold top venipuncture tubes. Measurements of AMH (p<0.001, d=0.22), FSH (p<0.001, d=0.29) and LH (p=0.008, d=0.13) were higher and E2 measurements were lower (p=0.012, d=0.34) in gold top compared to red top capillary tubes. No significant differences were found between red top capillary tubes and venipuncture tubes. Greiner Bio-One red top tubes produced moderately lower E2 measurements than BD (p=0.046, d=0.04), however no other significant differences were found.

Conclusion: This suggests that gold top, but not red top, capillary blood collection tubes yield significantly different measurements than venipuncture tubes and measurements are not manufacturer-dependent. Further investigation into capillary gold top tubes is required.

P123 The effect of aqueous lessertia frutescens extract on TM3 leydig cells exposed to TNF-? In vitro

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Background and objectives: Cytokines modulate Leydig cell function. Although TNF-α is known to induce Leydig cell dysfunction in acute and chronic inflammation, the mechanisms are poorly understood. Herbal extracts can act as TNF-α antagonists or potentially protect against TNF-α induced cytotoxicity. Lessertia frutescens (Lf), a Southern African medicinal plant, has immune modulating and antioxidant properties. However, the impact of Lf on Leydig cell function remains neglected. Hence, the aim of this study was to investigate the effect of an aqueous Lf leaf extract on Leydig cells exposed to TNF-α in vitro.

Methods: hCG-stimulated TM3 Leydig cells were exposed to different culture conditions: a) increasing concentrations of TNF-α (0.1, 1, 10, 100 ng/mL), and b) Lf extract (0.01, 0.1, 1, 10, 100 ng/mL), as well as c) co-exposure to 10 ng/mL TNF-α and Lf (0.01, 0.1, 1, 10, 100 ng/mL), for 24 hours. Outcomes analysed were cell viability, cytotoxicity, caspase 3/7 activation, testosterone concentration and intracellular superoxide concentration. The experiments were performed in triplicate.

Results: TNF-α exposure decreased Leydig cell viability and increased cytotoxicity, with early apoptosis and a down-regulation of testosterone. Lf extract protected against TNF-α-induced cytotoxicity and apoptosis, except at the highest experimental concentrations of 100 ng/mL where it was cytotoxic. Although TNF-α did not significantly decrease testosterone at most concentrations, culture of TNF-α with Lf significantly increased testosterone at higher concentrations. These effects were not mediated through a change in intracellular superoxide.

Conclusions: Although further investigations are warranted, our results suggest that aqueous Lf leaf extract may be useful in the protection of inflammation induced Leydig cell dysfunction.

P124 Eurycoma longifolia (Jack) improves serum total testosterone in men: A systematic review and meta-analysis

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Background and objectives: Male hypogonadism is a clinical disorder characterized by reduced serum testosterone in men. Although treatment using herbal medicines including Eurycoma longifolia (Tongkat ali), has been investigated, the benefits remain unclear. This study aims to investigate the efficacy of E. longifolia as a sole intervention to increase testosterone levels in males.

Methods: We conducted a systematic review and meta-analysis of randomized clinical trials (RCTs) according to the PRISMA guidelines. Relevant articles were retrieved from PubMed, Scopus, Web of Science, Cochrane, Ovid/Embase, and Google Scholar databases. For the RCTs included in this meta-analysis, the quality and the risk of bias was assessed using version 2 of the Cochrane risk-of-bias tool.

Results: After literature screening, a total of 9 studies were included in the systematic review, with 5 RCTs included in the meta-analysis. A significant improvement of total testosterone levels after E. longifolia treatment was mostly

reported, in both healthy volunteers and hypogonadal men. Random model effect revealed a significant increase (SMD = 1.352, 95% CI 0.565 to 2.138, P=0.001) in the total testosterone levels in men receiving E. longifolia supplementation, which was confirmed in the hypogonadism subgroup. **Conclusions:** This systematic review and meta-analysis of the literature supports the possible use of E. longifolia supplementation for enhancing testosterone production. Although more research is required before its use in clinical practice, this may represent a safe and promising therapeutic option, particularly in hypogonadal men.

P125 The management of age-related oxidative stress in male hypogonadism associated with non-communicable chronic disease

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Background and objectives: Hypogonadism is common among ageing males. As a risk factor and comorbidity, has a complex relationship with age-related noncommunicable chronic diseases (NCDs) such as obesity, metabolic syndrome, type 2 diabetes, and malignancy. Oxidative stress is a common feature among ageing, hypogonadism, and NCDs. Therefore, we aim to discuss the therapeutic options for the management of age-related oxidative stress in male hypogonadism associated with NCDs.

Methods: In this narrative review, we discuss the approaches for clinical management of NCDs associated hypogonadic men, including testosterone replacement therapy (TRT), metformin, weight management, and antioxidant supplementation.

Results: Pharmaceuticals used for TRT include testosterone cypionate, testosterone enanthate, or testosterone undecanoate. Their benefit and long-term safety are unclear, as they are contraindicated in prostate carcinoma and hyperplasia, and associated with cardiovascular events. Importantly, TRT is detrimental to male fertility. Metformin is an effective antiglycemic agent and have been found to improve reproductive function in diabetic males, such as semen parameters, testicular antioxidant function, and intratesticular and serum testosterone concentrations. However, it indirectly inhibits steroidogenesis through the downregulation of steroidogenic acute regulatory (StAR) protein. Weight management through lifestyle changes or bariatric surgery improves male fertility and endocrine function, sexual dysfunction and many comorbidities in obesity, metabolic syndrome, and diabetes. Natural antioxidants may be also beneficial in late-onset hypogonadism and the related degenerative diseases associated with ageing, for reducing testicular oxidative stress and Leydig cell apoptosis, and improving testosterone, male sexual behaviour, libido, and sexual function.

Conclusions: The relationship between ageing, oxidative stress, male hypogonadism, and NCDs in males is complex. Although TRT may be beneficial, alternative therapeutic approaches require consideration. Weight loss, through nutritional and lifestyle interventions, and the use of nutritional and phytomedicinal antioxidants, may provide novel therapeutic options in the management of age-related NCDs in males by alleviating oxidative stress and improving steroidogenesis.

P126 It takes two to tango: The involvement of endocrine disruptors in male infertility aetiology

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Purpose/Background/Objectives: Exposure to pollutants originated from (or used in) industrial processes may contribute towards reduced reproductive health status worldwide. This can be even more obvious in populations occupationally exposed and/or living in polluted areas. The Portuguese city of Estarreja encloses the 2nd largest chemical complex of the country and local contamination of heavy metal(loid)s was reported, with a preponderance of As and Hg. Despite the efforts made to mitigate such contamination, it is unknown if male fertility is compromised in this scenario.

Methods: Both in vivo(1) and in vitro(2) approaches were used.(1) 280 samples were collected from men who filled a comprehensive questionnaire. Several samples were excluded due to eligibility criteria and the remaining were divided in exposed (Estarreja;n=10) and control groups (n=88). As and Hg levels in seminal fluid were measured by ICP-MS. (2) Samples were exposed up to 24h (37°C, 5%CO2,n=8-10) with As and Hg doses found in (1), as well as others described in literature. Spermiograms were performed according to WHO guidelines. Sperm functional markers such as viability

(eosin Y), mitochondrial function(JC-1), chromatin/DNA status(Diff-Quik staining), acrosome integrity(PSA-FITC staining) and ROS production(DHE) were also analysed.

Results: (1)Comparisons between control and exposed groups showed no differences in semen volume, pH, sperm viability, concentration, motility and morphology; yet the sample size is still small. Similarly, no differences were obtained when looking for As and Hg levels (nM) between groups. Nonetheless, and although the values were smaller than reported in other regions of the world, (2) showed they were sufficient to, by acting synergistically, decrease motility, chromatin integrity and increase ROS production. Higher physiological doses induced a more pronounced effect.

Conclusions: The counteractive measures implemented in Estarreja decreased As and Hg exposures to levels similar to the general population. Yet, these seem to jeopardize male fertility. Special attention should be made to the potential adverse effects of mixtures in studies using relevant concentrations, as this seems to lack.

P127 Factors associated with deciding on elective single embryo transfer

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Purpose: To identify which factors should be considered when deciding on whether to use elective single embryo transfer (eSET) or multiple embryo transfer (MET).

Methods: The outcomes of in vitro fertilisation (IVF) from two national registries and three individual clinics were retrospectively analysed. A number of factors (the maternal age, number of oocytes collected, day of transfer, fresh embryo transfer or frozen-thaw embryo transfer (FET) and the use of pre-implantation genetic testing (PGT) or not) were evaluated for their impact on live birth rate (LBR), clinical pregnancy rate (CPR), cumulative live birth rate (CLBR), multiple live birth rate (MLBR) and miscarriage rate after eSET compared with MET.

Results: The retrospective study included 986,870 cycles (656,736 cycles between 2010-2018 from the British national registry, HFEA; 305,361 cycles between 2014-2018 from the French National Registry, and 24,773 cycles from three private clinics from Belgium and USA). eSET was associated with higher CLBR in patients ($p<0.05$), though these gains were only significant for patients under the age of 40 (OR 1.03, $p=0.74$). eSET also led to significantly higher CLBR, regardless of the number of oocytes collected ($p<0.05$). When compared to MET, the day of transfer, fresh embryo transfer or FET, and the use of PGT were not associated with a poor outcome after eSET. MLBR in eSET was significantly lower across all factors ($p<0.05$).

Conclusion: Maternal age, number of oocytes collected, day of transfer, fresh embryo transfer or FET, and whether or not embryos assessed by PGT did not favour MET over eSET, suggesting that even patients with relatively "poor" prognosis should still be considered for eSET. Using eSET was a reliable method for preventing multiple live births while preserving IVF success and these factors are not appropriate justifications for the use of MET.

P128 Visualising cleavage stage embryos in 4D using time-lapse images

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Introduction: 3D cellular arrangement in preimplantation human embryos has both clinical and biological implications [1]. However, assessing 3D cellular arrangement with confocal microscopes beyond t4 remains difficult to assess in-clinic due to cost and safety concerns. Our previous studies investigated 3D visualisation [2] and characterisation [3] of cell arrangement in clinical embryos at fixed points in time. The objective of this study is to examine 3D cellular arrangement over time (4D).

Methods: A deep learning system based on [2] was trained on a dataset of 443 cleavage stage embryos from four clinics (409 train/validation; 34 blind test) to detect individual blastomeres and their locations in Embryoscope focal stacks. The system was used to generate 3D models corresponding to each focal stack in a standard 2D time-lapse until the end of the cleavage stage. The 3D models were then combined sequentially to produce 3D time-lapses. Ten such 3D time-lapses were produced, loaded into a web-based interface alongside their 2D counterparts and evaluated on a scale of 1-5 (1=no resemblance to 2D time-lapse; 5=highly accurate) by two clinical embryologists.

Results: On individual frames, the system detected 95% of cells on average with a mean intersection-over-union of 0.81. The mean embryologist score was 3.6 and the percentage of images scored 1-5 was 0%, 10%, 35%, 40%, 15% respectively. Most images (55%) were scored 4 or 5 by clinical embryologists. In the few embryos graded 2 (10%),

embryologists highlighted issues pertaining to duplicate detections of blastomeres, inconsistent detection of fragments, and blastomeres jittering between frames.

Conclusions: We have introduced a tool that enables the visualisation of cleavage stage embryos in 4D using existing time-lapse images. The tool paves the way for non-invasive studies on cell contact, adhesion, movements, and fate using clinical embryos, as well as for more biologically grounded artificial intelligence systems for embryo assessment.

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P129 Semantic segmentation of blastocyst images: A multi-centre study

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Introduction: The analysis of blastocyst images is a challenging task for artificial intelligence models. Additional difficulties arise if the models have to analyse images from multiple clinics captured with equipment different to the ones used during training. We propose a robust deep learning model for the detection and segmentation of 5 blastocyst components: zona pellucida (ZP), trophectoderm (TE), inner cell mass (ICM), blastocoel (BL), and background. Our objective was to create a model that can generalise well across datasets from multiple clinics.

Methods: Two fully connected layers were added to the U-Net architecture to create additional high-level features for the decoder part of the network. The model was trained on dataset A, a publicly available dataset of 249 blastocyst images (train/validation/blind test: 179/35/35) captured by an Olympus IX71 inverted microscope [1]. A novel hybrid loss was used, combining Binary Cross Entropy (BCE) and Dice [2] losses. The input data was pre-processed and subjected to data augmentation to improve the generalisation capacity of the model and to create additional training examples. The model was evaluated using the Intersection over Union (IoU) score on dataset A and the additional dataset B of 244 blastocyst images from three clinics captured on Embryoscope incubators.

Results: The model achieved a mean IoU score of 86.56% on dataset A. The individual components scored: 97.66% (ZP), 76.70% (TE), 82.98% (ICM), 83.27% (BL), and 92.19% (Background). Dataset B only had ICM ground truth masks. The model achieved an IoU score of 70.16% for ICM segmentation on data it had never seen before.

Conclusions: We introduced a tool for automatic labeling of blastocyst components that has performance comparable to the existing state-of-the-art models and is capable of performing high-quality segmentation for unseen data from different equipment. The tool has the potential to aid blastocyst evaluation by enabling the automated measurement of key structures.

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P130 Integrated pseudo-time analysis of endometrial epithelium single-cell transcriptome reveals gene expression patterns as potential markers of receptivity

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Background: Understanding the lineage trajectory of epithelial cells through the endometrial cycle is the gateway to improving assisted reproductive technologies. Hypothesis: A non-linear regression model of luminal epithelium provides a continuous measure of transcriptomic changes through the endometrial cycle and allows the identification of gene expression patterns associated with the window of implantation (WOI).

Method: Pseudo-time analysis of single-cell RNA sequencing data was employed to reconstruct luminal epithelium trajectory through the endometrial cycle. The Leiden algorithm[1] allowed for the clustering of cells to provide an integrated, ordered, and continuous analysis of transcriptomic changes. Finally, a non-linear regression machine-

learning algorithm[2] was applied to identify key transcriptomic expression patterns and potential receptivity markers. Random forest[2] based on a categorical definition of the endometrial cycle was also computed to allow for comparisons downstream.

Results: Pseudo-time analysis of luminal epithelium identified four clusters of genes that demonstrated consistent patterns of expression through the phases of the endometrial cycle. The analysis also revealed a change in the luminal epithelial transcriptome, potentially signalling the WOI. Machine learning based on categorical definitions computed an area under the curve of 0.5, indicating no predictive value. In comparison, non-linear regression-based machine learning yielded a correlation coefficient of 0.78. Finally, feature importance analysis identified the downregulation of genes *VIM* and *MDK*to be potentially useful in estimating receptivity.

Conclusion: The comparison between categorical and regression machine learning approaches emphasises the endometrium as a dynamic and continuous tissue. Further work correlating transcriptomic and histological features of luminal epithelium can provide a platform for studying endometrial physiology and allow for clinical application.

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P131 Can the predictive power of an in-house live birth prediction algorithm be bettered by a modified version powered by machine learning-derived automated annotations?

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Background: Time-lapse imaging provides information on timings of embryo development morphokinetics (MK). MK algorithms generally rely on manual annotation of key MK variables to rank embryos according to outcome. Manual annotation is variable in quality, can be subjective and time-consuming. Utilising a machine learning tool, to automatically annotate has the potential to be time-saving, reproducible and improve accuracy.

Method: An in-house machine learning tool was developed using modern neural network architecture to automatically annotate time-lapse videos and generate MK variables. Thresholds for confidence in these MK outputs were set relative to their predictive ability, established by statistical modelling. Manual override of these values was programmed to be requested when the threshold was not met. To compare the machine learning approach with the established method, MK data from 900 time-lapse videos, for previously unseen blastocysts, blinded for livebirth outcome, was generated by both manual annotation and the machine learning tool. Manually derived MK outputs were used to predict livebirth using the established algorithm, which was also modified to receive the automatic annotations, consider thresholds and exception handing of data omissions. Both MK algorithms incorporated the same variables; t3, t4, t5, t8, tSB and tB. The live birth prediction of both approaches was compared by calculating the area under the receiver operating characteristic curve (AUC) for each.

Results: Manual override replaced machine learning-derived MK values for tSB and tB in 77 embryos and tB alone in 60 embryos accounting for 4% of an estimated 5400 auto-annotated variables from 900 embryos. The established algorithm driven by manual annotation yielded an AUC of 0.661, and for the machine learning approach, the AUC was 0.702.

Conclusions: Machine learning auto-annotation has the capacity to reduce the time to generate MK data. When combined with thresholds to facilitate manual intervention and exception handling, livebirth prediction can improve.

P132 Does the oil we employ for our culture affect our blastocyst arrival rate? A randomised control study

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Background: Different oils are found in the market. High-density oils are proposed as the best option for embryo culture due to their stabilising and protective properties. They buffer temperature, pH and osmolality changes caused by external unfavourable conditions. Our objective was to test several oils compared to the one routinely used at our laboratory and evaluate the best for our culture.

Methods: Randomised controlled trial conducted during 2021, 202 patients (n=98 egg-donor receptors and n=104 patients) were allocated into 3 groups: Ovoil™ (Vitrolife, Sweden), Ovoil Heavy™(Vitrolife, Sweden) and Hypure™ Oil Heavy(Kitazato BioPharma, Japan). Embryos were cultured with GLOBAL®TOTAL®LP (CooperSurgical, Denmark), at 37ºC and 6% CO2 in Geri® (GeneaBiomedx, UK) incubators. Embryo development was analysed by the same embryologist until day+5/+6. Gardner's-scoring system was used for blastocyst evaluation. The outcomes evaluated were:

fertilisation(2PN), embryo-division (Div), blastocyst-arrival(%BT), high-quality blastocyst(%HQ-BT) and pregnancy(%PR). As statistical analysis, Kruskal-Wallis and Yates corrected chi-square test was performed for continuous and categorical variables, respectively. A P-value>0.05 was considered not statistically significant.

Results: Amongst the egg donor receptors: 21 used Ovoil™(control), 39 Ovoil Heavy™ and 38 Hypure™. When analysing the %BT we observed that the control had 83.0 %, Ovoil Heavy™ 77.8% and Hypure™ 72.5%, there were no significant differences among groups(P>0.05). Similarly the %HQ-BT rate was statistically not different between groups(P>0.05) being 55.6% for the control group and Ovoil Heavy™ and 50.0% for Hypure™. Likewise with the pregnancy rates. Within the patients: 29 were cultured using the control oil, 36 Ovoil Heavy™ and 39 Hypure™ Heavy. Analysing the same outcomes as the egg donor receptor, no statistical significance was observed in any outcome(P>0.05).

Conclusions: Under our culture conditions, there are no differences on the embryo development and the main outcomes analysed regarding the oils tested. More embryos should be evaluated since the number of fresh embryo transfers was low. Individual analysis should be made when considering changing the oil employed.

P133 Could the practitioner preparing the culture dishes affect outcomes in the IVF laboratory?

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Care Fertility Group

Purpose/background/objectives: Embryos are routinely cultured in a small volume of the same culture medium for up to six days. Exceptional handling skills and speed are necessary during dish preparation to avoid evaporation and subsequent osmolality changes before overlaying with oil. The objective of this study was to assess if fertilisation and blastocyst utilisation rates were affected by the practitioner performing dish preparation.

Methods: Practitioner identification (1-4) was recorded during dish preparation on the day before oocyte collection in 75 ICSI cycles, where a total of 744 oocytes were collected and 562 were inseminated. Microdrop and timelapse dishes were prepared according to Standard Operating Procedures. Fertilisation rate was defined as the number of oocytes exhibiting two pronuclei (2PN) per total number of mature oocytes injected; blastocyst utilisation rate was defined as the number blastocysts utilised (transferred/cryopreserved) per number of 2PN. Chi-square test was used to determine significant differences in outcome measure between practitioners.

Results: No statistically significant differences were found in fertilisation rates between practitioners when preparing microdrop and timelapse dishes. Blastocyst utilisation rate was statistically significantly different for embryos cultured in microdrop dishes between practitioner 1 and 4 (49.56 % (n=28) vs 22.5% (n=8), p=0.01). Blastocyst utilisation rate was statistically significantly different for embryos cultured in microdrop dishes between practitioner 2 and 4 (45.80% (n=32) vs 22 .5% (n=8), p =0.025). No differences were found in blastocyst utilisation rate where embryos were cultured in timelapse dishes.

Conclusions: This analysis suggests that a practitioner driven effect on the preparation of dishes may affect blastocyst utilisation rates. This highlights the need for thorough training, swift and precise dish preparation and continued monitoring. Larger numbers, randomisation and consideration of confounders, such as maternal age or number of oocytes collected would strengthen this analysis.

P134 The relationship between ploidy status, morphology status and day of blastulation in frozen single embryo transfers

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Background: Transferring aneuploid embryos is a major cause of implantation failure. Therefore, identifying euploid embryos for transfer is crucial to potentially improve treatment outcomes. Morphological grading and day of blastulation are currently the main selective considerations, with questionable efficacy. Pre-implantation genetic testing for aneuploidy (PGT-A) is not recommended in current UK guidelines. This study investigated aneuploidy, outcomes, and morphological associations in embryos blastulation on day 5 (D5) and day 6 (D6).

Methodology: Blastocysts undergoing PGT-A between January 2019-June 2021 at the Lister Fertility Clinic (n=1502) were retrospectively analysed. The 247/1502 which underwent frozen-thawed single embryo transfer were subsequently analysed for outcomes. Data including morphological gradings, PGT-A results, day of blastulation, and outcomes were collected from the clinic database.

Results: Ploidy status and day of biopsy had a significant association (p<0.001, V=0.100). D5 blastocysts had slightly lower rates of aneuploidy (52.0% vs 58.8%) and higher rates of euploidy (38.9% vs 28.5%) compared to D6. Morphology and ploidy status had a significant association in D5 (p<0.001) but not D6 blastocysts (p=0.294). Euploid D6 blastocysts

had significantly lower implantation rates (p=0.027) but non-inferior live birth rates (LBR) (p=0.123) compared with euploid D5 blastocysts.

Discussion: The weak association between aneuploidy and day of biopsy suggests that slower embryo development does not strongly signify aneuploidy. The non-significant association between morphology and aneuploidy in D6 blastocysts suggests poor morphology D6 blastocysts should be considered similarly to those with excellent morphology, if validated with a larger sample size. The outcomes indicate euploid D6 blastocysts have slightly inferior outcomes. LBR likely did not reach significance due to sample size.

Conclusions: D5 blastocysts should not be considered substantially superior to D6 blastocysts in practice. PGT-A may be pertinent in D6 blastocyst assessment. This would maximise the number of healthy embryos considered for transfer in IVF.

P135 Does the decision to transfer C-grade blastocyst depend on its sub-grading or women's age?

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C-grade blastocysts have been reported to have the potential to result in live births. Although different practices on the usage of C- blastocysts have been described, the pregnancy outcome from each subcategory of C-blastocysts, relative to the maternal age has not been adequately explored. We performed a retrospective analysis of consecutive single blastocyst transfers. The study group consisted of blastocysts graded as C (n=406) for TE and/or ICM. Bb-grade blastocysts, which is the next higher grade (n=616), served as the control group. Autologous fresh IVF/ICSI cycles and frozen-thawed cycles were included, both adjusted in multivariate logistic-regression analysis, with women aged 18-44 years. The study period was 1st January 2018 to 31st December 2020. Data was obtained from Create clinics, sharing the same clinical protocols. Blastocysts were graded by the '(ACE) Embryo grading' criteria; where ICM and TE are both categorised as, A, B, C, or D. Except for age, no other prognostic factors were considered. Distinction between day-5 or day-6, or expansion of blastocysts, was not made. LBR was higher with Bb-blastocysts than with C-blastocysts (30.2% vs 19.7%, p=0.002) but MRs were similar (14.1% vs 14.2%). No significant difference in LBRs and MRs was found where the ICM or TE, or both, were classified of C-grade. There was no significant difference in LBRs between C-blastocyst and Bb blastocysts in women <35 years (29.1% vs 38.5%, respectively; p=0.1) as well as in women aged >40 years (12.1%vs 16.1%, p=0.54). However, in women aged between 35-39 years, LBRs were significantly lower (17.0% vs 29.2%, p=0.004). Nevertheless, our study reinforces the idea that C-blastocysts of any subtype should still be transferred or considered for freezing.

P136 Embryonic signal regulates the porcine trophoblast cell functions

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Early pregnancy establishment in all mammals requires proper communication between the conceptus and the maternal environment. The key processes in this period are maternal recognition of pregnancy and implantation. During the maternal recognition of pregnancy the developing embryo signalizes its presence by secretion of species-specific embryonic signals (in pigs it is estradiol-17; E2). Proper early pregnancy development in pigs requires two E2 secretion peaks. The first increase of E2 secretion occurs between days 10 and 13 (maternal recognition of pregnancy), while the second peak occurs between days 15 and 18 of pregnancy (beginning of implantation). This biphasic manner of E2 release by embryos affects the endometrium and prolongs progesterone production by the corpus luteum. However, it is not known whether the embryonic signal influences the development of the embryo. Therefore, the aim of the present study was to determine if E2 affects adhesion and proliferation of porcine trophoblast cells.

Trophoblast cells were isolated from porcine conceptuses collected from gilts (n=10) on day 15 of pregnancy. Confluent trophoblast cells (50-60%) were treated with vehicle or 100 nM E2, with/without 1 M of estrogen receptor antagonist (ICI182780) and 25 M PD098059, 25 M LY294002 (proliferation and adhesion assays), 2.5 M Akt1/2 kinase inhibitor or 50 nM rapamycin (only in proliferation assay) for 24 h. Cell proliferation and adhesion were assessed using colorimetric methods. Statistical analyses were performed using one-way ANOVA, followed by Tukey post-test.

Estradiol significantly elevated trophoblast cell proliferation ($p<0.05$) and adhesion ($p<0.05$) on days 15 of pregnancy. Co-treatment with ICI182780 and selected inhibitors diminished stimulating effect of E2 in both processes. Results of the present study indicate that E2 secreted by the conceptuses likely controls in an autocrine manner the processes crucial for the proper development of embryos and placenta.

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P137 Development of an NF-kB reporter assay system to detect the effects of trophoblast derived extracellular vesicles on endometrial cells

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Embryo-maternal communication is necessary for successful implantation and pregnancy. Recently, extracellular vesicles (EVs) have been considered as the mediator of trophoblast-endometrial communication during the implantation process. However, the mechanism of this mediation is still not yet elucidated. We hypothesized that EVs mediate trophoblast-endometrial communications through NF-kB dependent pathways. The aim of this study was to develop a secreted embryonic alkaline phosphatase (SEAP) based bioassay to detect NF-kB associated embryo-maternal communications through EVs. Ishikawa cells were transiently transfected with the pNifty2-SEAP commercial vector. Transfected cells were treated with different concentrations of Poly IC (20, 40, and 60 μg/ml) to trigger NF-kB activity. The conditioned media was collected 12, 24, and 48 hours after treatment, and SEAP absorbance was detected by QUANTI-Blue detection reagent. After confirmation of Poly IC triggered NF-kB activity detection via the developed reporter assay system, the cells were co-incubated with different concentrations of EVs derived from JAr cells which mimic trophoblast-derived EVs. A significantly ($p<0.01$) increased SEAP activity by Poly IC was observed at 24 and 48 hours after treatment indicating the establishment of SEAP-based NF-kB reporter assay activity. Moreover, 10^9 JAr EVs/ml significantly ($p<0.001$) induced NF-kB activity in Ishikawa cells at all the time points (12, 24, and 48 hours). These data suggested that SEAP-based NF-kB reporter bioassay can potentially be used to understand the involvement of trophoblast EVs in EV-mediated embryo-maternal crosstalk. However, further studies are needed for a better understanding of immune reactions induced by EVs during the embryo implantation process.

P138 Novel regulation of the secretory pathway by protein O-GlcNAcylation during trophoblast differentiation affects placental hormone secretion

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Background: During implantation and placental development, cytotrophoblast cells differentiate to form multinuclear syncytiotrophoblast (STB) which secretes essential pregnancy hormones. O-GlcNAcylation is a cytoplasmic post-translational protein modification in which O-GlcNAc moieties are added by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA)[1]. The effects of O-GlcNAcylation on trophoblast biology are largely unknown.

Aim: To investigate the function of dynamically O-GlcNAcylated proteins during STB differentiation and assess how O-GlcNAcylation impacts STB hormone secretion.

Methods: O-GlcNAcylated proteins were enriched by succinylated wheat germ agglutinin (sWGA)-binding from human trophoblast stem cell (TSC)[2] lysates at stages of induced differentiation to STB (TSC, early STB differentiation [day 2], and mature STB [day 8]). Enriched proteins were identified by mass spectrometry and refined by de-enrichment with the OGT inhibitor OSMI. Immunofluorescence microscopy of TSC cultures was used to localise candidate O-GlcNAcylated proteins. TSC-derived-trophoblast organoids[3]spontaneously form STB by day 8. ELISA was used to assess secreted hormones within the conditioned media from both organoids and STB induced from TSC in the presence and absence of TMG and OSMI.

Results: 403 proteins were differentially enriched by sWGA-binding between TSC, early differentiation and mature STB ($p<0.05$). OSMI diminished enrichment in 49/403 proteins, and of these 29 had mapped O-GlcNAc sites. A cluster of O-GlcNAcylated proteins in early differentiation included secretory COP1 complex delta subunit ARCN1 (5 GlcNAc sites). Immunofluorescence microscopy revealed ARCN1 association with the Golgi reduces during STB differentiation ($p<0.05$) and that OSMI treatment blocks this effect ($p<0.05$). OSMI-treated mature STB secrete dramatically less hCG compared to control ($p<0.001$). Furthermore, both OSMI-and-TMG-treated trophoblast organoids secrete less PIGF compared to control ($p<0.01$).

Conclusion: The COP1 coatamer complex regulates the return of vesicles from the Golgi to the rough endoplasmic reticulum (ER). The results suggest O-GlcNAcylation of ARCN1/COP1; is important for STB secretory pathway function. Stress-sensitive O-GlcNAcylation may influence

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P139 Prospective use of a patient stratification tool to guide embryo transfer policy and improve outcomes

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Gateshead Fertility

Gateshead Ferility stratify all IVF patients according to a bespoke RAG-rated system, using multiple clinical factors to predict the likelihood of success. The Gateshead RAG-system has been utilised since 2014 for retrospective audit purposes to interrogate laboratory performance in good, moderate and poor- prognosis patient groups. In January 2021 Laboratory Team proposed that Gateshead RAG-system should be use as a prospective tool to actively guide the number of embryos to transfer, to optimise clinical pregnancy rates in all patient-prognosis groups (RAG) whilst minimising the occurrence of multiple pregnancies. The data supported the targetted use of double-embryo transfers (DET) in specific circumstances, determined by the muti-factoral RAG-rating rather than only female age. The propective use of the Gateshead RAG-system in embryo transfer policy has resulted in; 1. The clinical pregnancy rate in all patient categories (RAG) has improved, in comparison to 2020. 2. The targeted use of DET in selected Amber patients has improved the overall CPR from 14% to 26.1%, 3. The overall multiple pregnancy rate in 2021 was 6%. However, 50% were monozygotic twins resulting from SET. This is below the HFEA multiple birth target of 10%. In conclusion, by basing the embryo transfer policy on the RAG-system rather than female age, Gateshead Fertility have improved outcomes in all patient groups and will continue to employ this strategy.

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P140 Comparison of fertilisation and blastocyst formation in cycles using microfluidic sperm devices to density gradient sperm preparation

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Background: Microfluidic sperm sorting may allow for better selection of sperm with less DNA fragmentation, potentially resulting in improved fertilisation and lower miscarriage rates1,2,3. Studies applying microfluidic sperm selection for ICSI have reported improved embryo quality and clinical outcomes (4,5).

Objectives: The principle aim of this study was to assess whether the use of a microfluidic sperm sorting device for sperm preparation improved fertilisation and embryo development over using standard density gradient preparation techniques.

Method: This was a retrospective study comparing ICSI and IMSI cycles that used a microfluidic sperm sorting device to those that used standard density gradient preparation. It focused on a cohort of patients who had ICSI or IMSI between October 2021 and June 2022 with over 5 mature oocytes collected and sperm counts of over 5x10-6/ml.

Results: Within these two patient populations, those that had microfluidic sperm selection and those that had density gradient preparation, 245 and 834 MII oocytes underwent ICSI or IMSI respectively. The fertilisation rate (70% vs 72%) and rate of good-grade blastocyst formation (37.2% vs 36.6%) were not significantly different (p= 0.367 and p= 0.388). There was no significant difference in the age between the two groups (37.2± 3.0 and 37.1±2.8). However, it should be noted that a higher proportion of those using microfluidic sperm sorting were not in treatment in their first cycle (44% vs 58%) and therefore may have been poorer prognosis patients.

Conclusion: There is no significant difference in fertilisation or good blastocyst formation using microfluidic sperm sorting compared with a density gradient in ICSI-IMSI cases. Both methods are promising options to optimise clinical outcomes and with the potential ease on laboratory workflow, the use of microfluidics warrants further investigation.

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P141 Intrauterine injection of human chorionic gonadotropin at the time of blastocyst transfer: A systematic review and meta-analysis

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Objective: To establish if intrauterine (IU) hCG injection prior to blastocyst-stage embryo transfer (ET) improves the outcome of livebirth rate (LBR), clinical pregnancy rate (CPR), miscarriage (MR) and implantation rates (IR).

Method: A systematic literature search was performed using Medline, Embase, the Cochrane Library, and Google Scholar databases for relevant randomized-controlled trials (RCTs) from inception until July 2022, evaluating IU hCG versus either a placebo procedure or no intervention, around the time of blastocyst-stage ET.

Results: 93 citations were identified, of which seven eligible RCTs were included. The incidence of LBR and, or ongoing pregnancy rate (OPR) per ET was 36.8% [410/ 1113] in the IU hCG intervention group and 37.1% [397/1070] in the control. Using the fixed effects model (I2<50%), the overall effect of IU hCG on combined LBR and, or OPR was not significant: z=0.04 (p=0.97). The incidence of CPR per ET in the intervention was 39.2% [372/948] and 41.6% [374/899] in the control. Using the fixed effects model; the effect of IU hCG on CPR was not significant: z=0.97 (p=0.33). The incidence of MR per ET in the intervention was 6.3% [70/1113] and 6.4% [69/1070] in the control group. When using the fixed effects model, the effect of IU hCG on MR per ET was not significant: z=0.18 (p=0.86). The incidence of MR per clinical pregnancy in the intervention was 14.1% [53/377] and 15.4% [58/377] in the control. The effect of IU hCG on MR per clinical pregnancy was not significant: z=0.76 (p=0.45). Using the random effects model, the patients in the intervention group 30.1% [332/1102] showed no significant difference to the IR in the control group 31.4% [343/91090]: overall effect z=0.45 (p=0.65).

Conclusion: There is no significant impact on assisted reproductive technology (ART) outcomes when comparing the IU hCG groups to controls.

P142 Embryo cohorts with exclusively slow blastocysts on day 5 should undergo elective freeze all on day 6 to significantly improve implantation rate

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Aim: The primary objective was to assess whether a blanket freeze all embryo (FAE) policy would improve implantation rate. Secondly, could certain groups of patients who might benefit from FAE be identified?

Method and results: Retrospective data analysis for all IVF cycles performed at one centre between January 2016 and December 2021 was performed. 1927 fresh and 1290 frozen cycles were eligible for analysis in which patients were divided into four age groups (<36, 36-37, 38-39 and 40-42). Significantly improved implantation rate was observed in frozen vs fresh cycles in all groups except ages 36-37(<36: 49.9 vs 34.4% (p<.05), 36-37: 34.2 vs 27.41% (p0.71), 38-39: 35.1 vs 20.7% (p<.05), 40-42: 26.5 vs 9.4% (p<.05). However, a number of biases might explain the difference in outcomes. Addressing this bias, data were reanalysed to include transfers using only a single top quality blastocyst. No significant difference in implantation rate was noted in fresh vs frozen cycles (<36, 44.3 vs 41.4%; 36-39, 34.6 vs 34.8%; 40-42, 12.9 vs 25.0%) suggesting cryopreservation has no minimal impact. Secondly, for patients under 38, reanalysis of implantation rate was performed based on ET grade: (I)Fresh good quality blastocyst(47.4%), (II) vitrified blastocyst (44.6%), (III)expanded reduced quality blastocyst (37.0%), (IV)slow (22.7%) (V)cleavage (25.0%). Patients who underwent ET with cleavage stage or slow blastocyst had significantly reduced implantation rates when compared to patients who had a fresh or frozen expanded blastocyst replaced regardless of quality (p<.05).

Conclusion: Freeze all can be applied for patients with exclusively slow blastocysts on day 5 in an attempt to improve implantation rate within the same embryo cohort. Implantation rates should be between 37.0 and 47.4% (based on group II and III) and significantly higher than the 22.7% chance if replaced on day 5.

P143 Male and female embryos display differences in trophectoderm development with implications for routine morphological grading and the development of morphokinetic algorithms for embryo selection

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Background: Previous studies have suggested that male and female embryos may have subtle differences in their rates of preimplantation development. However, this possibility remains controversial. Apart from being of scientific interest, the question of whether the sex of an embryo can affect its growth trajectory is of clinical importance. Morphological grading, the primary method used by most clinics when deciding which embryo to prioritise for transfer, assumes that developmental rates are independent of sex. Similarly, morphokinetic strategies for embryo evaluation, using data gathered from time-lapse incubators, are likely to be compromised if male and female embryos behave differently. We sought to clarify whether the sex of embryos truly influences morphological grading.

Methods: A large number (n=1,241) of embryos underwent PGT-A at the blastocyst stage on either day-5 or day-6, using a highly validated method, and were found to be chromosomally normal. These embryos were divided into male and female groups and morphological grades were compared.

Results: The proportion of embryos biopsied on day-5 versus day-6 was identical for males and females (68% day-5 for both). No difference was observed in blastocyst expansion or inner cell mass grading on day-5. However, a highly significant difference was noted in the distribution of trophectoderm grades (A,B,C,D) (P<0.0001), characterised by a disproportionate representation of high grades amongst male embryos. 21.6% of male blastocysts had trophectoderm graded 'A', compared to 14.9% of females.

Conclusions: While most aspects of blastocyst morphology seem identical regardless of embryo sex, males tend to achieve superior trophectoderm grades. Since male embryos do not have greater viability than female embryos, this finding implies that different criteria should be used for grading trophectoderm in males and females. These results also suggest that the sex of the embryo may need to be considered when fine-tuning morphokinetic algorithms developed for embryo selection.

P144 Multi-centre assessment of the efficacy of CHLOE-EQ (fairtility) in automatically assessing zygotes on day 1

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Introduction: To assess how well CHLOE-EQ, an artificial intelligence (AI) based embryo assessment support tool, is able to assess the number of pronucleates (PN) in a zygote compared to embryologists, and whether this capability is generalised across different clinics.

Method: Time-lapse images of 6048 zygotes from three different clinics (Clinic 1: n=518, Clinic 2: n=307, Clinic 3: n=5223) were prospectively assessed by clinical embryologists on day 1 as per routine clinical procedures. Blind to human assessment, all zygote time-lapse videos were retrospectively assessed by CHLOE-EQ (Fairtility). Number of PNs was categorised as 0,1,2,3+ and the level of agreement was quantified in two was: (1) accuracy = total agreement / total number of zygotes assessed; (2) Kappa agreement across all categories (Kappa score +- 95% confidence interval). Data was assessed for each individual clinic as well as overall. Accuracy was further assessed for 2PN specifically.

Results: Overall level of agreement across all clinics was 94% (5664/6048), with similar (p<0.05) levels of agreement between the three clinics (1: 95%, 491/518; 2: 90%, 275/307; 3: 94%, 4898/5223). The overall accuracy for 2PNs was 95% (5761/6048) which was similar (p<0.05) between the three clinics (1: 96%, 499/518; 2: 91%, 278/307; 3: 95%, 4984/5223). The kappa agreement overall was almost perfect [0.834(0.819-0.850)]. This was consistent across the individual clinics which had at least substantial agreement between CHLOE-EQ and embryologist PN assessment [1: 0.846 (0.791-0.900); 2: 0.748 (0.668-0.827) ; 3:0.839 (0.822-0.855)]. The agreement observed was significantly higher than the agreement expected by chance (chance vs actual: 1:66%vs95%; 2:59%vs90%; 3:61%vs94%;p<0.001).

Conclusion: CHLOE-EQ has at least strong level of agreement with the PN assessment by human embryologists. Further studies will assess the nature of the few disagreements observed. The high agreement allows for increased consistency between operators, automatic EMR data entry, and automatic KPI assessment.

P145 Validating CHLOE-EQ as a tool to support embryo assessment automatically

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[1]Kings Fertility; [2]Fairtility

Introduction: The purpose of this study was to validate the effectiveness of CHLOE-EQ in supporting embryologists in assessing embryos for morphokinetics and selection for treatment.

Methods: From January 2021 to June 2021, following ICSI/IVF, 1762 inseminated oocytes were cultured in a time-lapse incubator and cultured to the blastocyst stage as part of routine treatment. Embryos were manually annotated daily by experienced embryologists using the Embryo Viewer software and embryos were selected for transfer based on

morphology and KIDSCORE. Retrospectively, the time-lapse data from the same embryos were automatically assessed using CHLOE-EQ , CHLOE BLAST and CHLOE RANK (three AI-based tools to assist embryologists in the assessment of human embryos), to automatically annotate embryos and predict at 68hpi their ability to reach the blastocyst stage, predict whether the embryos would be utilised (transferred or cryopreserved) or selected for transfer. The level of agreement in annotation between embryologists and CHLOE-EQ was quantified using Intracorrelation coefficient, with an ICC of 0.6 or above considered strong agreement. Prediction of embryo viability was assessed using binary logistic regression, and quantified using the area under the curve (AUC).

Results: The level of agreement between embryologists and CHLOE-EQ was strong (tPNf 0.66, t2 0.74, t4 0.76, t5 0.73, t6 0.69) and very strong (t3 0.84, t7 0.8, t8 0.83, tsB 0.92, tB 0.95). CHLOE-EQ was predictive of embryo utilisation (AUC=0.96), blastulation (AUC=0.98) and selection for transfer (AUC=0.81). CHLOE BLAST was predictive of blastulation (AUC=0.92) and CHLOE RANK was predictive of utilisation (AUC=0.81).

Conclusion: There was overall strong agreement between embryo viability assessment by human embryologists and CHLOE-EQ. CHLOE-EQ has the benefit over manual annotation of being completely automatic and objective, saving precious embryology time and increasing embryo assessment consistency.

P146 CHLOE-EQ score: A novel biomarker of embryo viability

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Introduction: Artificial Intelligence (AI) based tools have promised to improve embryo viability prediction. There is a need to validate these promises before introducing AI technologies into clinical practice. The objective was to validate the ability of CHLOE-EQ to predict embryo utilisation, decision for transfer, ploidy and clinical pregnancy.

Methods: CHLOE EQ score combines morphological and morphokinetic AI algorithms, trained on over 100,000 embryo videos, to assist in embryo selection. From January 2021 to July 2022, 8368 embryos were cultured in embryoscopes across four different clinics: clinic 1 (n=362), clinic 2 (n=5591), clinic 3 (n=653), clinic 4 (n=1762). Efficacy of prediction of CHLOE-EQ score for embryo utilisation, decision for transfer, ploidy and clinical pregnancy for each individual clinic was assessed using Binary logistic regression and quantified using the area under the curve (AUC). Data presented as (mean AUC across the four clinic ± standard deviation: clinic 1, clinic2, clinic 3, clinic 4). Ploidy and clinical pregnancy data was only available for clinic 2. CHLOE RANK (proposed ranking in order of priority for transfer) and CHLOE BLAST score were assessed relative to blastulation and utilisation.

Results: CHLOE-EQ score was predictive of embryo utilisation (0.89±0.01: 0.90, 0.88, 0.88, 0.96), decision for transfer (AUC=0.75±0.12: 0.64, 0.72, 0.89, 0.81), ploidy (AUC=0.60) and clinical pregnancy (AUC=0.72). CHLOE BLAST score was predictive of blastulation (0.88±0.02: 0.91, 0.87, 0.87, 0.92) and decision for transfer (0.86±0.08: 0.91, 0.9, 0.76, 0.74). CHLOE RANK was predictive of utilisation (0.88±0.01: 0.89, 0.92, 0.82, 0.81). There was no significant difference in the efficacy of prediction between the different clinics for CHLOE EQ, CHLOE BLAST or CHLOE RANK ($p>0.05$).

Conclusion: CHLOE-EQ is consistently predictive of embryo viability across different clinics, suggesting that CHLOE-EQ could be a valuable biomarker to support clinical decisions regarding transfer, cryopreservation or discarding.

P147 Comparison of metabolite signatures of sibling embryos inseminated by conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) using sensitivity enhanced NMR spectroscopy

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There is an increasing trend in ICSI usage irrespective of the etiology demonstrating the overuse of this insemination technique. As ICSI bypasses natural barriers, it is important to understand the embryonic behavior in relation to the mode of insemination in non-male factor infertile patients. Embryo morphology or morphokinetic evaluation give quick but not enough sensitive biomarkers about embryo developmental capacity. Hence, the main objective of this study is to understand metabolic physiology and behavior of sibling human embryos inseminated by IVF and ICSI using metabolomic approach.This prospective study included 19 infertile couples with non-male factor infertility undergoing ART treatment. The sibling oocytes from each patient was randomly inseminated by IVF and ICSI. Spent culture media (SCM) collected during 96 hours of culture along with medium control samples were profiled using sensitivity enhanced NMR spectroscopy (800MHz) equipped with cryogenically cooled micro-coil (1.7 mm) probe. A significant reduction in

the intensity of pyruvate, citrate, glucose and lysine observed in both IVF and ICSI sibling embryos compared to medium control. Further, histidine and valine level were found lower in ICSI embryos compared to medium control during 96 hours of in vitro culture. Notably, no significant differences found in metabolite intensities between IVF and ICSI SCM samples. ICSI and IVF derived sibling blastocysts have comparable SCM metabolomic signature. Keywords: non-male factor infertility, embryo metabolomics, sensitivity enhanced nuclear magnetic resonance spectroscopy, ICSI.

P148 A quantitative inner cell mass shape classification system based on symmetry and eccentricity

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Introduction: Currently, clinical ICM classifications are based on qualitative assessments of cell abundance and compaction leading to subjectivity and inconsistency. This study aimed to devise and evaluate a novel quantitative ICM classification scheme that objectively classifies ICM morphology according to symmetry and eccentricity.

Methods: 239 images of tEB embryos from 3 clinics were retrospectively annotated and computationally assessed for symmetry and eccentricity (a measure of circularity) using MATLAB software. ICMs were classified as spheres (3+ lines of symmetry), ellipses (2 lines of symmetry) or others (1 lines of symmetry). The relationship between these ICM shape parameters and maternal age, BMI, aneuploidy, implantation, live birth and embryologist-provided Gardner scores was assessed.

Results: Clinics 1 (21%:36%:43%) and 2 (21%:27%:52%) had similar distributions of sphere:ellipse:others whilst clinic 3 (44%:28%:28%) had more spheres and fewer others (p=0.02). Age, BMI, implantation and live birth were not associated with ICM shape or symmetry (p>0.05). Spheres were more likely to be aneuploid than ellipses (57% vs 37%, risk ratio=1.56, p=0.048). Embryos with 0 lines of ICM symmetry (87%) were two times more likely to be aneuploid compared to 1 line (41%, p=0.004); 2 lines (46%, 0=0.008) and 3+ lines of symmetry (50%, p=0.02). Eccentricity was not associated with implantation, live birth or ploidy (p>0.05). BMI correlated with eccentricity (R2=0.19, p=0.008). Gardner grade C ICMs were more likely to be classified as 'other' than grade B (p=0.0015) or A (p<0.00001). Moreover, embryos with Gardner grade A trophectoderm was more likely to have ICMs classified as 'ellipse' than grades B (p=0.018) or C (p=0.0084).

Conclusion: ICM shapes based on symmetry and eccentricity are directly associated with Gardner grade ICM, trophectoderm quality and ploidy.

P148 A quantitative inner cell mass shape classification system based on symmetry and eccentricity

Elaina Lausic[1]; Chloe He[1]; Neringa Karpavičiūtė[1]; Thomas Fréour[2]; Marcos Meseguer[3]; Nikica Zaninovic[4]; Ryan Miller[4]; Céline Jacques[1]; Jérôme Chambost[1]; Cristina Hickman[1]

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Introduction: Currently, clinical ICM classifications are based on qualitative assessments of cell abundance and compaction leading to subjectivity and inconsistency. This study aimed to devise and evaluate a novel ICM classification scheme that objectively classifies ICM morphology according to symmetry and eccentricity.

Methods: 239 images of tEB embryos from 3 clinics were manually annotated in a retrospective setting using the Computer Vision Annotation Tool software (Intel, Santa Clara California). The resulting annotations were computationally assessed for symmetry and eccentricity (a measure of circularity) using MATLAB (MathWorks, Natick, MA). The ICMs were classified as spheres (3+ lines of symmetry), ellipses (2 lines of symmetry) or others (1 lines of symmetry). The relationship between ICM shape parameters and maternal age, BMI, aneuploidy, implantation, live birth and embryologist-provided Gardner scores was assessed.

Results: Clinics 1 (21%:36%:43%) and 2 (21%:27%:52%) had similar distributions of sphere:ellipse:others whilst clinic 3 (44%:28%:28%) had more spheres and fewer others (p=0.02). Age, BMI, implantation and live birth were not associated with ICM shape or symmetry (p>0.05). Spheres were more likely to be aneuploid than ellipses (57% vs 37%, risk ratio=1.56, p=0.048). Embryos with 0 lines of symmetry (87%) were two times more likely to be aneuploid compared to 1 line (41%, p=0.004); 2 lines (46%, 0=0.008) and 3+ lines of symmetry (50%, p=0.02). Eccentricity was not associated with implantation, live birth or ploidy (p>0.05). BMI correlated with eccentricity (R2=0.19, p=0.008). Gardner grade C ICMs were more likely to be classified as 'other' than grade B (p=0.0015) or A (p<0.00001). Gardner grade A trophectoderm was more likely to be classified as 'ellipse' than grades B (p=0.018) or C (p=0.0084).

Conclusion: ICM shapes based on symmetry and eccentricity are directly associated with Gardner grades for ICM and trophectoderm quality, as well as with embryo ploidy.

P149 Zinc finger protein 81; a potential extracellular vesicle linked mediator of embryo maternal communication

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Zinc Finger protein 81 is encoded by ZNF81 gene and is known as a potential DNA binding transcription factor. Previously we have shown that JAr trophoblast spheroid derived extracellular vesicles (EVs) can specifically down-regulate the ZNF81 gene expression in receptive endometrial cells in vitro (1). However, the exact subcellular localization of ZNF81 protein and its functional role in the human endometrium are yet to be described. We investigated the ZNF81 protein expression and subcellular localization in receptive endometrial epithelial cell (EEC) analogue RL95-2 cells and trophoblast cell analogue JAr cells using confocal microscopy. The expression of ZNF81 protein in EEC under the eostrogen and progesterone concentration combinations mimicking follicular and luteal phases of the menstrual cycle was also investigated. The confocal analysis revealed that ZNF 81 protein mainly has a perinuclear cytoplasmic localization. The localization was significant in perinuclear intracellular vesicles and weak in the cytoplasm in both EEC and JAr cells. JAr cells had high abundance of ZNF81 protein compared to RL95-2 cells ($p<0.05$). ZNF81 protein expression was significantly higher in hormone treated groups compared to the untreated controls ($p< 0.05$). However, no significant differences of ZNF81 protein expression was detected between luteal and proliferative phase mimics ($p>0.05$). The perinuclear localization of ZNF81 protein hints to its possible role as a transcription factor. As ZNF81 was responsive to oestrogen and progesterone, it could be differentially regulated in vivo in different phases of the menstrual cycle. Thus, studying the changes of this protein in human endometrium during menstrual cycle is warranted. Further studies are required to decipher exact functional role and mechanism of ZNF81 protein in EV mediated human endometrium and embryo communications.

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P150 Several factors (position, compaction, ICM cell number, focus) impact ICM detection and therefore quality evaluation, however getting the ICM in focus reduces the disparities in ICM detection

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Purpose: To study the impact of four factors (position, compaction, ICM cell number, focus) on the variation of ICM detection by embryologists and AI models and to observe the reduction of disparities when the factor with the greatest impact is reduced.

Methods: The study was conducted on two datasets DbA/DbB. ICM detection variations were evaluated with Intersection over Union (IoU).
DbA contains 201 blastocyst images extracted from timelapses from a French clinic (2008-2010) and annotated by three embryologists (E1, E2, E3). E1 added to the DbA annotations information concerning ICM positioning relative to embryo, focus, compaction, ICM cell number.
The publicly available dataset DbB, published by [1] with ICM annotations of 249 blastocyst images, was annotated again by E2.
Two AI models (DeepLab/resnet101) were trained: first (AI1) on DbA split into train(141 embryos)/test (60) subsets, second (AI2) on DbB split into train(199)/test(50) subsets.

Results: On DbA_test, the inter-operator variation was high according to the average IoU: E1vsE2: 0,52; E2vsE3: 0,46; E1vsE3: 0,51, with similar results for AI1: AI1vsE1: 0,54; AI1vsE2: 0,47; AI1vsE3: 0,54. All four factors have the same impact on AI1-human and inter-operator variation: Position (IoU middle/side: HvsH: 0,41/0,65, AI1vsH: 0,46/0,59) ; Compaction (Compacted/Mostly compacted/Mostly dispersed: HvsH: 0,69/0,58/0,46, AI1vsH: 0,70/0,56/0,45) ; ICM Cell Number (Lots/In between/Few: HvsH: 0,70/0,49/0,48 AI1vsH: 0,67/0,49/0,48) ; Focus (In Focus/Partially/Not in focus: HvsH: 0,68/0,58/0,39, AI1vsH: 0,64/0,58/0,41).
The largest variation is obtained when ICM is out of focus. Images from DbB were selected to have good focus on ICM which leads to much lower variation: HvsH: 0.84, AI2vsH: 0.80.

Conclusions: When training embryologists or AI models, all four factors should be considered, but especially having the ICM in focus. Choosing the focal image with ICM in focus can reduce variation, but is longer than assessing one image. Using AI becomes essential, although it should be checked and corrected by experts if necessary.

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P151 Kolaviron protects the hippocampus of fetal wistar rats against aluminium chloride-Induced toxicity in-utero

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Introduction: Aluminum is an ubiquitous metal that is able to generate reactive oxygen species and cross both placenta and blood- brain - barrier to exert its effect. Kolaviron is an ethanolic extract of Garcinia kola which contains bi-flavonoids rich in antioxidants and anti-inflammatory properties.

Aim: This study investigated the role of kolaviton on aluminium chloride- Induced toxicity on the hippocampus of fetal Wistar rats in-utero.

Materials and methods: Female Wistar rats (25) were randomly selected, mated and then assigned into 5 groups of 5 (n=5) once mating was confirmed through vaginal smear. Group A received distilled water, group B; 0.6mls of corn oil, group C; 200 mg/kg of Kolaviron, group D; 100 mg/kg of aluminium chloride and group E; 100 mg/kg of aluminium chloride + 200 mg/kg of kolaviron. Administration was done orally in the 2nd week of gestation from days 8-10. Pregnant animals were sacrificed on day 20 of gestation; fetuses, their brains and hippocampi were excised respectively. Hippocampal tissues of fetuses were homogenized in 0.25M of sucrose solution for biochemical assay which included acetylcholinesterase, cytochrome -- c oxidase, glucose-6-phosphate dehydrogenase while some were fixed in 4 % paraformaldehyde for immunohistochemical studies.

Results: The group treated with kolaviron showed significant decrease in acetylcholinesterase levels while cyt-c oxidase and glucose-6-phosphate dehydrogenase were improved compared to the aluminium chloride only group. A reduction in glial fibrillary acidic protein positive cells and neuron specific enolase expression were also observed in the kolaviron-treated group.

Conclusion: This study demonstrated that kolaviron could serve as a therapeutic tool in the treatment of aluminium chloride -- induced toxicity associated with fetal hippocampal degeneration in--utero owing to its antioxidative and anti-inflammatory properties.

Key words: hippocampus, fetus, kolaviron, oxidative stress, aluminium chloride

P152 Ovine conceptuses utilise FGF23-KL signalling to regulate phosphate homeostasis and cellular proliferation

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Purpose: Despite the appreciation of an essential role of phosphorus in conceptus development, the mechanisms regulating phosphate homeostasis at the maternal-conceptus interface remain poorly understood. Fibroblast growth factor 23 (FGF23) is a regulator of phosphate homeostasis postnatally through interactions with Klotho (KL), and we have recently demonstrated abundant expression of both molecules at the ovine maternal-conceptus interface. This study investigated the utilisation of FGF23-KL signaling by ovine conceptuses to regulate phosphate homeostasis, cell proliferation, and expression of extracellular matrix, apoptosis, and inflammation regulatory mRNAs.

Experimental Design: An ovine trophectoderm cell line (oTr1) was cultured in medium containing phosphate (1, 2, 5 or 10mM), recombinant FGF23 (1, 10, 50 or 100ng/mL) or KL (2g/mL). Phosphate and calcium in medium were quantified spectrophotometrically, expression of candidate mRNAs was quantified by qPCR, and cell proliferation was quantified.

Results: Treatment with 10mM phosphate increased proliferation of oTr1 cells 18% ($P<0.05$), upregulated expression of fibronectin (FN) ($P<0.05$), and downregulated expression of FGF23 ($P<0.001$), SLC20A1 ($P=0.069$) and SLC20A2 ($P<0.0001$) (sodium-dependent phosphate transporters), BCL2 associated X (BAX; $P<0.05$), caspase 3 (CASP3; $P<0.0001$), and transforming growth factor beta (TGFB; $P<0.05$) mRNAs. In contrast, oTr1 cells cultured with 5mM phosphate had greater expression of FGF23 ($P<0.01$) and SLC20A2 ($P<0.01$) mRNAs. FGF23 and KL supplementation significantly altered phosphate and calcium release into the medium and increased proliferation oTr1 cells in a dose dependent manner. oTr1 cells cultured with 10ng/ml and 50ng/ml FGF23 had lower expression of CYP24 (encodes 24-hydroxylase; $P<0.05$) and SLC20A2 ($P=0.06$) mRNAs, respectively. Additionally, oTr1 cells cultured with 50ng/mL and 100ng/mL FGF23 had greater expression of TGFB ($P<0.05$) and FN ($P=0.10$) mRNAs, respectively.

Conclusion: These findings suggest oTr1 cells utilize FGF23-KL signalling to regulate phosphate homeostasis, proliferation, and expression of apoptosis, inflammation, and extracellular matrix regulators; suggesting an important role for FGF23-KL signalling in conceptus development.

P153 Chemerin in egg albumen and viable cells number of germinal disc: Potential biomarkers of the embryo development for genetic selection in birds

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Background: One of the breeding companies goal is the production of viable and robust chicks. A major issue is the embryo mortality that represents 5 to 40% according to avian species. New fertility biomarkers are necessary to improve reproductive performances and genetic selection.

Objectives: We investigated two potential molecular markers of embryo development: chemerin concentration in albumen and the number of viable cells in the germinal disc at the oviposition time.

Methods: We studied two layer breeds, two broiler breeds and one duck breed. Eggs from 50 females were collected during one week laying at three periods of the laying cycle (before, after laying peak and at the end of laying period). For each egg, an albumen sample was collected to measure chemerin concentration by a home made ELISA assay. The germinal disc was dissected to count viable cells. For each biomarker, the different breeds were compared by one-way ANOVA. Then, concentration of chemerin in albumen and viable cells number of germinal disc were correlated with reproductive performances by Pearson correlation.

Results: The variability of chemerin concentration in albumen is higher inter-hen compared to intra-hen for each breed. We observed significant different concentrations of chemerin in albumen between breeds during the laying cycle. Chemerin concentration in albumen is positively correlated with laying, fecundity and fertility rates for layer breeds. Moreover, the number of viable cells of germinal disc is correlated: -negatively with laying and positively with fecundity rates for layers -positively with laying and hatchability rates for broiler -and negatively with fertility and hatchability rates for duck.

Conclusions: Chemerin concentration in egg albumen and viable cells number of germinal disc may improve some reproductive parameters that's why we will study these biomarkers on a 2nd generation. These biomarkers could be involved in embryo development in chicken/duck and used in genetic selection.

P155 Ovarian torsion in IVF patients - a case series

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Introduction: Ovarian torsion is a gynaecological emergency which can lead to significant morbidity, including loss of fertility. Diagnosis can be challenging, with imaging often inconclusive. We present a case series of patients who were diagnosed with torsion following IVF treatment over a 14 month period in our unit.

Methods: Paper and electronic records for all patients who had an ovarian torsion after IVF treatment from August 2020 to October 2021 were reviewed. Data collected included demographics, risk factors for torsion, IVF drug regimen and response, blood results, ovarian imaging, operative intervention and pregnancy outcome.

Results: Four patients were affected, with one patient (A) having contralateral torsions in two successive IVF cycles. Three out of four patients (A,B and C) had risk factors for ovarian torsion (polycystic ovary syndrome or ovarian hyperstimulation syndrome) and two of them (A and C) had a large discrepancy in ovarian sizes during serial ultrasound scans. Patients A, B and C underwent a right salpingo-oophorectomy whilst Patient D underwent a laparoscopic detorsion and retention of the left ovary. Patient A had a subsequent laparoscopic detorsion of the left ovary with viability of the ovary retained. In four cases of torsion, the patients were all ≤13 weeks pregnant at the time of operative intervention. No detrimental effect on pregnancy outcomes attributable to the ovarian torsion was noted, with all four patients having a live birth between 36+1 and 40+3 weeks gestation.

Conclusions: Learning points from this case series include: * A large discrepancy in ovarian size cannot be attributed to ovarian stimulation alone. * Torsion can occur in the absence of raised inflammatory markers. * Consider elective buserelin trigger in patients at high risk of OHSS. * Early intervention may allow detorsion and preservation of ovary. * Data are reassuring regarding the effect of torsion on pregnancy.

study in humans.

P157 Age related fertility decline: Is there a role for elective ovarian tissue cryopreservation?

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Background: Age related fertility decline (ARFD) is a prevalent concern amongst western cultures due to the increasing age of first-time motherhood. Elective oocyte and embryo cryopreservation remain the most established methods of fertility preservation (FP), providing women the opportunity of reproductive autonomy to preserve their fertility and extend their childbearing years to prevent involuntary childlessness. Whilst ovarian cortex cryopreservation has been used to preserve reproductive potential in women for medical reasons, such as in pre or peri pubertal girls undergoing gonadotoxic chemotherapy, it has not yet been considered in the context of ARFD.

Aims: In this review we summarise the current literature on elective oocyte cryopreservation (EOC) and introduce the prospect of elective ovarian cortex cryopreservation (EOTC) as an alternative method of FP for ARFD.

Methods: A narrative literature search of major databases (Pubmed, Medline, Google Scholar, Web of science) was performed. The literature was critically appraised by world renowned experts in the field of reproductive medicine. Results: The clinical application of EOTC is undoubtedly feasible as a method of fertility preservation for medical indications and with more than 200 reported livebirths, is no longer considered an experimental procedure. EOC is not without risks, including those associated with controlled ovarian stimulation and being restricted to store a finite number of oocytes giving a reasonable probability of achieving a livebirth based on the woman's age. EOTC provides the opportunity to preserve hundreds of primordial follicles at once, thereby not restricting women to a finite number of oocytes cryopreserved.

Conclusions: Evidence strongly suggests that EOTC could provide an alternative option to EOC, overcoming some of the aforementioned challenges, by facilitating spontaneous conception and not being curtailed by a limited number of oocytes for cryopreservation.

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P158 Exploring the knowledge, attitudes and perceptions of women of reproductive age towards fertility and elective oocyte cryopreservation in the United Kingdom

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[1]Imperial College London; [2]Lister Fertility Clinic; [3]Centre for Reproductive and Genetic Health

Objective: To evaluate the knowledge, perceptions and attitudes towards fertility and elective oocyte cryopreservation (EOC) in women in the UK.

Methods: An online cross-sectional survey was published on the social media platform Instagram, between 25th February 2021-11th March 2021. All women aged 18-50 years old from the UK were invited to participate.

Results: 5,482 women fulfilled the inclusion criteria. The mean age of participants was 35.0 years old (±10.25 SD; range 16-52). Over half (n= 3,158; 57.7%) agreed or strongly agreed they were prepared to become pregnant at a later age, despite associated risks of advanced maternal age. Three quarters (74.1%; n=4,055) disagreed or strongly disagreed they felt well informed regarding the options to preserve their fertility, in case of a health related problem or ARFD. Almost three quarters (n=4,007; 73.2%) reported an awareness of EOC. Only 10.4% (n=566) believed a single cycle of ovarian stimulation would be adequate enough to retrieve sufficient oocytes for cryopreservation and 11.0% (n=599) believed EOC may pose significant health risks and affect future fertility. Less than half agreed or strongly agreed the lack of awareness regarding EOC has impacted the likelihood of them pursuing this method of fertility preservation further (n=2,259; 41.4%).

Conclusion: Awareness of ARFD and EOC has improved significantly compared to studies carried out almost a decade ago. However, inconsistencies in knowledge remain regarding the rate of miscarriage and likelihood of spontaneous conception amongst specific age groups. Further education regarding the financial costs of undergoing EOC and the optimal age to undergo the procedure to increase the chances of successful livebirth are also required. Clinicians should encourage earlier fertility counselling, to ensure that EOC is deemed a preventative measure of ARFD, rather than an ultimate recourse to saving declining fertility.

P159 Mechanisms of ovarian aging in mammalian species: A systematic literature review

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Background: Ovarian aging is a multi-factorial process that is characterized by the age-related progressive decline in the quality and quantity of ovarian primordial follicles. The objective of this study was to systematically analyse current literature on the mechanisms of ovarian aging in mammalian species.

Methods: Five databases used for the search included EMBASE OVID (1947 onwards), MEDLINE OVID (1946 onwards), Web of Science, Scopus, and The Cochrane Library. The search ran from 1st January 2000 to 1st December 2021 using keyword search terms: 'ovar*' and 'reserve' and 'aging' or 'senescence' and 'decline' or 'loss' and 'folic*'. Search parameters included original research articles in English that explored ovarian aging in human and mammals. Reviews, book chapters, case reports, male reproductive aging, and non-mammalian animal models were excluded.

Results: A total of 188 studies were accepted. These studies suggested that the leading molecular mechanisms of ovarian aging are the independent but mutually inclusive accumulation of oxidative stress damage and DNA mutations in follicular cells. These mechanisms were induced by endogenous (gene mutations, epigenetic modifications, DNA damage and repair, telomere shortening and telomerase inactivity, X-chromosome alterations, mitochondrial and metabolism dysfunction, inflammation, HPO axis dysfunction, intra-ovarian morphological changes) and exogenous (poor maternal nutrition, obesity, cigarette smoking, environmental pollutants, chemotherapeutic agents, and drugs) factors that impact signalling pathways and cellular processes involved in follicle development and survival. These include metabolism, cell cycle, autophagy, chromosome segregation, folliculogenesis and steroidogenesis, and DNA repair. Thus, leading to follicle senescence and apoptosis. Preliminary evidence suggested that exercise, calorie restriction, antioxidants, and anti-ovarian aging agents may alleviate ovarian aging phenotypes.

Conclusions: Insight is provided into the molecular, structural and functional mechanisms underlying mammalian ovarian aging. This knowledge will prove invaluable for the development of interventions to preserve the ovarian reserve and so alleviate ovarian aging phenotypes.

P160 Transcriptomic profile of the ovarian cortex & the effect of whole ovary cryopreservation on DNA methylation

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Background: Whole ovary cryopreservation (WOCP) and transplantation in a study led by Campbell shows restoration of ovarian function and natural fertility resulting in multiple live births in sheep (1). This technique promises to restore complete ovarian reserve for women seeking to preserve their fertility for social or medical reasons. It is therefore necessary to study the possible variations in epigenetics that might be induced by the procedure. Employing microarray and DNA methylation ELISA, this study takes a step to investigate these immediately following WOCP and thawing.

Method: A total of three (3) reproductive tissues (control, CT, n=3 ovaries and WOCP, n=3 ovaries) from abattoir-sourced sheep was used. The WOCP group was cryopreserved by slow freezing in the method described by Campbell (1) in a previous study and thawed after which the cortex was obtained for RNA and DNA extraction for microarray by Affimetrix and DNA methylation respectively.

Results: The complete gene list was a total of 24596. After applying p-value <0.05, a total number of 2557 genes remained with 726 being unidentified with 1831 being identified genes. Using the Partec software, with FDR no genes were generated, however with no FDR and excluding all unknown genes, a total number of 114 genes was generated with 25 genes of p-value <0.05. Using Webgetstalt, genes including GNRH, BAX & MRPL24 involved in cell maturation, apoptosis and RNA binding were identified to be upregulated with significant enrichment scores. After the ELISA, the concentration of 5mC was 0.47, 0.61 and 0.62 for the controls and 0.83, 0.77 and 1.12 for the WOCP samples.

Conclusions: These results indicate that whole ovary cryopreservation by slow freezing does show variation in transcriptomic RNA and DNA methylation compared to the control.

Keywords: Whole ovary, Cryopreservation, Slow freezing, Microarray, DNA Methylation

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P161 Can donor age affect thawed donor oocyte cycle outcomes?

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Reduced pregnancy rates are correlated with advanced maternal age and increased incidence of aneuploidy. Donor oocytes can improve the chances of a successful outcome in patients of advanced maternal age. HFEA legislation restricts oocyte donor ages from 18 to 35 to optimise recipient outcomes. However, a study by Roca-Feliu et al (2022) concluded that donors aged 25 and below had significantly lower live birth rates, compared to donors that were aged above 25. This study aimed to assess the impact of donor ages on treatments using frozen donor oocytes. This retrospective study analysed treatments using frozen donor oocytes from January 2020 to May 2022. The ages of the donors were categorised into three groups; donors aged under 20 (group 1), donors aged between 20 and 25 (group 2) and those aged above 25 (group 3). Survival rate (SR), fertilisation rate (FR), positive hCG rate (POS), clinical pregnancy rate (CPR), blastocyst formation rate (BR) and utilisation rate (UR) were analysed using Chi-squared testing to identify significant differences. Of the 751 cycles assessed 81.3% SR, 72.9% FR, 57.3% POS, 42.9% CPR, 66.8% BR and 47.4% UR were achieved. A significantly higher SR was observed for group 1 (90.8%) compared with groups 2 (81.1%) and 3 (80.9%) ($p<0.005$, $p<0.001$ respectively). Significantly higher BR were observed for group 2 (67.6%) and 3 (67.0%) compared with group 1 (60.9%) ($p<0.01$, $p<0.01$ respectively). Significantly higher UR were observed for group 2 (47.4%) and 3 (48.1%) compared with group 1 (36.4%) ($p<0.05$, $p<0.05$ respectively). No other differences were observed. Oocytes from donors aged under 20 achieve significantly higher SR, however oocytes from donors aged 20 and above observe significantly higher BR and UR. Although, CPRs are not significantly different, oocytes from donors aged 20 and above have a higher UR, hypothetically leading to higher cumulative CPRs.

Roca-Feliu, M., Clua, E., García, S., Polyzos, NP., Martínez, F., Recipient outcomes in an oocyte donation programme: should very young donors be excluded? Reproductive BioMedicine Online Volume 44, Issue 5, May 2022, Pages 867-873.

P162 A 2-year follow-up study of ovarian reserve in female survivors of childhood cancer

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Aim: The overall likelihood of a woman achieving a pregnancy following cancer treatment is reduced by nearly 40%. There is limited prospective data examining changes to ovarian reserve in young survivors over time. We sought to quantify AMH and antral follicle count in a cohort of young adult female survivors of childhood cancer.

Methods: Female survivors of childhood and adolescent cancer were invited to attend for fertility assessment and consultation. As measures of ovarian reserve, antral follicle count (AFC) and serum Anti-Mullerian Hormone (AMH) levels were measured. Demographic and treatment details were recorded.

Results: AMH levels at first consultation ranged from a critically low 0.3pmol/L up to 64.3pmol/L. A similarly broad range was noted in the AFC, which ranged from 3-33 follicles. Of 22 patients who attended for assessment we had complete follow up data for 13 patients. This demonstrated a reassuring trend in AMH levels, with 12 patients demonstrating either an increase or stability of AMH and AFC.

Discussion/Conclusion: Our study will enable us to provide reassurance to female survivors of cancer treatment, even those who have undergone high-risk gonadotoxic treatment. In our study, the majority of the female survivors had reassuring results. We hope that by highlighting this service, care providers will be encouraged to refer such survivors for assessment.

P163 Application of a mouse model to investigate the effect of high-grade serous ovarian cancer on the ovaries

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Introduction: It is estimated that one in fifty women will be diagnosed with ovarian cancer in their lifetime, of which 12% will be within their reproductive years (1). A routine treatment of ovarian cancer involves the surgical removal of the ovaries and fallopian tubes, as the latter may be the major site of origin of this cancer (2,3). Novel mouse models have been designed to investigate ovarian cancer and the mechanisms involved (4). We investigated the incidence of ovarian cancer in a high-grade serous ovarian cancer (HGSOC) mouse model for future application for ovary-focused fertility research.

Methods & materials: Ovaries were provided from mouse models of HGSOC (from mice injected intraperitoneally with HGSOC cell-lines HGS2, HGS3, and 60577 as previously described by the Balkwill group) (4). Gross anatomical evaluation of ovaries was performed to identify suspected tumours and potential necrosis.

Results: Ovarian-specific tumours were identified in 21% (13/62) of ovaries collected from the HGSOC-model mice injected with the HGS2 cell-line (n = 31). No tumours were present in the ovaries of mice injected with the HGS3 (n = 3) nor 60577 (n = 4) cell-lines. Necrosis was present in 7.9% (6/76) of total ovaries, but not within the ovarian tumour.

Discussion: Despite the nomenclature and being a well described model of ovarian cancer, few of the total HGSOC-model ovaries appeared cancerous even with a significant tumour burden at time of death, probably because the bursa acts as an anatomical barrier. It is important to note that ovarian cancer diagnosis does not require the primary tumour to be present within the ovary (5). Therefore, although this model is valuable for investigating the tumour microenvironment, it was not ideal for investigating cancer within the ovaries due the low ovarian tumour incidence following intraperitoneal injection.

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P164 The epoxiconazole fungicide impairs human granulosa cells proliferation and steroidogenesis with contrasted effects in obese, normo-weight and PCOS women: Role of aryl hydrocarbon receptor expression?

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Introduction: Epoxiconazole (EPOX) is among the most widely applied fungicides in conventional agriculture in worldwide. It was found in 2021 in soils (up to 151 nM), surface water (up to 0.8 nM) and groundwater (up to 1.4 nM) in France. Polycystic ovary syndrome (PCOS) and obesity are one of the main causes of infertility, affecting 10-20% of women of childbearing age in France. The objectives of our study were to investigate the EPOX effects and the associated potential molecular mechanism on cell proliferation and steroidogenesis (progesterone (Pg) and estradiol (E2) release) of granulosa cells (HCG) from four groups of patients: normo-weight and non PCOS (N), normo-weight and PCOS (PN), obese non PCOS (O) and obese with PCOS (PO).

Methods: We exposed HCG to EPOX doses ranging from 12.5 µM to 100 µM. Cell proliferation and steroid secretion were determined by BrdU and ELISA assays, respectively.

Results: In response to exposure to EPOX at higher doses, a greater decrease in cell proliferation was significantly observed in HCG from PN and PO (31.5 ±4.6 and 36 ±0.85 %) as compared to N group. The secretions of Pg and E2 were also more significantly inhibited in response to EPOX in PN, O and PO groups than in N group. We have also shown a significant increase in the expression of the gene encoding the nuclear receptor, aryl hydrocarbon receptor (AhR) at the EPOX higher doses in PN, O and PO as compared to N cells. We next investigated the effect of EPOX in the human KGN granulosa cells line invalidated for AhR (AhR-/-). AhR-/- cells had a lower decrease in Pg and E2 secretion in response to EPOX exposure.

Conclusion: In HCG, EPOX impairs steroidogenesis partly through AhR expression.

P165 Microvesicles composition from follicular fluid changes according the metabolic status and the PCOS syndrome

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Background: Gamete maturation in mammals requires extensive signaling between germ cells and their supporting somatic cells. Extracellular vesicles are very small vesicles that circulate in fluids such as blood or follicular fluid. Few studies have been carried out in women, but some of them have shown the importance of microvesicles in oocyte quality. The objective of this project is to obtain a "global" view of the content of microvesicles from follicular fluid in

women with a BMI <25 or BMI>30 without or with PCOS syndrome. In addition, we compared microvesicles from plasma versus follicular fluid in the same patients to investigate their differential protein composition.

Methods: Microvesicles were individually purified from a total of 38 patients with either normal weight (n=20) or obesity (n=18) and/or PCOS syndrome (n=12) in both follicular fluid and blood plasma. Microvesicles morphology and concentration was analyzed by nanosight. Protein analysis was performed by Digestion FASP protocol and trypsin then Analyze nanoLC-MS. Identification of protein was performed by MaxQuant v1.5.8.3 and Statistical and comparative analysis with Perseus software 1.6.10.43.

Results: In all patients, microvesicles present similar morphology and diameter. In microvesicles from follicular fluid, 1195 proteins were identified with 5% of specific protein from translation protein family detected in PCOS patients as compared to control. In addition, 154 proteins are only detected in obeses patients which are mainly proteins from the adaptative immune system. Finally, the comparison in same patient of microvesicles purified from blood and follicular fluid has shown 45% of proteins specific from follicular fluid which are involved in lipid and steroid synthesis and transporters.

Conclusion: In conclusion, the analysis of proteome has identified that a half proteins are specific from follicular fluid and several proteins could be used as specific markers of the PCOS status or change in the metabolism.

P166 Effect of Ocimum gratissimum leaf extract on ovarian follicle development in Wistar rats

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Background: This study investigated the effect of Ocimum gratissimum leaf extract (OG) on ovarian follicle development in Wistar rats.

Method: Fifteen female Wistar rats (130-150g) with regular oestrus cycles were randomly divided into three (3) groups (n=5). Group 1 (control) received distilled water (0.5 mL/Kg) while groups 2 and 3 were administered 300 mg/kg and 400 mg/kg of OG respectively for 21 days. In anasthetized rats, blood was collected for serum assay of progesterone, estradiol and Tumor Necrotic Factor-alpha (TNF-alpha). Right ovary was obtained and fixed for histomorphometry determination of developing follicles and graffian follicular counts while the left ovary was homogenized for determination of Malondialdehyde (MDA) level, Catalase and Superoxide Dismutase (SOD) activities. Data were summarized as mean ± SEM and analyses using one-way ANOVA at $p<0.05$.

Results: Progesterone significantly decreased in groups 2 and 3 compared with the control while estradiol was not different across all groups. Graffian follicle count was reduced by OG in groups 2 and 3 compared with the control while the number of developing follicles was not statistically different across all groups. Superoxide dismutase activity decreased and catalase activity increased in group 3 compared with the control while MDA was not different across all groups. Decreased TNF-alpha level was observered in the two OG treated groups.

Conclusion: Findings from this study indicate that antioxidant activities of Ocimum gratissimum may perturb ovulatory process in female Wistar rats.

P167 Effect of androgen signalling on the transcriptome of mouse preantral follicles

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Background: Polycystic ovary syndrome is a condition that affects >5% of women of reproductive age and is often associated with ovarian dysfunction due to excessive androgen production. Androgens can act on small, preantral follicles to stimulate growth and development via actions on granulosa cells (GCs). The aim of this study is to extend earlier studies (1) and gain a fuller picture of the androgen-induced transcriptomic changes that occur in mouse preantral follicles using next-generation sequencing.

Methodology: Forty-eight preantral follicles were dissected from a 16-day old mouse and equally distributed between the following groups in culture: dihydrotestosterone (DHT; 10nM), DHT+flutamide (10nM+20µM), flutamide (20µM) and control (no supplementation). Follicles were imaged every 24H for analysis of growth and at 72H were pooled and processed for RNA library preparation and sequencing (n=6 mice). Data was processed in Galaxy and differential expression and ontology analyses were carried out using EdgeR (Degust) and WebGestalt, respectively.

Results: Follicles increased significantly in size throughout culture regardless of treatment group, due to GC expansion. DHT promoted a significant increase in GC growth relative to all other groups at 48H and 72H ($P<0.05$). Sequencing identified 6177 transcripts that were consistently expressed in all samples in at least one treatment group, with 539 being differentially expressed (FDR<0.05). The majority of differentially expressed transcripts (DETs) in the DHT group were inversely related to those in the other groups. Gene set enrichment of DETs in the DHT group revealed strong

associations (FDR<0.05) with metabolic processes and ovarian steroidogenesis, specifically highlighting upregulation of androgen biosynthesis enzymes.

Conclusions: Preantral follicles exposed to elevated androgens promote GC proliferation and differentiation thereby creating a self-propagating mechanism of androgen hypersecretion. This study solidifies earlier findings but also provides a broader picture of the molecular changes that may occur in early follicles in an environment of androgen excess.

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P168 Optimisation of the energy requirements and tissue dimensions needed to support preantral follicle development in ovarian cortex in vitro

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Background: The therapeutic production of in vitro-derived follicles has been proposed for fertility restoration in oncofertility patients. Pilot experiments were conducted using ovine cortex culture as a model to optimise the energy requirements and tissue dimensions needed to support preantral follicle development in vitro.

Methods: Abattoir-derived ovine cortex biopsies were cultured over 15-20 days at 38.5C in humidified air. Initial experiments cultured five 2.5mm cortex biopsies per well, 6.89 0.28 mg (0.27 0.01 mg/mm2, n=6) on Millipore PTFE inserts, in 500l of defined, serum-free -MEM media containing 5.5 mM (n=3) or 16.65 mM (n=3) glucose. The impact of reduced tissue thickness and weight (< 2mg of cortex/500l media, n=3) on follicle growth was then tested in media containing 5.5 mM glucose. Tissue was weighed before and after culture. Growing preantral follicle content was visualized in situ at the end of culture and confirmed by mechanical or enzymatic follicle isolation. Media changes occurred at 24 hours, and 48-72 hours intervals. Media glucose and lactate content were analysed by fluorometric assays.

Results: No significant interaction was detected between time or media glucose concentration (P>0.05) and cortex glucose or lactate metabolism/mg tissue/hr. Lactate production/mg tissue/hr decreased significantly (P<0.05) over time. No significant (P>0.05) interaction occurred between culture conditions and glycolytic indices, but the latter decreased significantly (P<0.05) over time. A strong negative correlation (P<0.001) existed between cortex starting weight (0.23 0.02mg/mm2, n=9) and weight loss/day in culture (-0.11 0.02 mg/day, n=9). Neither 5.5 mM nor 16.65 mM glucose supported preantral growth in large cortex pieces. Reducing cortex mass to 1.64 0.09 mg/well, (0.23 0.01 mg/mm2, n=3) yielded growing follicles (189.1 9.93 m diameter, n=14) in 50.0% of cultured tissues.

Conclusion: These pilot studies demonstrate that optimal energy substrate provision and cortex dimensions are needed to minimize tissue stress and maximise follicle growth potential.

P169 Relationships between Insulin like growth factors (IGF1 and IGF2) genes and steroidogenesis in ovine ovarian antral follicles

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Background: Reproductive inefficiency in sheep is critical source for reduction of the livestock profitability worldwide. The importance of insulin-like growth factor 1 and 2 belonging to insulin-like growth factors system were identified as pivotal key roles in the ovarian follicular development and steroidogenesis in different species (1). This study aimed to detect the IGF-1 and IGF-2 genes and measure the viability in the ovine small and large antral follicle, as well as to identify putative biological functions using bioinformatics approaches.

Methods: Sheep ovaries (n=50) were collected from an abattoir and healthy follicles were dissected out based pre-established criteria (2,3) and their diameters measured using Vernier Calliper. IGF-1 and IGF-2 genes and Follicular oestrogen level were detected using PCR and ELISA, respectively. Construction of protein-protein interaction network and gene enrichment analysis were obtained using String software.

Results: Our results showed that IGF-1, IGF-2 and CYP19A1 were markedly present in small and large antral follicles of the local sheep ovary. On the other hand, the level of oestrogen was significantly different among these ovarian follicle types. String database, suggested the strong relationships between IGFs and CYP19 which in turn involved in regulation of steroidogenesis. Functional annotation (GO terms) revealed that IGFs and CYP19 genes were mainly associated with response to hormone and insulin growth factor bindings. In addition, KEGG pathways mapped to ovarian steroidogenesis and Ras signaling pathways.

Conclusion: Overall, these findings are consistent with the critical roles of IGF-1 and IGF-2 in regulating steroidogenesis, growth and viability of follicular cells in the sheep ovary, evaluation of IGFs genes can be useful for the understanding of reproduction performance in ewes.

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P170 Outcome of ART cycles with oocytes containing smooth endoplasmic reticulum aggregates

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Background: SERs are organelle clusters evident in 10% of IVF/ICSI cycles (1). The clinical relevance of these dysmorphisms has been a subject of debate. It has been reported that SER+ oocytes as well as other oocytes derived from that same ART (assisted reproductive technology) cycle may impact embryological and clinical outcomes. The Istanbul consensus recommended not to inject/inseminate SER+ oocytes due to adverse foetal outcomes reported in the literature (2). More recent studies, however, have refuted this (3). The aim of this study was to analyse IVF/ICSI cycles in our population where SERs were noted in one or more oocytes.

Study Design: Retrospective review of 2019-2020 ART cycles. SER positive oocytes were identified and their cycles were analysed with respect to oocyte maturity, fertilisation rates, and pregnancy outcomes. Results were compared to those of the total clinic population over the same time period.

Results: 952 ART cycles were commenced during study period. 72 cycles had at least one SER identified, with a total of 135 SER+ oocytes. There were 104 embryo transfers from SER+ cycles, of which 53% had a positive pregnancy test, compared with 49.6% positive pregnancy tests for all ART cycles. The clinical pregnancy rate and live birth rates per embryo transfer for SER+ cycles were 46% and 29% respectively compared to 41% and 32.7% in the background population. In terms of embryology, the SER+ cycles had a fertilisation rate of 59.9% and a high-quality blastocyst rate of 25.9%.

Conclusion: These findings suggest that SER positive cycles have similar outcomes to those of the general population, though higher numbers are needed to fully examine this. Ongoing work will examine these outcomes in more detail, including neonatal outcomes and analysis of time-lapse morphokinetic data of embryos from SER+ cycles.

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P172 Trapezoids, polygons & inverted trapezoids: Can stimulation profile shape predict the number of oocytes collected? A proof of concept study to determine sample size for a planned multi-centre study

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Apricity

Introduction: Current artificial intelligence (AI) tools to assist with ovarian stimulation decisions are unable to classify the growth of follicles in a transparent manner so that the clinician can understand the decision being suggested by the AI. To overcome this, we propose a novel follicle profile classification based on the shape of the distribution of follicle sizes seen in a folliculogram on a given day. Our objective was to determine the sample size required to assess in a multi-centre study the effectiveness of this classification system in predicting outcome.

Methods: Stimulation profiles from follicle tracking data from 136 patients on day 11 of stimulation were categorised into the shape of follicle distributions according to the size of follicles: trapezoid (most follicles <12mm), polygon (most follicles: 12-19mm) and inverted trapezoid (most follicles >19mm). The follicle distributions were compared with patient factors (AMH, BMI, Age and number of eggs collected) to identify differences in variable averages between each follicle distribution.

Results: A polygon distribution led to the highest number of eggs collected at the end of stimulation (17) but this difference was not statistically significant (p=0.2). Patient factors did not affect follicle distribution (AMH: p=0.2, BMI:

p=1.0, Age: p=0.4). A power analysis determined that a sample of n=395 patients would be required to observe an 11% difference in the number of oocytes collected between different shapes, assuming =0.05.

Conclusion: A multi-centre study assessing the stimulation profile from 395 patients undergoing IVF will be required to establish the clinical relevance of stimulation profile shapes. Stimulation profile shape is a novel and simple method of categorising follicle tracking data which could be assessed automatically using artificial intelligence.

P173 Region- and culture period-specific collagen signature across the human ovarian cortex

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Background: Despite significant progress made in culturing human follicles in vitro, the process remains challenging, particularly due to inefficient primordial follicle activation. Although the physical environment surrounding quiescent follicles within the cortical tissue influences their ability to grow, the ovarian stroma remains poorly defined. Here, we explored the dynamics of collagen, the main component of the ovarian extracellular matrix regulating tissues' stiffness, in each region of the ovarian cortex during culture.

Methods: Fresh ovarian cortical biopsies were obtained from 6 women aged 28-38 years (mean SD: 32.7 4.1 years) at elective caesarean section. Biopsies were cut into fragments of 4 1 0.5 mm and cultured for 0, 2, 4 or 6 days. Collagen content and fibre characteristics (density, width, length, straightness, angle and alignment) were quantified within each cortical sub-region, namely the outer cortex, the mid-cortex, and the cortex-medulla junction, at all time-points using PicroSirus red staining and CurveAlign/CT-Fire image analyses.

Results: Collagen content was uneven across the cortex and during culture, being maximal in the outer cortex and the lowest in the mid-cortex (69.4 1.2% vs 53.8 0.8% positive area, $p < 0.0001$), and decreased from day 0 to day 2 (65.2 2.4% vs 60.6 1.8%, $p = 0.033$) then stabilised. Assessment of collagen architecture revealed distinct signatures according to the cortical sub-region, with differences between fibres from the outer cortex and from the cortex-medulla junction (width: 1.272 0.003m vs 1.230 0.004m; $p < 0.0001$; length: 6.80 0.08m vs 6.16 0.03m; $p < 0.0001$; straightness: 0.9473 0.0004 vs 0.9450 0.0003; $p < 0.0001$; density: 5.13 0.07 vs 5.58 0.03 fibres/100m2; $p < 0.0001$) whilst mid-cortex fibres shared features from both compartment.

Conclusion: Collagen deposition and architecture are spatiotemporally remodelled across the ovarian cortex and during culture, likely conferring the tissue with a stiffness gradient controlling follicle activation.

P174 Follicle-like structures in southern white rhinoceros ovaries

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Background: Every female rhinoceros is born with an ovarian reserve that consists out of immature oocytes in primordial follicles. Establishing methods to grow and culture these oocytes in the laboratory would increase the chance of successful in vitro embryo production. In order to establish these methods, the follicle development process needs to be revealed. Development of advanced reproductive techniques are the only option for saving the northern white rhinoceros, which is functionally extinct with only two females alive.

Methods: This study examined the structural and molecular characteristics of follicle-like structures observed in two adult southern white rhinoceros ovaries. Ovarian sections were stained with H&E and molecular analysis was performed by detection of hyaluronic acid, collagen I, Ki-67, DDX4 and TUNEL assay to detect apoptosis.

Results: Preliminary studies showed the presence of normal follicles at all stages of development in the southern white rhinoceros. However, histological analysis revealed several structures that resembled follicles, but they deviated from the expected appearance. We identified them as follicle-like structures (FLS). Structurally, those FLS were built up out of a collection of cells organised around fluid islands with or without an antral cavity. Rarely any oocyte was found. No extreme rates of proliferation or apoptosis were observed.

Conclusions: Several FLS appear in the rhinoceros ovary. Although resembling a normal follicle, the molecular signature could not give a definite answer on the function or stage of these structures. Future research will have to focus on the functionality of those FLS. Since the southern white rhinoceros is the closest related animal to the northern white, we expect translation of results between the two subspecies. Mapping of ovarian tissue might benefit all rhinoceros species contributing the development and evaluation of in vitro culture techniques in endangered wildlife species.

P175 Effect of PKA inhibition on angiogenesis, progesterone production, cell proliferation, and steroidogenic enzyme expression in bovine luteinising follicular cells

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Background: Hypoxia and cAMP regulate HIF1A expression in human granulosa and bovine luteinised granulosa cells. In the peri-ovulatory period, LH increases follicular HIF1A activity by increasing intracellular cAMP and activating protein kinase A (PKA). It has been proposed that this stimulates subsequent luteal angiogenesis. Thus, this study investigated the interaction between hypoxia and PKA on HIF1A responsive genes, endothelial cell (EC) network formation and progesterone production.

Methods: Dispersed cells from granulosa and theca layers (bovine follicles; >10mm) were co-cultured (n=4) in either high (20%) or low (3%) oxygen. Cells were then treated with control or PKA inhibitor (H89). On day 5 of culture, EC networks were quantified by von Willebrand factor immunohistochemistry and image analysis. Spent media were assayed for progesterone by ELISA. Expression of steroidogenic acute regulatory protein (STAR) and HIF1A-responsive BNIP3 in cell lysates was determined by Western blot.

Results: Organised, intricate EC networks were observed in control and H89 treated cells. The number of branch points ($p<0.05$) and degree of branching ($p<0.01$) were reduced by H89. Moreover, H89 reduced the total area of EC networks but only in 20% oxygen ($p<0.05$). Progesterone concentrations increased ($p<0.001$) during culture but were unaffected by H89 and oxygen on day 3. On day 5, progesterone production was reduced (1.2-fold, $p<0.01$) by 3% oxygen but not in the presence of H89. BNIP3 was upregulated ($p<0.05$) by 3% oxygen while STAR was downregulated ($p<0.05$). Both STAR and BNIP3 were unaffected by H89.

Conclusion: PKA signalling increased endothelial cell branching and EC network formation but only in 20% oxygen. BNIP3 expression was oxygen sensitive but was unaffected by PKA inhibition. In conclusion, PKA signalling can modulate angiogenesis during the peri-ovulatory period, particularly when oxygen concentration is high. Funded by TETFUND, Nigeria

P176 Fibronectin type III domain containing 3A (FNDC3A) expression in bovine ovary and in vitro effect on bovine granulosa cell proliferation and steroidogenesis

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Introduction: The modern dairy cow requires substantial energy intake to sustain milk production, and many cattle cannot meet the energy demands post-partum from the diet alone. This induces a state of negative energy balance (NEB) and mobilization of adipose tissue, which results in altered adipokine secretion. Adipokines such as leptin are known to affect ovarian function. A recently characterized family of adipokine-myokines is the Fibronectin Type III domain containing (FNDC) proteins, including irisin that we have shown to be upregulated in adipose tissue post-partum. Another FNDC protein whose expression is increased during NEB is FNDC3A, which is expressed in the testis and is essential for spermatogenesis in mice. We therefore hypothesised that FNDC3A is expressed in the ovary and impacts follicle development.

Methods and results: Using RT-qPCR, we detected FNDC3A mRNA in cortex, granulosa and theca cells, cumulus cells and oocyte of the bovine ovary. Relative mRNA abundance was higher in granulosa cells than in the cortex ($p<0.01$), and FNDC3A mRNA abundance in COCs decreased during in-vitro maturation ($p<0.001$). Addition of human recombinant FNDC3A to cultured granulosa cells increased cell viability and proliferation ($p<0.001$), and decreased basal and IGF1-stimulated progesterone secretion ($p<0.05$) but had no effect on oestradiol secretion.

Conclusion: Taken together, these data show that FNDC3A is expressed in the ovary and modulates granulosa cell viability and function and may play a role in oocyte maturation.

P177 Mycotoxins deoxynivalenol and its major metabolite de-epoxy deoxynivalenol differentially activate ER-stress and autophagy in bovine granulosa and theca cells

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Background: The common mycotoxin deoxynivalenol (DON) causes cell death through the ribotoxic stress response in numerous cell types including ovarian granulosa cells(1), and the 'non-toxic' metabolite de-epoxy-deoxynivalenol (DOM-1) has activated pathways downstream of endoplasmic reticulum (ER) stress in theca cells(2). The three main ER-stress pathways involve activation of protein kinase R-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring kinase 1 (IRE1)(3). The objective of this study was to determine if DON and DOM1 activate ER-stress in granulosa and theca cells, respectively.

Methods: Bovine granulosa (GC) and theca (TC) cells were cultured in serum-free conditions and GC were treated with DON and TC with DOM-1 for different time points. After treatments, total RNA was extracted for qPCR, protein for western blot and cells for FACS.

Results: DON and DOM-1 significantly increased abundance of phosphorylated PERK in GC and TC, respectively, at 12 and 24 h after exposure. Both toxins increased ATF6 activation at 8 h of exposure, which declined to control levels by 24 h. DOM-1 caused a short-term increase in IRE-1 protein levels in TC whereas DON gradually increased protein levels in GC. As PERK activation is associated with autophagy, we measured autophagy markers in treated cells: DOM-1 resulted in a significant increase in LC3 protein cleavage in TC without an increase in Beclin1 mRNA abundance, whereas DON caused a modest increase in LC3 protein cleavage in GC but with a significant increase in Beclin1 mRNA abundance. Treatment of GC with DON increased the proportion of dead cells, and inhibition of autophagy with ULK1 further increased cell death. However, DOM-1 increased the proportion of dead TC but inhibition of autophagy did not further alter the proportion of dead cells.

Conclusion: We conclude that both toxic DON and 'non-toxic' DOM-1 activate ER-stress in follicular cells.

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P178 Optimising media to support In vitro activation/IVA and In vitro growth/IVG of bovine primordial/preantral follicles

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Background: Bovine primordial follicles can be activated in vitro (IVA) and preantral follicles in vitro grown (IVG) to antral stages (1) but there is no consensus on the basic media required to support these processes. In this study we compared 4 basic media (M199, McCoy's5a, MEM, and Waymouth's) to support activation of primordial, and growth of preantral bovine follicles.

Methodology: The cortex of abattoir-derived bovine ovaries was thinly sliced and cut into strips (4 x 2 x 1 mm) and cultured in either M199, McCoy's5a, MEM, and Waymouth's all containing glutamine (3 mM), bovine serum albumin (Fraction V 0.1%), penicillin G (0.1 mg/ml), streptomycin (0.1 mg/ml), transferrin (2.5 g/ml), selenium (4 ng/ml), insulin (10 ng/ml) sodium ascorbate (50 g/ml), and follicle-stimulating hormone (1 ng/ml) for 6 days (n=7 strips/group). The number of primordial and growing follicles were analysed by histology before and after culture. Secondary follicles (100~200 m) were manually isolated from fresh ovarian tissue and cultured in each media as above with the addition of 100 ng/ml of Activin A (n=25~21 follicles/group) and their growth monitored for 6 days.

Results: The proportion of primordial follicles (day 0=86.9%) decreased following culture in all groups (76.4-82.5%, P 0.05) with the activation rate highest in McCoy's5a (23.6%, M199=19.1%, MEM=17.5%, Waymouth's=21.0%, P 0.01). The proportion of secondary follicles present (Day 0=0.7%) increased following culture in McCoy's 5a (2.6%), MEM (2.1%), and Waymouth's (1.8%), but no differences were observed with M199 (1.2%). No difference in growth rate of isolated secondary follicles (18~26 m, P 0.01) was detected between groups.

Conclusion: Cortical strips cultured in McCoy's5a showed the highest activation rate of primordial follicles, and comparable growth of secondary follicles compared to other media tested. McCoy's5a is an effective basic medium for bovine primordial follicle activation and the growth of secondary follicles.

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P179 Validation of housekeeping genes under low and high O2 tension in the presence or absence of melatonin for long term cultured bovine GCs

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Housekeeping gene (HKG) expression varies depending on cell type and metabolic status, and between certain experimental conditions. Selection of reference genes is essential for normalization of target gene expression. To obtain more accurate results in mRNA analysis and increase the gene specificity, TaqMan primers and probes were used. Based on previous studies (Baddela et al., 2014; Khan et al., 2016) β-2 Microglobulin (B2M), TATTA box binding protein (TBP), Ribosomal protein large, P0 (RPLP0), Ribosomal protein L19 (RPL19), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Tyrosine 3-monooxygenase(YWHAZ), Succinate dehydrogenase complex, subunit A (SDHA) and β-actin (ACTB) were proposed to be stable HKGs in granulosa cells (GCs). In the current study bovine GCs were cultured under atmospheric (20%) or low (5%) O2 in the presence or absence of the antioxidant melatonin (0 and 2 ng/mL). Total RNA from four biological replicates were extracted from GCs at 48, 96 and 144 h of culture. The stability of reference genes was analysed by general ANOVA and RefFinder. There was no significant effect of culture conditions (i.e., oxygen tension, melatonin and duration of culture) on TBP expression. However, mRNA levels of B2M increased (P=0.004) with time in culture. RPL19 expression was greater (P=0.019) at 20% than 5% O2. Oxygen tension tended to interact (P<0.06) with duration of culture to affect RPLP0 transcript expression. SDHA, ACTB, GAPDH, YWHAZ were unstable over time in culture (P<0.001), with oxygen tension (P<0.001) and oxygen tension over time (P<0.05) In conclusion, the HKGs assessed in this study, strengthen the importance of selecting best normalizing genes to analyse mRNA expression under specific experimental conditions. TBP was the most stable reference gene in GCs subjected to different oxygen concentrations during extended culture and in the presence/absence of melatonin.

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P180 The effect of lipopolysaccharide on bovine luteal angiogenesis and gene expression in vitro

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Purpose/Background/Objectives: Lipopolysaccharide (LPS) acts through its receptor TLR4 to negatively affect luteal function. This study determined the effect of LPS on luteal gene expression and cytokine production; and assessed the role of TLR4 signalling in regulating the expression of critical luteal genes in vitro using TAK242 (a specific TLR4 inhibitor).

Methods: Bovine luteal cells were treated with LPS on days 1 and 3 of culture (n=3 independent cultures). On days 3 and 5, LPS treated luteal cells were collected for RNA sequencing, pathway analysis and RTqPCR. Conditioned media was collected for cytokine arrays.

Results: Differential gene expression analysis using a log2 fold change of <-1 or >1 revealed 33 downregulated genes and 18 upregulated genes on day 3. On day 5, 4 genes were down regulated while 33 genes were upregulated in LPS treated cells. RNA sequencing and RTqPCR analyses revealed decreased expression ($p<0.05$) of ROBO4, EGFL7, SCG2 and OAS1Y and decreased expression ($p<0.01$) of DLL4, PECAM1 and CXCL5 in LPS treated cells on day 3. There was decreased expression ($p<0.05$) of CDH5, LHCGR and decreased expression ($p<0.01$) of PECAM1, CXCL5 and SCG2 in LPS treated cells on day 5. TLR4 inhibition using TAK242 reversed the effect of LPS on the expression of DLL4 and PECAM1 on days 3 and 5. The effect of LPS on the expression of ROBO4 and EGFL7 on day 3, LHCGR, CDH5, SCG2 and CXCL5 on day 5 was partially reversed following TLR4 inhibition. Cytokine arrays revealed an increased ($p<0.05$) concentration of VEGF in conditioned media from LPS treated cells on days 3 and 5.

Conclusions: LPS acts through TLR4 to decrease the expression of critical genes which promote luteal cell function and angiogenesis while also causing increased expression of cytokines, chemokines, and pro-apoptotic genes.

P181 Prokineticin 1 stimulates angiogenesis in the porcine corpus luteum

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Prokineticin 1 (PROK1) is a secretory protein exerting multiple functions in various organs, including the reproductive tract. We recently indicated that PROK1 acting on porcine endometrial and trophoblast cells contributes to pregnancy establishment. However, little is known about the role of PROK1 in the porcine corpus luteum (CL) during pregnancy. Hence, the aims of the present study were: (1) to evaluate the localization of protein expression of PROK1 and its receptors (PROKR1 and PROKR2) in the porcine CL during the estrous cycle and early pregnancy and (2) to determine the effect of PROK1 on angiogenesis in the CL. Corpora lutea were collected from gilts on either day 12 and 14 of the estrous cycle or pregnancy (n=5 per group). Localization of PROK1, PROKR1, and PROKR2 protein expression was assessed by immunohistochemistry. To investigate the effect of PROK1 on luteal angiogenesis an in vitro models involving precision-cut CL tissue slices and capillary-like structure formation assay with luteal endothelial cells were used. PROK1, PROKR1, and PROKR2 were expressed in steroidogenic cells and blood vessels of the CL during the estrous cycle and pregnancy. Prokineticin 1 stimulated gene expression of angiogenin ($p<0.05$) on day 12 of pregnancy in the CL tissue slices when compared to control. Furthermore, PROK1 increased the secretion of vascular endothelial growth factor A by CL tissue slices collected on day 12 of pregnancy ($p<0.05$) and on day 14 of the estrous cycle ($p<0.01$) and pregnancy ($p<0.05$). In addition, PROK1 stimulated formation of capillary-like structures by luteal endothelial cells by increasing number of nodes, junctions, meshes, segments, and total master segments length ($p<0.05$) on day 12 of the estrous cycle. To conclude, the presented data indicate that PROK1 is an important factor involved in the regulation of angiogenesis in the CL. Supported by National Science Centre (project No. 2019/35/N/NZ9/03986).

P182 The effect of cisplatin on the fetal mouse ovary in vitro

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Introduction: Around 3,000-5,000 pregnant women receive a diagnosis of cancer every year in Europe alone. Whilst chemotherapy treatment is considered safe to administer after the 1st trimester of pregnancy, it brings about concerns as chemotherapy drugs are known to cause damage to gonads in both children and adults. In addition, studies to determine whether and how chemotherapy drugs can affect the future fertility of the fetus have been limited. In this project, we investigated whether the chemotherapy drug cisplatin affects germ cell number, apoptosis and DNA damage within the fetal mouse ovary in vitro.

Methods: Paired ovaries were collected from female fetuses of pregnant mice at embryonic day 13.5 (E13.5), with one ovary from each pair being cultured under control (saline) conditions, and the other with cisplatin (3 M) added to the media. Ovaries were cultured for either 8 h, 16 h or 24 h, for time-course analysis. Following tissue fixation, ovaries were histologically processed, embedded in wax and sectioned. Apoptosis was examined in ovaries from all time-points using immunohistochemistry for the apoptotic marker cleaved-caspase 3 (CC3). Germ cell number and double-strand DNA damage were examined at 24 h time-point only, using immunohistochemistry for the germ cell marker ddx4, and a marker for double-strand DNA breaks, H2AX, respectively.

Results/Discussion: Cisplatin exposure induced a 5.3 fold increase in CC3 expression ($p<0.05$) 16 h after drug-exposure. This corresponded with a 54.7% loss of germ cells at the 24 h ($p<0.001$), at which point H2AX expression was also found to be significantly higher ($p<0.001$). These findings suggest that the observed loss of germ cells is likely due to an

increase in apoptotic cell death, where cisplatin exposure increases the level of double-stranded breaks in fetal ovaries, potentially resulting in increased DNA damage within the germ cells.

P183 How does background humidity of the laboratory affect IVF outcomes?

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Purpose/background/objectives: Dry culture conditions have been shown to increase culture media osmolality which can significantly impact embryo quality and clinical outcomes (Swain et al., 2012), but most IVF laboratories do not monitor background humidity regularly. This study aimed to investigate the impact of background humidity on IVF outcomes and whether any deleterious effects could be overcome by a day-3 dish change.

Methods: Patients were randomly allocated for embryo culture using either humidified incubation (BT37 MarkII (Planer) and/or Geri® (Genea Biomedx)) (n=58), or dry incubation (Miri® Multiroom (ESCO Medical) and/or EmbryoScope+ (Vitrolife)) (n=59). Dry-culture patients with ≥6 embryos underwent a day-3 dish change of half the embryos (n=17). Background humidity in the laboratory was recorded daily.

Results: When background humidity was considered acceptable (>20-25%), there was no significant difference in blastocyst utilisation between humidified culture (148/297 (49.8%)), dry culture (32/63 (50.8%)) and dry culture with a dish change (138/288 (47.9%)). However, clinical pregnancy rate was 15.8% lower in dry-culture embryos with a dish change (8/28 (28.6%)) compared to humidified culture (12/27 (44.4%)) (p=0.27). When background humidity was considered low (<20-25%), humidified culture trended towards higher blastocyst utilisation compared to dry conditions (41/82 (50.0%) vs. 7/17 (41.2%), p=0.6), and performing a dish change produced similar blastocyst utilisation rates to humidified culture (17/35 (48.6%)). Due to observed reduced blastulation rates, the non-dish change arm was suspended resulting in limited data in this group.

Conclusions: Although further work is required, this research supports the hypothesis that low laboratory humidity levels (<20-25%) may negatively affect blastocyst utilisation and clinical pregnancy rates and embryos should be preferentially cultured in humidified incubators. If unavailable, a day-3 dish change may negate the effects of a dry environment, however, this should be reconsidered if background humidity levels are acceptable (>20-25%) as the additional manipulation step may negatively affect clinical outcomes.

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P184 In the absence of personalised progesterone testing, implantation rates in medicated frozen embryo transfers are improved with a combination of sub-cutaneous and pessary luteal progesterone support

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Background: There has been increased focus in optimising luteal phase support (LPS) for frozen embryo transfer (FET) cycles. Routine performance monitoring in 2021 identified an underperformance in our short hormone replacement therapy (HRT) FET cycles. A quality improvement project was designed to modify LPS in HRT/FET cycles from twice daily 400mg pessaries to once daily 25mg sub-cutaneous (c/s) injection (morning), together with once daily rectal 400mg pessary (evening). Progesterone serum monitoring is not currently routinely performed for all patients.

Methods: A prospective audit of 131 single blastocyst FET cycles assessed the effectiveness of the change in three different FET protocols; long HRT, short HRT and natural cycle (NC). Implantation rate (IR) (pregnancy sacs/blastocyst transferred) was used as the audit standard, with competence set at >35%1. Satellite cycles, female over 40, donor gametes and PGT were excluded. Audit results were compared with the 24-month period preceding the change to practice. NC FET's were referenced as controls.

Audit results: All three protocols exceeded the audit standard. Using Chi-square, IR was significantly improved in short HRT cycles (28% (92/327) vs. 45% (18/40): p=0.027) and in long HRT cycles (41% (94/230) vs 54% (49/91): p=0.035). IR in NC remained unchanged (45% (386/852) vs 42% 82/195): p=0.40).

Audit recommendations: LPS was increased to twice daily 25mg s/c progesterone without pessaries and re-audited after 6 months.

Re-audit results: When comparing the initial audit to the re-audit, no further improvements in IR were observed: long HRT: 45% (18/40) vs 43% (23/53): p=0.22, short HRT cycles: 54% (49/91) vs 38% (25/65) p=0.5), NC: 42% (82/195) vs. 47% (45/96): p=0.43).

Conclusions: In the absence of personalised progesterone testing, which requires staffing and additional clinic visits, a daily combination of 25mg s/c progesterone with 400mg pessary worked best in terms of clinical improvement and cost effectiveness for HRT/FET's.

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P185 Fertility outcomes in women with BMI >30 investigated for tubal patency with hysterosalpingogram (HSG) between 2016-2017

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Introduction: Obesity has been proven to have detrimental effects upon female reproductive health. Menstrual irregularities, anovulation and polycystic ovaries are at a higher incidence in women with BMI >30. In addition, infertility, conception rates, miscarriage rates and pregnancy complications such as gestational diabetes (GDM), hypertensive disease and risk of venous thromboembolism are also increased in this population. 124 women underwent hysterosalpingogram (HSG) for investigation of subfertility. Thirty-three (33) women with BMI >30 was identified, and fertility outcomes determined.

Methods: Electronic care records were searched on NIECR from April 2016-2017 to identify women who underwent HSG whilst undertaking fertility investigations. From this data women with BMI >30 were selected and investigated.

Results: Thirty-three women with BMI >30 was identified. BMI range 30-55, average 36.63. Twenty-one (64%) had never been pregnant, seven (21%) have previously been pregnant and had live births, six (18%) have had a previous miscarriage and no live births. Sixteen (48%) HSG results were abnormal. Seventeen (52%) had subsequent live births following HSG. 11 (65%) of these births were spontaneous conception. 6 (35%) required assisted conception, 4 (67%) required IVF and 2 (33%) required ovulation induction with Clomid. Sixteen (48%) did not conceive and had no subsequent live birth.

Conclusion: In one year, women identified with BMI >30 had a live birth following HSG. Over half (52%) of this cohort underwent spontaneous conception, whereas 35% required assisted conception.

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P186 The impact of extracellular vesicles derived from watermelon on placental function and pregnancy outcomes in an in vivo model

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Background: FGR is a common pregnancy complication associated with adverse outcomes for the offspring. Limited treatment options necessitate research into potential treatments that pose a minimal risk to pregnancy. Our group has successfully isolated extracellular vesicles (EVs) from watermelon as a potential plant-derived therapeutic supplement and shown that these EVs influence key aspects of placental cell behaviour in vitro (1). This study aimed to investigate their impact on pregnancy outcomes in vivo.

Methods: Female C57BL/6J mice were time-mated with visualisation of the vaginal plug designated as gestational day (GD) 0.5. Pregnant mice were randomly allocated to receive PBS control, low-dose (5 x 109 particles/mL) watermelon EVs or high-dose (5 x 1010 particles/mL) watermelon EVs by oral gavage once daily from GD7.5 -- GD13.5. Mice were subsequently harvested 24 h later at GD14.5 or close to term at GD17.5. Litter size, number of resorptions, maternal organ weights and fetal and placental weights were recorded. Maternal food and fluid intake was monitored throughout pregnancy. Maternal bodyweight throughout pregnancy was also recorded.

Results: Treatment with watermelon EVs had no significant impact on maternal feeding or maternal bodyweight during pregnancy. No effect of treatment was observed with respect to maternal organ weights, litter size, resorption number, sex distribution or fetal weight at either harvest time-point. Whilst placental weight was unaffected at GD14.5, a significant dose-dependent increase in placental weight was observed at GD17.5 in response to watermelon EV treatment ($P<0.05$).

Conclusion: The lack of effect on maternal bodyweight, litter size and maternal food and fluid intake suggests treatment with watermelon EVs does not appear to adversely affect pregnancy progression. Increased placental weight in response to watermelon EVs suggests potentially beneficial effects in vivo. It remains to be determined whether EV supplementation in rodent models of pregnancy complications including FGR will impact positively on fetal weight.

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P187 Effects of maternal high fat diet consumption during only preimplantation or whole gestation/lactation on mouse offspring locomotory, exploratory and anxiety- like behaviour and working memory

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Introduction: Altered maternal intrauterine and early postnatal environment due to maternal high fat diet (HFD) negatively affects neurodevelopment, cognitive function, and emotional behaviour of offspring in later life. We hypothesise, in the absence of obesity, a maternal HFD during gestation/lactation or only during the preimplantation period affects offspring locomotor, explorative, anxiety behaviours and memory.

Method: After mating, female-MF1 were allocated to one of three groups: Embryonic-HFD (EHFD: HFD up to E3.5, chow-diet after); HFD and control group (NFD), consuming HFD and chow-diet throughout pregnancy/lactation periods, respectively. Open field test (OFT) for 10min at 4 (OFT1) and 10 (OFT2) weeks old; and elevated plus maze test (EPM) for 5min at 5 (EPM1) and 11 (EPM2) weeks old; and T-maze at week 8 were performed. OFT data were analysed as the first-5 and last-5min. Male and female offspring from HFD(n=9), EHFD(n=8), and NFD(n=9) groups were compared using a hierarchical linear regression model.

Results: EHFD males but not females, significantly travelled less (p=0.043), rested more (p=0.026), and had fewer ambulatory (p=0.023) and jump counts (p=0.018) than controls in the first 5min of OFT1. HFD males had fewer jump counts (p=0.027) while HFD females spent more time rearing (p=0.001), compared to respective controls in the second 5min of OFT1. Moreover, the number of closed arm entries was higher in HFD females (p=0.020) compared to NFD females in EPM1. There were no significant differences observed in T-maze, OFT2 or EPM2.

Conclusions: Maternal HFD exposure during only the preimplantation period particularly decreased juvenile male offspring locomotor and exploratory behaviour. Juvenile female offspring from mothers who consumed HFD for the whole gestation/lactation, showed anxiety-like behaviour. There were no differences in their working memory. This confirms that diet changes during the preimplantation or gestation/lactation can affect offspring future health. Also, exposure to a normal diet can rescue the phenotype in adulthood.

P188 Establishing the impact of paternal diet on maternal cardio-metabolic health in the late gestation mouse

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Background: While the connection between poor paternal diet and perturbed fetal development is becoming established, the underlying mechanisms remain undefined. Studies have suggested that maternal health during pregnancy can be compromised by paternal factors at the time of conception, impacting subsequent fetal development. This study aimed to define the impact of poor paternal diet on maternal cardio-metabolic health in late gestation.

Methods: Male C57/BL6J mice were fed either a control (CD: 18% casein, 10% fat, 21% sugar), a low-protein (LPD: 9% casein, 24% sugar, 10% fat), a Western diet (WD: 19% casein, 34% sugar, 21% fat) or an LPD or WD supplemented with methyl donors (termed MD-LPD and MD-WD respectively) before mating with C57BL/6J females. At embryonic day 17.5 dams were culled for the analysis of maternal hepatic cholesterol, free fatty acids, and triglycerides as well as serum glucose and insulin using commercially available assays. Maternal fecal samples were collected for microbiota analysis by Illumina HiSeq sequencing of the hypervariable bacterial V3-V4 region of the 16S gene. Maternal cardiac expression of a panel of 86 cardiovascular disease-related genes was conducted using the commercially available RT2-profiler (Qiagen) array.

Results: No significant differences in the levels of hepatic cholesterol, free fatty acids, and triglycerides or serum insulin and glucose levels were observed in late gestation. Furthermore, no difference in the diversity or relative abundance of bacterial species in the maternal gut in response to the paternal diet was observed. Finally, no significant differences in the expression of a panel of 86 cardiovascular disease-related genes in the maternal heart were observed.

Conclusion: Our results indicate that poor paternal diet at the time of conception has minimal impact on maternal cardio-metabolic health in the late gestation mouse. However, further studies are required to define the consequences for the long-term health and well-being of his offspring.

P189 Could corpulence index be a better prognostic marker than body mass index for live birth rate following assisted reproductive technology cycles?

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Objective: To compare Body Mass Index (BMI) and Corpulence index (CI) as prognostic markers for live birth rate (LBR) following In vitro fertilisation (IVF) and frozen embryo transfer (FET).

Setting: A retrospective cohort study based on the clinic records of 1916 IVF and 1436 FET cycles between 2017-2020 at a private fertility clinic.

Methods: BMI categories were classed as underweight (BMI <18.5 kg/m2), normal weight (BMI 18.5-24.9 kg/m2), overweight (BMI 25-29.9 kg/m2), class 1 obesity (BMI 30- 34.9 kg/m2) and class 2 obesity (BMI ≥35 kg/m2) categories. CI was classified into four quartiles, Q1 (9-13.4 kg/m3), Q2 (13.5-17.9 kg/m3), Q3 (18-22.4 kg/m3) and Q4 (22.5-27 kg/m3). Data was collected on the eggs fertilized, clinical pregnancies and LBR. Line graphs and Receiver Operating Characteristic (ROC) curves were constructed to visualise the relationship between BMI/CI and LBR.

Results: There was no significant difference in LBR between different classes of BMI in the IVF cycles (Underweight=23.8%, Normal weight=22.25%, Overweight=18.43%, Class 1 obesity=21.18%, Class 2 obesity=18.45% and in the FET cycles (Underweight=34.62%, Normal weight=34.8%, Overweight=35.51%, Class 1 obesity=30%, Class 2 obesity=33.64%). Similar was noted for CI in the IVF cycles (Q1=22.7%, Q2=20.53%, Q3=20.61%, Q4=14.29% and in the FET cycles (Q1=37.23%, Q2=34.58%, Q3=30.92%, Q4=28.36%. There was a higher area under the curve (AUC) using ROC for CI (AUC for IVF/ICSI=0.531, AUC for FET=0.528) than for BMI (IVF/ICSI=0.525, FET=0.526) indicating that CI might be a slightly better prognostic marker than BMI. Practically, both CI and BMI would be poor predictive markers for LBR as they had AUC under 0.6.

Conclusion: CI and BMI are both weak predictors of IVF/ICSI and FET outcomes. However, CI had a slightly greater AUC. Further analysis is underway to examine whether CI performs better than BMI at extremes of height where BMI traditionally performs poorer as a marker of adiposity.

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P190 Dairy herd reproduction management strategies for improved efficiency

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Optimum dairy herd economic performance is generally achieved by maintaining a calving interval of 365 days. Mitigating the environmental impact of dairy production requires efficient reproductive performance. Statham et al. modelled reduction in greenhouse gas emissions intensity when reproductive management strategies such as fixed-time insemination and sensor technologies were deployed, modelled the effects of fertility on emissions linking changes in fertility to herd structure, number of replacements, milk yield, nutrient requirements and gas emissions. Improving submission rate from 50 to 70% could reduce emissions of methane by up to 24% by reducing the number of heifer replacements required to maintain herd size. A range of strategies are available to boost submission rates, however adequate conception risk requires effective management of the root causes of poor reproductive efficiency. Oestrus synchronisation techniques offer an effective approach to increased submission rate, but require intensive hormonal intervention such as 'Ovsynch' and variations Co-synch, Heat-synch, Select-synch and Pre-Synch. Inclusion of progesterone-releasing devices has offered an alternative approach. Automated heat detection systems utilising sensors based on cow activity, positioning or temperature sensors can accurately predict oestrus as can low milk progesterone from in-line milking parlour systems. Root causes of poor reproductive performance require a holistic herd-based approach and reproductive challenges such as early and late embryonic death may still have a significant effect on reproductive outcome and herd performance. Both female and male factors should be considered at herd level and include nutrition, infectious disease & environmental factors such as heat stress. Screening semen quality using objective multimodal systems offers an important opportunity to address variations in male factors. Perturbations in time-series data from sensors such as milk meters and activity meters offer emerging opportunities for managing the resilience and efficiency of dairy herds, ranking cows on those parameters to manage herd status over time.

P191 The effect of clinical and subclinical mastitis and on the subsequent reproductive performance of cows at Nottingham dairy centre

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Background: Mastitis has negative impact on the reproductive performance in high yielding dairy cows in the UK during the last decade.

Aim: This study assessed the effect of clinical mastitis, subclinical mastitis on the subsequent reproductive performance in high yielding dairy cows.

Methods: Data was collected from 184 multiparous Holstein dairy cows. Binomial logistic regression analyses were performed to determine the incidence rate of clinical mastitis and subclinical mastitis between parity, calving year seasons of the year. The same model used to determine the association between clinical or subclinical mastitis and the probability of a cow to get pregnant at 1st, 2nd or 3rd service. The probability of a cow getting pregnant from 30-60 days postpartum or 61-90 days postpartum was analysed by using Binomial logistic regression. Linear mixed model analyses were performed to determine the effect of clinical and subclinical mastitis on reproductive performance including Day to first service (DFS); Calving to conception interval (CCI) and Calving interval (CI).

Results: The incidence of clinical mastitis was different between calving years (25%, $P<0.01$). Cow with clinical mastitis or subclinical mastitis had longer DFS (9 days, $P<0.05$), CCI (21 days, $P<0.05$). Cows with clinical mastitis had a lower rate to get pregnant within 20-30 days postpartum compared to healthy cows ($P<0.05$). Cows with higher somatic cells count, (especially cows with greater than 399,000 cells/mL of milk), had a higher number of services compared to cows with a lower number of individual cow somatic cell counts.

Conclusion: The study has quantified the negative impact of clinical and subclinical mastitis on the reproductive performance in high yielding dairy cows.

P192 Can serum hCG levels at early pregnancy (days 14 to 16 post ovulation) predict the risk of still birth, preterm birth and low birth weight after fertility treatment?

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Background: Pregnancies conceived after fertility treatment have higher perinatal risks of still birth (SB), preterm birth (PTB) and low birth (LBW). We planned to assess whether serum hCG levels at very early pregnancy (days 14 to 16 post-ovulation) might help predict the risk of SB, perinatal death, LBW and PTB. (1-3)

Methods: This retrospective cohort study analysed data from 2205 Assisted Reproductive Technology (ART) cycles, 1124 Frozen Embryo Transfer (FET) cycles and 149 Intrauterine Insemination (IUI) cycles performed between 2006 and 2021 at a private fertility clinic where hCG levels had been measured at least once between 14 and 16 days post-ovulation. Levels were compared to pregnancy and perinatal outcomes. ROC analysis determined an optimal cut-off to predict early-pregnancy-loss (EPL) and biochemical-pregnancy (BP) and the risks of SB, perinatal death, LBW and PTB were compared between low and high hCG groups. Maternal age and body mass index (BMI) were adjusted for as confounders.

Results: On 15 days post-ovulation a hCG cut-off of 70IU/L was effective for predicting EPL (95.0% sensitivity) and BP (94.0% sensitivity). There was an increased SB rate (6.81% v 0.61%, p<0.001) and perinatal death rate (6.81% v 1.09%, p=0.006) when the hCG level was under 70IU/L. There was a trend to rise in LBW and PTB, however this was not statistically significant. Further subgroup analysis of hCG levels was performed for days 14 and 16, and separately for ART, FET and IUI cycles; whose results appear to confirm these trends. However, when adjusting for maternal age and BMI, these increases in perinatal morbidity were no longer significant indicating that the perinatal impact of hCG levels was influenced by maternal age and BMI.

Conclusion: Our study has demonstrated that hCG levels days 14-16 post-ovulation could help prognosticate pregnancy outcomes. This is likely modulated by the impact of maternal age and BMI.

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P193 Can anti-mullerian hormone levels predict delivery outcomes in recurrent pregnancy loss?

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Background: Anti-Mullerian Hormone (AMH) levels have been shown to be lower among women who have experienced recurrent pregnancy loss (RPL) compared with the general population1-3. However, it is unclear whether it can predict the ultimate outcome in the RPL setting, a livebirth. This study aims to determine whether AMH can predict the likelihood of a livebirth in women with RPL.

Methods: Retrospective analysis of a consecutive cohort of women undergoing investigation for RPL in a tertiary referral centre over a three year period (December 2018 -December 2021). Analysis was done using logistic regression models adjusting for maternal age and previous livebirth. Exclusion criteria included abnormal parental karyotype and abnormal pelvic ultrasound scan. Pregnancy outcome was defined as livebirth or further pregnancy loss.

Results: 477 women underwent investigation of RPL during the study period. Of these, 63.7% (n=304) conceived while attending the clinic. The majority of women (71.7%, n=218) proceeded to have a livebirth. There were no differences in median AMH levels between the livebirth group and the further pregnancy loss group (11 pmol/L vs 9 pmol/L respectively (p=0.083). AMH ≥1 pmol/L was associated with higher clinical pregnancy rates (p<0.01, 95% CI= 3.01 [2.07, 4.37]) but no difference in pregnancy outcome (p=0.36, 95% CI= 0.32 [0.03, 3.71]).

Conclusion: Although AMH levels may have some utility in counselling of some couples with RPL, these contemporaneous data indicate that low AMH does not negatively influence pregnancy outcome in women with recurrent pregnancy loss.

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P194 Cessearan myomectomy for giant fibroid in toxemic patient. Case report

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Fibroid, myoma, and leiomyoma are synonymous to define the commonest benign solid tumor of the female genital tract. It is commonly encountered in pregnancy. It may end up in several obstetric complications. Management of these women presents great challenges. Cesarean myomectomy is a debatable procedure because of higher risk of associated morbidity. Adequate management of these women, improves their quality of life. Here we presents a case of primigravida with multiple giant fibroids who underwent successful cesarean myomectomy resulting in healthy baby and multiple degenerated fibroid weighing more than 10 kg with remaining healthy uterus.

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P195 Complement and inflammatory marker analysis in women with antiphospholipid syndrome (APS) and recurrent pregnancy loss (RPL)

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Background: Antiphospholipid syndrome (APS) causes recurrent pregnancy loss (RPL) and is associated with other serious adverse pregnancy outcomes including pre-eclampsia (PET), preterm birth and stillbirth. Increasing evidence suggests the pro-inflammatory complement pathway is dysregulated in primary APS and is a cofactor in aPL-associated thrombosis[1]. The principal aim of this study is to investigate whether detailed aPL and complement pathway analysis aids pre-conceptual risk stratification for women with APS and RPL. Identification of women with RPL and APS at greatest risk for complications would permit a more targeted therapeutic approach.

Methods: Serum samples were obtained from women with RPL and APS (aPL positive OAPS;N=24), RPL but no APS (RPL;N=25), and healthy non-pregnant controls (controls;N=25). C3a, C5a, SC5b-9, and Annexin V levels were measured alongside cytokine, chemokine, and cytolytic molecule concentrations via ELISA.

Results: SC5b-9 concentrations were increased in the OAPS and RPL cohorts (p=0.0036), whilst C3a and C5a levels appear unaltered. The RPL group displayed reduced Annexin V, compared to controls (p=0.0096), whilst for OAPS a non-significant decrease in Annexin V was seen. IL-5 levels were elevated in OAPS compared to RPL (p=0.0044), whereas Granzyme B was reduced in RPL compared to controls (p=0.0266), being non-significantly decreased in OAPS.

Conclusions: Preliminary findings suggest immune dysregulation in RPL, with and without APS. The SC5b-9 increase in OAPS and RPL, indicates that RPL may be associated with dysregulation in the complement cascade, potentially inducing elevated SC5b-9-mediated cell lysis and pro-thrombotic endothelial changes. As prothrombin activation is inhibited by Annexin V, its reduction could elevate thrombotic risk. This data is part of a larger prospective risk stratification study (ASSIST) aiming to identify women with increased subsequent pregnancy loss risk, via aPL profile assessment and prospective pregnancy outcome investigation.

Chaturvedi S, Braunstein EM, Yuan X, et al. Complement activity and complement regulatory gene mutations are associated with thrombosis in APS and CAPS. Blood. 2020;135(4):239-251.

P196 Cohort profile: Anhui maternal-child health study in China

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Purpose: The Anhui Maternal-Child Health Study (AMCHS) aims to examine determinants of reproduction, pregnancy and post-partum maternal and child health outcomes in Chinese women who received assisted reproductive technology (ART).

Study design and Participants: AMCHS is an on-going cohort study, starting from May 2017. It recruits participants from all couples who sought ART treatment in the hospital. The participants are interviewed to document baseline socio-demography, lifestyles, dietary intake and environmental exposure. Their clinical characteristics are obtained from hospital records. Samples of blood, follicular fluid and semen are collected at clinic. Participants receive a standard long pituitary downregulation or a short protocol with an antagonist for the treatment. They are followed up from preconception to delivery, or discontinuation of ART treatment. Details of children health are documented through a questionnaire focussing on developmental status and anthropometry measurement.

Findings to date: Until April 2021, AMCHS has recruited 2042 couples in the study. Of women, 111 withdrew from the study, 19 failed to retrieve oocytes.Among 1475 confirmed pregnancy, 146 had miscarriage or termination of pregnancy, 9 had stillbirth, and 263 were ongoing pregnancy. The implantation failure increased with maternal age; adjusted odds ratio was 1.43 (95% CI 1.16-1.77) in age of 31-35 years, 1.97 (95% CI 1.46-2.66) in 35--39 years and 6.52 (95% CI 3.35-12.68) in ≥40 years compared to those aged 20-30 years. Among the 1057 couples with successful ART who were followed up for delivering baby, 576 had their children examined at age 30-42 days, 459 at 6 months, and 375 at 12 months.

Future plans: The AMCHS will identify comprehensive risk factors for poor ART outcomes and explore potential interaction effects of multiple factors including socio-psychologic aspects environmental exposure, dietary intake and genetics on maternal and child health.

P197 Seroprevalence and risk factors of specific toxoplasma gondii antibody among pregnant women

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Toxoplasmosis during pregnancy has an adverse effect on pregnant women, fetus and neonatal. The infection is generally asymptomatic but can cause severe effect on the fetus and immunocompromised women. Control study conducted on 77 pregnant women categorized according to the risk factors influencing toxoplasmosis infection. Specific anti Toxoplasma gondii IgG and IgM were evaluated using Enzyme-linked immunosorbent assay (ELISA). Among 77 pregnant women,(57.1%) women had toxoplasmosis, seropositive for latent infection with specific Toxoplasma gondii immunoglobulin G (IgG) antibodies were (54.54%), whereas acute infection immunoglobulin M (IgM) were only in (2.59%) cases. The seroprevalence of Toxoplasma was higher in older pregnant women (> 60%) than younger ones (< 50%). The specific IgG antibody was higher in pregnant women working in farms (65.7 vs 45.23; P= 0.053). Also, the seropositive IgG antibody was low in the first trimester and high in rural areas. Pregnant women need to educate more about toxoplasmosis and prevention to exposure in order to reduce the risk of congenital toxoplasmosis.

P198 RNAseq analysis identifies PPAR signalling and calcium binding as key players in the hypoxic mouse placenta

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Introduction: Hypoxia during intrauterine development increases the risk of pregnancy complications and adversely affects fetal growth. Adequate oxygen (O2) is required by the fetus for growth and metabolism, and by the placenta, as the site of materno-fetal substrate exchange. In mice, late-gestational exposure to maternal inhalation hypoxia causes severity-dependent reduction in fetal weight and altered glucose and amino acid transport (1). However, little is known about the molecular mechanisms governing the feto-placental response to hypoxia.

Method: Mice were exposed to inhalation hypoxia (10% or 13% O2) from day 14-19 of pregnancy. RNA-sequencing (RNAseq) was performed on dissected placental transport zone (labyrinth zone; LZ) of male fetuses exposed to 13% O2 or normoxic controls (21% O2) (n=4/group). Differentially expressed genes (DEGs) were assessed in LZ samples of both sexes from normoxic controls and those exposed to 13% O2 and 10% O2 by qPCR and western blotting (n=5-7/sex/group). Whole placentas from each group were sectioned for histology. Data were analysed by Two-way ANOVA (treatment and sex).

Results: RNAseq analysis identified 21 DEGs in LZ from mice exposed to 13% O2, with gene ontology analysis showing these were linked to calcium binding and PPAR signalling. Histological staining showed elevated calcium deposition in

the placental LZ, which was colocalised with increased fibrosis, in both 10% and 13% O2. Ntrk2was significantly upregulated in both 10% and 13% O2, and correlated with fetal weight in 10%, but not 13% or 21% O2. Eight DEGs harbour peroxisome proliferator-activated receptor (PPAR) binding sites and western blot analysis showed decreased PPAR; abundance in both severities of hypoxia.

Conclusion: Gene expression analysis identified pathways involved in the placental response to hypoxia including PPAR signalling and calcium binding. Further investigation of these pathways could help identify diagnostic markers for conditions that feature placental hypoxia, to improve fetal outcomes and future offspring health in complicated pregnancies.

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P199 Pregnancy outcomes following recurrent miscarriage

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Background: Recurrent pregnancy loss affects 1-2% of the population(1). Literature around recurrent pregnancy loss has largely focused on the causes, treatment options and live birth rate following recurrent pregnancy loss(2). Recently research has shown that patients with recurrent pregnancy loss may be at increased risk of adverse pregnancy outcomes(3).

Objective: This study aimed to assess the reproductive outcomes for patients who attended a specialist clinic for investigation and treatment. Methods Prospective analysis of all patients who attended a recurrent miscarriage clinic from January 2014 to January 2021. Data was gathered on a total of 488 patients from paper and electronic records.

Results: Of the 488 patients who attended a specialist clinic, 318 had a further pregnancy with 299 included in this study. The median age of patient was 37 years, with 55.6% having a previous livebirth. The subsequent live birth rate was 75.3%, 22.0% had a further pregnancy loss, 1.7% had an ongoing pregnancy and 1% attended another institution after second trimester. Patients were prescribed progesterone in 58.2% (n=174), eltroxin for hypothyroidism in 14.4% (n=43), aspirin in 23.7% (n=71) and LMWH in 14.0%(n=42). The majority of patients had an early pregnancy scan 93.0% (n=278). The rate of preeclampsia was 2.2%, pregnancy induced hypertension 2.2%, fetal growth restriction 5.3%, preterm birth ≤34 weeks was 1.8% and preterm birth >34 weeks < 37 weeks was 6.6%. Regarding mode of delivery 58.0% (n=131) had a vaginal delivery, 4.0% (n=9) an operative vaginal delivery and 37.2% (n=84) had a caesarean section. The majority of patients, 73.9% (n=221) attended consultant led antenatal care.

Conclusions: From this review of pregnancy outcomes recurrent pregnancy loss is not associated with an increased risk of adverse pregnancy outcomes. Patients who attend a recurrent pregnancy loss clinic have a high livebirth rate in a subsequent pregnancy.

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P200 Age considerations in recurrent miscarriage

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Background: Recurrent miscarriage (RM) affects up to 5% of women.[1,2] The incidence of RM is increasing, likely due to improved case recognition and advancing maternal age.[3] Most guidelines advocate a generalised approach to RM in all age groups.[4,5] We compare the aetiologies of RM and subsequent pregnancy outcomes in women aged <35 years versus >40 years to assess whether a tailored age-based approach to care may be used.

Methods: We performed a retrospective review of RM between 2014 and 2021. Data on demographics, clinical features, investigations performed, management and obstetric outcomes in a subsequent pregnancy were separated into female age group: (1) <35 and (ii) >40 years.

Results: There were 488 cases of RM with complete data during our study period. Approximately one quarter of our cohort were aged <35 years (n = 126), while almost one third were >40 years (n = 152). Where cytogenetic testing of products of conception (POC) was performed, abnormal results were more common in the >40 year old group compared to the <35 year old group (84.8% vs. 41.1%; p=0.0001). There were no differences in the rates of positive

parental karyotype results (8.6% (n=5/58) vs 1.8% (n=1/55); p=0.2), Antiphospholipid Syndrome (7.3% vs 9.3%; p=0.66) or thyroid dysfunction (16.4% vs 13.2%; p=0.49) between the two groups. Subsequent conception rates were lower in the >40 year old group (51.3% vs. 77.8%; P=0.0001), however live birth rates were comparable (70.4% vs 61.5%; P=0.26).

Discussion: Cytogenetic testing of POC should be prioritised in women >40 years. This approach may be used to reduce delays in the work up and treatment of women with RM to optimise conception rates. Women may be reassured that live birth rates are similar in both age groups if they subsequently conceive.

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P201 Uterine transplantation: Legal and regulatory implications in England

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Uterus transplantation (UTx) is fast evolving from an experimental to a clinical procedure, combining solid organ transplantation with assisted reproductive technology. UTx, alongside IVF, presents a transformative option for treating women who are unable to gestate such as those with absolute uterine factor infertility (AUFI) which affects approximately 1 in 500 women of reproductive age worldwide. UTx has ushered in a new clinical arena in the field of transplantation and assisted reproduction. Since the first livebirth following UTx in 2014, women with AUFI may now have an alternative option to adoption and surrogacy in starting a family. To date there have been over 70 UTx procedures and 24 live births achieved, with detailed outcomes from 17 births reported in the literature. The commencement of the first human uterus transplant trial in the United Kingdom leads us to examine and reflect upon the legal and regulatory aspects closely intertwined with UTx from the process of donation to potential implications on fertility treatment and the birth of the resultant child. As the world's first ephemeral transplant, the possibility of organ restitution also requires consideration. As UTx transitions to a clinical procedure, policies in parallel to other SOT's must be considered for its incorporation into practise. This includes the allocation of deceased donor uterus' which importantly may need to factor the ageing recipient, in addition to regulatory policies on living UTx donors. Furthermore, by recognising the uterus as a solid organ transplant it can assimilate to the same consent policy for organ donation after death. Especially, as unlike composite transplants such as the face or limbs, there is no physical disfigurement on procurement of the uterus.

P202 Attitudes, knowledge, and perceptions amongst women toward uterus transplantation and donation in the UK

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Objective: To assess the motivations, perceptions and number of the general public in the UK toward donating their uterus for Uterus Transplantation after death (UTx).

Design, setting and population: A cross sectional survey-based study consisting of a 32-item electronic questionnaire conducted over a four-month period. 159 participants over the age of 16, living in the UK consented and took part in the study.

Main outcome measures: The motivations and perceptions towards UTx amongst the general public in the UK including the willingness to donate and barriers preventing donation.

Results: A total of 159 women completed the questionnaire. The majority of respondents had never heard of UTx (n=107, 71%) and most of the participants were not aware the uterus could be donated after death (n=130, 92%). Almost half of the cohort were willing to donate their uterus after death (n=57, 43%). Only a minority stated they

wished to donate their organs but not their uterus (n=10,8%). Approximately a third of women (n=42;30%) believed the child born following UTx would have genetic links to the donor. Over half of the respondents (n=65, 51%) strongly agreed or agreed they would feel joy in the knowledge that donation would lead to bringing a new life into the world. A quarter of respondents strongly agreed or agreed (n=45, 25%) that the use of their uterus by another woman would feel like an extension of life.

Conclusions: The findings indicate a favourable opinion towards UTx and a positive attitude towards donation of the uterus after death amongst the general public in the UK. The findings also highlight the need for education around UTx now the option is available.

P203 Endometrial autotransplantation in the rabbit model- exploring neovascularisation models for ashermans syndrome

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Introduction: Ashermans Syndrome (AS) is the responsible aetiology in 5% of infertile women. Some women with AS have complete absence of functional endometrium devloping absolute uterine factor infertility (AUFI). Endometrial transplantation (ETx) may present a valuable treatment option in this cohort. Previously, our team has demonstrated the feasibility of autologous ETx but a livebirth was not achieved. The addition of platelet rich plasma (PRP) was proposed for its ability to harness the intrinsic regenerative capacity of the endometrium as proven in both in vivo and ex vivo models.

Objective: To investigate the role of PRP in neovascularisation at the endometrial-myometrial interface and to compare the effect of PRP treated rabbits with a control group undergoing ETx with no additive therapy.

Study design: Autologous PRP was prepared using the Endoret®(prgf®) technology (BTI System IV/V; BTI Biotechnology Institute, Vitoria, Spain). Six New Zealand Rabbits underwent ETx in one of the two uterine horns. Five rabbits were allocated to the treatment arm and one rabbit to the control group. PRP was injected at the endometrial-myometrial interface immediately following transplantation. Four weeks post operatively, each rabbit underwent IVF with the transfer of six early blastocysts to each uterine horn. Ultrasound was used to assess the endometrium prior to embryo transfer.

Results: Preliminary results are presented. The mean operative time per case was 94 minutes. Four rabbits were alive at 2 weeks post operatively, one rabbit died on day 2 due to a peritoneal haematoma. 1 rabbit achieved a successful implantation in the operated horn as seen on laparoscopy on Day 11 post transfer. No live births have been achieved.

Conclusion: The use of PRP in the rabbit model of ETx does not appear to improve the implantation nor clinical pregnancy rate. Histological studies are outstanding to assess for endometrial regeneration.

P204 Therapeutic potential of platelet rich plasma in infertility

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The use of cell-based therapies such as platelet rich plasma (PRP) have gained considerable momentum over the last decade in their ability to promote tissue regeneration through cell differentiation and trophic activities. Therapeutic applications of PRP within the human uterus are either via the intrauterine (intracavity) or subendometrial route. This review provides an up-to-date analysis of the use of PRP to improve fertility. PRP has been explored both in vivo and ex vivo in the human endometrium model in its ability to harness the intrinsic regenerative capacity of the endometrium. The first study to demonstrate its potential displayed successful endometrial expansion and pregnancies in women treated with intrauterine autologous PRP infusions. Other studies have reported patients with thin endometria treated with intrauterine PRP infusions 36-48 hours prior, displaying increased endometrial thickness >7mm and successful pregnancies. Another delivery technique which has been explored is direct injection of PRP beneath the superficial endometrium during hysteroscopy- the subendometrial route. In women with recurrent implantation failure and thin endometrium, subendometrial injection has been proven to improve endometrial thickness and vascularity. Another exciting potential is the application of PRP for premature ovarian insufficiency (POI). In vitro studies demonstrate intraovarian injection of PRP supports the growth of preantral follicles. In ovaries which are responding poorly to stimulation, PRP has demonstrated an increase in the number of follicles and egg yield at collection.

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P205 Feasibility study assessing normothermic perfusion of the rabbit uterus; implications for uterus transplantation

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Background: In organ transplantation, normothermic perfusion (NMP) has been used to facilitate transplantation of livers, kidneys, hearts, and lungs. Storing organs at physiological conditions offers multiple advantages over cold storage: most notably the minimisation of cold ischaemic injury. It also allows organ monitoring and permits the addition of intravenous therapy ex-vivo, which can help determine organ functionality or optimise the organ pre-transplantation.

Methods: Two feasibility studies were undertaken to determine if NMP can be utilised in a rabbit uterus.

Results: A fluid circuit was created using silicone tubing. The two uterine arteries were cannulated with 22G cannula. Phosphate buffer solution was pumped through each uterine graft using a roller pump. An in-line pressure sensor allowed the pressure to be monitored and controlled. A temperature sensor was used to monitor the temperature, which was warmed by a reservoir using a water bath set to 38 degrees celsius. Custom control and a switching circuit served as an interface for the sensor inputs and motor control output. During the first case, the ovarian arteries and veins were ligated, and the uterine veins left open, but the perfusion was suboptimal. To improve venous outflow the ties on the ovarian vessels were released and perfusion improved. For the second case, the ovarian artery was ligated but the ovarian veins left patent. Introduction of dye demonstrated optimal perfusion the uterus.

Conclusion: This study confirmed NMP is feasible in rabbit uteri and has established a circuit and proposed cannulation sites for further studies. In the context of UTx, prolonged preservation in the absence of a clinically indicated waiting list, would facilitate the nationwide optimisation of tissue matching between donor and recipient. Longer preservation periods would also permit more efficient allocation and scheduling of clinical personnel and resources.

P206 Investigation study into transplantation of the uterus (INSITU); screening of uterine transplant recipients in the UK

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Background: A uterine transplantation (UTx) is a feasible fertility restoring intervention for women with absolute uterine factor infertility (AUFI). Whereas AUFI affects one in 500 women of childbearing age,[1] there is limited data on demand and suitability to undergo UTx in this population. The aim of this study was to gauge interest amongst women with AUFI, characterise their background demographics, and determine their reasons for considering UTx over adoption and surrogacy.

Methods: A cross-sectional survey utilising a questionnaire amongst women with AUFI who have enquired about UTx.

Results: 210 women participated. The most common aetiology of AUFI was MRKH (68%; n=143) whereas 29% (n=62) had previously undergone hysterectomy. 48% (n=30) were undertaken because of cancer, the most common of which was cervical cancer (n=22; 73%). In those undertaken for benign disease (n=32), the most common indication was massive obstetric haemorrhage (n=13; 41%). 16% (n=33) of women were parous. 63% (n=132) of the cohort had previously considered adoption, 5% (n=11) had attempted it, and two (1%) had successfully adopted. The most common reason cited to undergo UTx over adoption was to experience gestation (n= 63; 53%), while 37% (n=44)

wanted a biologically related child. 76% (n=160) of participants had previously considered surrogacy, 22 (10%) had attempted it and two (1%) had successfully become mothers using a surrogate. The most common reason to undergo UTx over surrogacy was to experience gestation (n=77; 54%). 15% n=21) were concerned about the legal implications, 14% (n=20) identified the financial cost as a barrier and 8% (n=12) could not consider it due to religious reasons.

Conclusions: This is the largest and most detailed screening analysis of women with AUFI who have expressed interest in UTx. Our findings highlight important novel insights into such women, including their perceptions toward UTx and alternative options to acquire motherhood.

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P208 Adenomyosis is not rare, it is rarely looked for: A systematic review and meta-analysis on the prevalence of adenomyosis in women with subfertility

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Background: Currently, a rising number of adenomyosis cases are diagnosed in women aged 30 to 40 years presenting with subfertility (1-3). Yet there remains a paucity of epidemiological data on the burden of adenomyosis in women with subfertility. The aim of this systematic review and meta-analysis was to provide a comprehensive up-to-date synthesis of the prevalence of adenomyosis diagnosed in women with subfertility.

Methods: We conducted systematic searches in MEDLINE, Embase, EBSCOhost CINAHL plus, Google Scholar, PsycINFO, and Web of Science Core Collection from database inception to October 2022. Studies evaluating the prevalence of adenomyosis in women with subfertility, with or without endometriosis and uterine fibroid(s), were included.

Results: Among 21 longitudinal studies evaluating 25,600 women, the pooled prevalence of isolated adenomyosis was 10% (95% confidence interval [CI] 6% to 15%). The pooled prevalence was 10% in women with coexisting fibroids (95% CI 2% to 25%; 8 studies), 18% in women with coexisting endometriosis (95% CI 9% to 28%; 18 studies) and 17% in women with coexisting endometriosis and fibroids (95% CI 5% to 34%; 9 studies). The prevalence of isolated adenomyosis varied substantially according to geographic location, with Australia exhibiting the highest pooled prevalence of adenomyosis (19%, 95% CI 12% to 27%) and Asia the lowest (5%, 95% CI 1% to 13%). The pooled prevalence of isolated adenomyosis diagnosed using a combination of direct and indirect USS features was 11% (95% CI 7% to 16%) whereas it was 0.45% (95% CI 0% to 1%) where only indirect features were used as a diagnostic criterion.

Conclusions: One in ten women with subfertility have a diagnosis of isolated adenomyosis and the prevalence varies by whether there is coexisting endometriosis or fibroids.

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P209 The effect of uterine disease on the reproductive performance in high yielding dairy cows from commercial UK dairy herds

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Background: It has been reported that fertility in high yielding dairy cows has declined over the past five decades corresponding with increased milk production. Uterine health is an important factor with endometritis prevalent in high-yielding dairy cows.

Aim: This study aimed to assess the effect of uterine disease on reproductive performance in 78 commercial UK dairy herds.

Methods: Data was collected from 2000-2009, and the number of lactations included this study was 59 118 lactations (n=29 157 cows). The data included presence of uterine disease, calving date, and insemination information. Linear mixed model analyses were performed to determine the effect uterine disease on the conception rate, days to first insemination (DFS), calving to conception interval (CCI) and calving interval (CI). The proportion of animals culled was compared using χ2-test.

Results: The incidence of endometritis in this study was 12% per lactation. Cows with uterine diseases has longer DFS, CCI and CI by 7 (P<0.05), 20 days (P<0.001) and 26 days (P<0.001) in their lactation. The extension in the CCI was, in part, explained by a lower first service conception rate (P< 0.0001) in cows that had post-partum uterine disease (24.3%) compared to controls (38.0%). Furthermore, there was an increase of 0.8 services per conception (P<0.001). The culling rate in cows that experienced uterine disease was greater (24.9%; P<0.001), compared with the control group (21.2%).

Conclusion: This study has quantified the negative impact of uterine disease on the reproductive performance in UK commercial dairy herds, with the biggest association performing to occur on the ability of cows to re-conceive.

P210 Estradiol-17 and PGE2 regulate gene and protein expression of enzymes involved in DNA methylation in porcine endometrium in vivo

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During early pregnancy in pigs, conceptuses secrete elevated amounts of estradiol-17(E2) which is their primary signal. However, the pattern of enhanced synthesis and secretion of prostaglandin E2 (PGE2) in porcine endometrium and conceptuses is similar to the pattern of embryonic E2 secretion. We showed that E2 together with PGE2 significantly alters endometrial transcriptome in pigs and regulates expression of genes involved in pregnancy establishment. One of the mechanisms by which E2 may regulate gene expression is related to DNA methylation processes. The present study aimed to determine whether E2 and PGE2 may affect the gene and protein expression of enzymes regulating DNA methylation: DNMT1, DNMT3A and DNMT3B. We applied an in vivo model of intrauterine E2 and PGE2 infusions. Gilts on day 12 of pregnancy and the estrous cycle were used. Western blot and qPCR techniques were employed in gene and protein expression studies. E2 significantly (p<0.05) decreased endometrial abundance of DNMT3A and DNMT3B mRNA. Lower levels of DNMT3A (p<0.05) were observed in endometrial samples from gilts on day 12 of pregnancy compared to day 12 of the estrous cycle. No effects of PGE2 and E2 on endometrial expression of DNMT1 and DNMT3A proteins were found. However, E2 administered simultaneously with PGE2 significantly reduced endometrial abundance of DNMT1 and DNMT3A proteins (p<0.05). Likewise, expression of DNMT1 and DMNT3A proteins was significantly lower in endometrial samples collected from gilts on day 12 of pregnancy compared to day 12 of the estrous cycle (p<0.05). Both PGE2 and E2 (833 ng/infusion) administered alone and simultaneously significantly decreased endometrial levels of DNMT3B protein (p<0.05). Our results indicate that DNA methylation likely controlled by estradiol-17and PGE2 may be a novel potential mechanism regulating the expression of genes in porcine endometrium during early pregnancy. Funded by National Science Centre, Poland, grant no. 2017/27/B/NZ9/03014.

P211 NK cells in reproductive failure: In-depth analysis of uterine NK cells subsets

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Background: In a significant proportion of women with reproductive failure (RF), the cause of the failure is unexplained and immunological cause is thought to be contributory. Uterine Natural Killer cells (uNK) play an important role in placental development in first trimester pregnancy. However, pathophysiology of uNK in RF is not fully elucidated. Our meta-analysis showed elevated total uNK level in women with RM and RIF compared to fertile controls [3]. More recently, single-cell RNA sequencing in first trimester pregnancy found uNK to consist of three subpopulations (uNK1, 2, 3) [1] with different roles throughout the reproductive cycle [2]. We aim to assess these uNK subsets in endometrium of women with RF compared to controls.

Methods: Here, we used flow cytometry to assess proportion, phenotype and function of matched peripheral NK cells (pNK) and uNK subsets in the endometrium of women with RF (including unexplained subfertility (US), recurrent miscarriage (RM) and implantation failure (IF)) compared to controls. Mann-Whitney U, t-test or one-way ANOVA were performed where indicated. p<0.05 is considered significant.

Results: For phenotype, we found lower KIR2DL1/S1 and LILRB1 receptor expression in pNK, uNK2 and uNK3 in RF group. These receptors are important for interaction with HLA-C and HLA-G molecules on extravillous trophoblast cells (EVT) [4]. For function, we found TNF-; production to be reduced in all stimulated uNK subsets in RF group. Unexpectedly, IFN-; is higher in unstimulated uNK2 in IF subgroup. For proportion, we found no difference in uNK subsets, although pNK Bright was significantly lower in RF group.

Conclusion: Taken together, our findings showed that uNK of women with RF have reduced KIR2DL1/S1, LILRB1, and TNF-; which may contribute to problems with implantation and/or placentation. This serves as a platform from which

relationship between uNK and reproductive failure can be investigated, which will facilitate identification of potential therapeutic targets.

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P212 MiR-185-5p regulated cadherin binding proteins: A potential pathway involved in endometrial receptivity to implantation in humans?

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Previous work by our group has established miR-185-5p as an evolutionarily conserved regulator of endometrial gene expression in placental mammals with divergent implantation strategies. Proteomic analysis of transfected human endometrial epithelial cells revealed significant changes to protein abundance in response to miR-185-5p mimic (n=1304) and inhibitor (n=363), or both (n=146). Comparison of these data with published data on endometrial gene expression and miR-185-5p online target prediction revealed functional enrichment for cadherin binding and cell adhesion molecule binding to be significantly represented functions. We tested the hypothesis that members of the cadherin binding family of transcripts would be dysregulated in endometrial biopsies from individuals that experienced recurrent implantation failure (RIF). Endometrial biopsies were obtained following informed consent and ethical approval from Tommy's National Reproductive Health Biobank. Luteal phase biopsies from individuals with a history of RIF (n=10) and patients with proven fertility following a previous live birth (LB; n=9) were processed for quantitative real-time PCR analyses of the following transcripts involved in cadherin binding: annexin A1 - ANXA1, capping actin protein of muscle Z-line subunit alpha 1 - CAPZA1, cadherin 1 - CDH1, catenin beta 1 - CTNNB1, casein kinase 1 delta - CSNK1D, isocitrate dehydrogenase (NADP(+)) 1 - IDH1, junction plakoglobin - JUP, tight junction protein 1 - TJP1 and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta - YWHAZ. All transcripts were detected in the endometrium while expression of CSNK1D and TJP1 was lower in biopsies from the RIF group compared to the LB group (p=0.036 and p=0.079, respectively). These data provide preliminary evidence to support a regulatory role for miR-185-5p and its targets in contribution to endometrial function during implantation.

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P213 Surgical treatment of caesarian scar defect: The effect of each approach on clinical manifestations and fertility

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Background: Caesarian scar defect or isthmocele is a long-term occult complication of cesarean section, which has become alarmingly prevalent due to the increase in cesarean sections performed. It can be the cause of irritating symptoms, severe complication during pregnancy and a cause of infertility. Therefore, treatment of isthmocele is a

research field of growing interest, with many approached being tested for optimal results. In this systematic review we will examine and compare the efficacy of laparoscopic, hysteroscopic and vaginal approach in fertility improvement.

Methods: A systematic search of the available literature on PubMed, Scopus and Web of Science was conducted and the PRISMA 2020 guidelines were followed. Eligible studies were further assessed for risk of bias with tools specific for each study's design.

Results: Ultimately, 37 studies were eligible for inclusion and were evaluated as of an acceptable risk of bias, although of significant heterogeneity. Based on extracted data, pooled clinical pregnancy rates were calculated for laparoscopic, hysteroscopic and vaginal approaches: 49%, 56% and 59% respectively, however without any statistically significant difference between the three.

Conclusions: Cesarean scar defect is a considerable cause of secondary infertility following cesarean delivery, along with other, possibly life-threatening complications. Among the three different therapeutic techniques we examined, no statistically significant differences arose. Although, the significant methodological and statistical heterogeneity among the studies must be noted, along with the fact that pregnancy rates are not always indicative of trends in live births rates as well. Further research on this hotly debated issue is required to better guide the clinicians and fertility specialists that will be facing this affliction more and more frequently in practice.

P214 Role of the endometrial receptivity array and endometrial microbiome in recurrent implantation failure patients

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Background: Successful implantation of a blastocyst requires a receptive endometrium. The Endometrial receptivity array (ERA) gives information on the receptivity status of an endometrial sample and recommends adjusting time of frozen embryo transfer (FET) for patients with non-receptive results. A non-Lactobacillus dominant endometrial microbiome has been associated with poorer reproductive outcomes. The Endometrial Microbiome Metagenomic Analysis (EMMA)/Analysis of Infectious Chronic Endometritis (ALICE) test reveals the microbial composition of an endometrial sample and advises appropriate treatment prior to FET.

Methods: A retrospective cohort study of recurrent implantation failure (RIF) patients was performed. All ERA and EMMA/ALICE patients had an endometrial biopsy. ERA patients with non-receptive results had timing of FET within their cycle altered. EMMA/ALICE patients with abnormal results underwent recommended treatment prior to FET. Statistical analyses were performed using SPSS.

Results: Patients who had ERA testing had higher live birth rates (38.9% v 32.1%, p=0.298) and lower miscarriage rates (15.7% v 19.8%, p=0.436) compared to controls, although differences were not statistically significant. Patients who had EMMA/ALICE testing led to higher live birth rates (41.2% v 32.1%, p=0.173) and lower miscarriage rates (15.7% v 19.8%, p=0.557), although differences were not statistically significant. There was no significant difference between reproductive outcomes of patients with receptive or non-receptive ERA result or normal or abnormal EMMA/ALICE result, although those with abnormal result had a lower LBR.

Discussion: We observed a non-significant trend that ERA and EMMA/ALICE tests improved reproductive outcomes. This study was underpowered to detect a 5% difference at 80% power. The poorer LBR of those with an abnormal EMMA/ALICE result might suggest issues with the current recommendations offered by the test. Further studies with larger sample sizes should be conducted to investigate these findings.

Conclusion: Use of the ERA and EMMA/ALICE tests may improve reproductive outcomes in RIF patients although further studies with larger sample sizes should be conducted for conclusive.

P215 Endometrial receptivity analysis (ERA) before embryo transfer in women undergoing assisted reproductive therapies: A review of the literature

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Aim: Endometrial Receptivity Analysis (ERA) is a state-of-the-art technology developed to characterise the endometrium and optimise timing of embryo transfer for women undergoing assisted reproductive therapies (ART). The aim of this project is to evaluate the effectiveness of this new method and identify conditions where it would be most applicable.

Methods: A literature review was conducted to collect current data on ERA used at the time of (ART). Articles studying the efficiency of ERA were selected and divided into three categories (high, medium and low quality) based on the level of evidence provided on the topic. Data was reviewed as an umbrella analysis.

Results: In total, eighteen out of the thirty-three relevant articles were identified in the literature and were selected for an umbrella review analysis. Thirteen out of the eighteen studies showed improved outcomes post ERA and personalised embryo transfers. In the appropriate population ERA is associated with optimal identification of the window of implantation and higher pregnancy and live birth rates. ERA could provide beneficial information to optimise transfer for cases of embryo shortage, history of recurrent implantation failures, or infertility that could be explained due to a displaced window of implantation.

Conclusion: ERA allows for guided, personalised embryo transfers. There is no statistical significance in regular use of ERA for the optimisation of routine IVF. However, in targeted populations results appear promising, but more focused high-quality studies need to be completed to collect more conclusive evidence.

P216 Impaired decidualisation in obese mice is associated with the upregulation of leptin signalling modulators Socs3 and Ptpn2

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Maternal obesity is associated with pregnancy complications and predisposition to the development of obesity and other comorbidities in offspring. A key event in early pregnancy is decidualisation of the uterine endometrium, but despite its importance, little is known about the molecular mechanisms compromising decidualisation in obese mothers. Here, we hypothesise that impaired decidualisation in obese mice is mediated by the disruption of leptin signalling in endometrial stromal cells (MESCs). We tested this in a mouse model of diet-induced obesity in which mice were fed chow-diet (CD) or high-fat diet (HFD) for 16 weeks prior to mating. First, we confirmed the decreased expression of decidualisation markers Dtprp1, Bmp2, Hand2, Hoxa10 in MESCs collected from obese mice 3.5 days post-coitum and decidualised in vitro. Then we collected decidua at gestational day 6.5 (G6.5) and found that the same decidualisation markers were downregulated in decidua from HFD mice ($p<0.05$). By immunofluorescence, we also observed increased staining of the proliferation marker Ki67 and progesterone receptor PgR in the primary decidual zone (PDZ) in HFD mice, whereas in CD the aforementioned markers were already visible in the secondary decidual zone (SDZ). Furthermore, pSTAT3, a major mediator of decidualisation as well as of leptin signalling, was localised in mesometrial decidua of CD mothers, while in HFD mothers pSTAT3 was still in the antimesometrial area. Together, these observations suggested a delay in decidualisation in HFD mice. Subsequently, we found that both mRNA and protein levels of leptin signalling inhibitors SOCS3 and PTPN2 were upregulated in MESC decidualised in vitro ($p<0.05$), as well as G6.5 decidua from HFD mice ($p<0.05$). Finally, siRNA silencing of Socs3 and Ptpn2 in MESCs from HFD mice led to the upregulation of the decidualisation markers Dtprp1 and Bmp2 ($p<0.05$). In conclusion, our results suggest that the delay in decidualisation observed in obese mice...

P217 Characterisation of immune cells at the endometrium of day 17 pregnant and non-pregnant ewes

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Before pregnancies can be guaranteed in the mammalian species, the uterine environment must tolerate the semi-allogenic fetus, a phenomenon which is achieved through an interplay of several activities which are endocrinological, physiological and immunological in nature. Unlike the invasive placentation that is known with the murine and humans, ruminants have a rather less invasive placentation and thus possess a peculiar mechanism of achieving maternal tolerance. Whereas there are records of the characterization of the immunological cells in the endometrium of a cow at implantation, there is a dearth of information on the characteristics of endometrial cells in the ewe during this phase of reproduction. And, unlike in rodents and humans where the specific roles of uterine natural killer cells have been determined, little is known on the availability, quantity and functions of these group of cells in the ovine species. Uterine horns were harvested from pregnant and non-pregnant ewes seventeen days after mating. The horns were opened for confirmation of pregnancy by presence of elongating morula. Endometrial scrapings taken specifically from the caruncular and intercaruncular areas were also stored at -80oC before serially processed for mRNA quantification. Quantitative PCR (qPCR) results showed that the caruncular areas of pregnant ewes had significant expression of CD2, CD3, CD4, and LAG3, whereas there was no statistically significant difference ($p>0.05$) in the values of CD8 and CTLA4 between the pregnant and non-pregnant animals. However, none of these genes was significantly expressed in the intercauncular areas of the pregnant ewes when compared to the non-pregnant ones. Overall, some of the genes were significantly higher ($p<0.05$) in the pregnant ewes compared to the non-pregnant ones.

P218 The secretome of the decidualizing endometrial stroma is altered in women with early onset pre-eclampsia

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Background: Pre-eclampsia results from inadequate spiral artery remodelling, a process reliant on the interaction between the extravillous trophoblast and decidualizing endometrium, mediated by the local immune environment. Decidualization involves phenotypic changes to endometrial stromal cells in response to progesterone, and leads to a nurturing and immunotolerant environment that supports embryo implantation and placental development. Evidence suggests pre-eclampsia is associated with defective decidualization, suggesting a maternal contribution to pre-eclampsia pathogenesis which could account for disease recurrence.

Methods: Endometrial stromal cells were isolated from non-pregnant endometrial biopsies from women with previous early-onset pre-eclampsia and compared to parous women with no history of prior pre-eclampsia. Cultured endometrial stromal cells underwent 8-day decidualization in vitro (0.5mM cAMP, 1µM medroxyprogesterone) and culture supernatant was collected 48 hourly. On day 8, serum-free media then conditioned the cells for 24 hours. Insulin-like growth binding protein-1 (IGFBP-1), a marker of decidualization, was quantified by ELISA across decidualisation (n=9). Arrays detecting 108 cytokines profiled the conditioned medium (n=8), in which Automated Protein Array Analysis determined raw pixel density data.

Results: Endometrial stromal cells from women with recurrent early-onset pre-eclampsia had reduced secretion of IGFBP-1 on day 6 and 8 of decidualization ($p<0.05$). Decidualization induced secretion of over 40 cytokines in women with previous pre-eclampsia. Cytokines secreted in abundance across all biopsies included Serpin E1, Dkk-1, ST2, GDF-15, RBP-4, angiogenin and osteopontin. With those showing differential secretion, 6 immunomodulatory cytokines had reduced secretion in pre-eclamptic samples; FGF-19, IFN-γ, IL-1α, thrombospondin-1, M-CSF and TfR ($p<0.05$). Enriched biological processes were centred on macrophage chemotaxis and differentiation.

Conclusion: Endometrial stromal cells from women with recurrent early-onset pre-eclampsia exhibit hampered decidualization and secrete an altered cytokine profile in comparison to women without previous pre-eclampsia. These findings support the hypothesis that aberrant decidualisation underpins recurrent pre-eclampsia pathogenesis, potentially through altered regulation of endometrial macrophage populations leading to dysfunctional placentation.

P219 Investigating the enzymatic activity of endometrial DPPIV in the molecular adhesive pathways at implantation using an in-vitro co-culture model

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In the UK, 1 out of 7 couples are infertile and 10% of them suffer from unexplained recurrent implantation failures1. The clinical success rate has stagnated at 25-30% per cycle for the last decade and the knowledge of molecular events at the embryo implantation remains limited, hindering further increase in clinical pregnancy success rates. An endometrial receptor, dipeptidyl peptidase-4 (DPPIV), involved in cell-cell adhesion via a fibronectin-binding domain, was identified as upregulated at implantation2-3. However, its enzymatic function remains unclear at implantation. Therefore, we aim to investigate it at the trophoblastic-endometrial interface.

In endometrial Ishikawa cells, DPPIV was heterogeneously detected at the apical and lateral cell membranes. The enzymatic activity of DPPIV was measured in-vitro and significantly inhibited by a potent DPPIV inhibitor, diprotin A. Using bovine serum albumin-, poly-L-lysin- or fibronectin- coatings, BCECF-AM-treated endometrial cells were plated for single-cell attachment assays. The fibronectin coating improved endometrial cell attachment. However, a 50g/mL Diprotin A pre-treatment of endometrial cells significantly decreased their cell attachment to fibronectin (36%). Therefore, the inhibition of DPPIV appeared to impair its adhesive function in-vitro.

Using an in-vitro model of implantation, monolayers of ishikawa cells were pre-treated with diprotin A or not and thus, trophoblast-derived BeWo spheroids were co-cultured onto the monolayer. A 32% reduction in spheroids attachment was significantly triggered by the inhibition of DPPIV with 50g/mL of diprotin A (compared to control). Moreover, clustered DPPIV-positive endometrial cells were observed at the spheroid attachment sites.

In this study, combined with its subcellular localisation at spheroids attachment sites, we demonstrated that the inhibition of the enzymatic activity of DPPIV had a negative impact on the DPPIV-specific binding to fibronectin in ishikawa cells, leading to the impairment of the trophoblast/endometrial attachment in-vitro. This data set the scene for the investigation of DPPIV enzymatic activity and its prescribed inhibitors at implantation

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P220 Differential expression of evolutionarily conserved, progesterone regulated microRNAs facilitates attachment during implantation in humans

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Early pregnancy events like implantation strategies are diverse amongst placental mammals. Some molecules involved signal in conserved manners, such as progesterone for establishing uterine receptivity to implantation across the Eutheria. Others are species-specific, such as those involved in maternal recognition of pregnancy. MicroRNAs are a key set of molecules which may be involved in the conservation or diversification of early pregnancy events. Recent work from our lab has identified a set of microRNAs which arose concurrent with placental mammals and have subsequently never been lost. Three of these (miR-340-5p, -542-3p and -671-5p) are differentially expressed in response to progesterone. Proteomic analysis following transfection with microRNA of interest mimics and inhibitors revealed alterations in proteins involved in many pathways that are important for implantation, such as response to endoplasmic reticulum stress - which is crucial for decidualization. We hypothesised that these microRNAs may be involved in implantation. Endometrial epithelial cells (Ishikawa cells, n=5) were transfected with microRNA mimics and inhibitors for the 3 microRNAs. Spheroids were produced from trophoblast cells (BeWo cells) and incubated on the epithelial monolayer for 1 hour. Wells were imaged before fixation using formalin, washed, and a second image was taken. Before and after counts of BeWo spheroids were taken to calculate attachment rate. An ANOVA followed by Bonferroni correction was carried out to test for statistical significance ($p<0.05$). MiR-542-3p mimic significantly decreased attachment rate compared to the non-targeting mimic. MiR-340-5p mimic significantly increased attachment rate in comparison to either miR-542-3p or miR-671-5p mimic, which both appeared to decrease attachment compared to controls. These results demonstrate that miR-340-5p, -542-3p and -671-5p play a role in facilitating attachment during implantation in humans.

P221 The effect of prolactin on postpartum uterine disease pathophysiology in dairy cattle

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Postpartum dysbiosis of the uterine microbiome and endometrial inflammation causes uterine disease in dairy cows, resulting in poor fertility and reduced production. High milk yielding cattle are at increased risk of developing uterine disease. Milk production is initiated by the hormone Prolactin (PRL) so, in a pilot study,we sought to determine whether increased PRL concentrations increase the risk of uterine disease, and whether PRL affects the immune response of uterine and ovarian cells to infection. We measured serum PRL concentration in postpartum cows (n = 158) diagnosed as healthy or diseased based on vaginal mucus assessment. Bovine uterine endometrial epithelial and stromal cells, and ovarian granulosa cells were isolated from abattoir tissue. Cells were challenged with E.coli lipopolysaccharide (LPS) in the presence or absence of PRL. TNFa and IL1-b concentrations in culture supernatants were measured using ELISA. Cows who developed uterine disease had higher serum PRL concentrations in the first week postpartum than healthy cows (61.4 ± 5.3 vs. 54.7 ± 10.9 pg/ml, P=0.09). IL-1b production was increased in bovine endometrial stromal cells challenged with LPS compared to unchallenged cells (8.4 ± 3.4 vs, 0 pg/ml, P=0.03). In the presence of PRL, LPS induced IL-1b production was reduced (1.97 ± 1.48 vs. 8.4 ± 3.4 ng/ml, P=0.1). Granulosa cell TNFa production was increased with both LPS, and LPS+PRL treatment (164.8 ± 17.3 and 159.8 ± 14.9 vs. 104.7 ± 26.56 pg/ml, respectively P=0.1). Our results suggest the role of PRL in modulating the postpartum uterine immune response, and thus the influence of PRL on the pathophysiology of uterine disease in dairy cows, deserves further scrutiny. This work was funded by SRF's Return to Research Award.

P222 Embryo signals regulate DNA methylation in the porcine endometrium in vivo

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Estradiol (E2) is the primary porcine embryonic signal which is secreted in a biphasic manner on days 11-12 and 15-30 of pregnancy. During this period, porcine embryos secrete abundantly also prostaglandin E2 (PGE2). We recently

characterized changes in the endometrial transcriptome in response to pregnancy and estradiol effect in vivo and established that estradiol together with PGE2 regulate the endometrial expression of pregnancy-related genes in vivo. Interestingly, studies on tumor growth and endometrial pathologies revealed the great potential of E2 in induction of changes related to DNA methylation involving DNA methyltransferases (DNMTs). The present study aimed to examine the effect of pregnancy, E2 and PGE2 on: (1) activity of factors regulating DNA methylation, i.e. DNMT1, DNMT3A and DNMT3B and on (2) CpG methylation patterns of selected genes expressed in porcine endometrium. An in vivo model of intrauterine infusions of E2 and PGE2 was applied. Moreover, endometria collected from gilts on day 12 of pregnancy and the estrous cycle were used. DNA methyltransferase activity was determined by ELISA and local DNA methylation was studied by bisulfite pyrosequencing. Activity of DNMT enzymes in endometrium was significantly elevated during pregnancy and in response to E2 treatment in vivo. Treatment with E2 alone and/or simultaneously with PGE2 changed endometrial DNA methylation of CpG sites of BGN, PSAT1 and WNT5A. Different CpG methylation patterns of BGN, RASSF1 and WNT5A were detected in the endometrium on day 12 of pregnancy vs. day 12 of the estrous cycle. Significant correlations were found between CpG methylation and gene expression for BGN, PSAT1 and WNT5A. Our findings indicate a contribution of embryonic signals in regulation of DNA methylation as putative physiological mechanisms regulating endometrial gene expression in pregnancy. Funded by National Science Centre, Poland, grant no. 2017/27/B/NZ9/03014 and the Institute grant no. 2/FBW/2022.

P223 Impact of the use of RI Witness electronic witnessing system on the IVF laboratory staff and patient experience

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Aim: Electronic witnessing systems have been used for over a decade. Increased media reporting of errors and overall stress placed on laboratory staff has led to recognition of wider benefits beyond their witnessing function. We designed a survey to investigate the impact of the utilization of RI WitnessTM (CooperSurgical, USA) a radio frequency identification (RFID) based electronic witnessing system, on the experience of laboratory staff and their perceptions of patient experience.

Methods: An 11-question anonymous self-administered online survey was developed using Qualtrics® software (Qualtrics, USA). 52 respondents were recruited to complete the survey from IVF laboratories in the UK and Ireland. Using Likert scale survey questions, recipients were asked their opinion on several statements, including how RI Witness impacted their daily work experience and patient care. Responses were analysed using descriptive statistics and Chi-square automatic interaction detection in SPSS.

Results: Embryologists made up 98% of respondents. 95% of respondents found RI Witness easy to use, rising to 100% of trainee/junior embryologists. Similarly, 91% of respondents, and 100% of trainee/junior embryologists, found the system intuitive. Job related stress was reduced in 81% of all respondents. Additionally, RI Witness increased confidence in 100% of trainee/junior embryologists that they had not made any potential errors. The majority of respondents agreed patients were aware their clinic was using RI Witness and this group believed patients were more likely to have treatment at their clinics (79%) and were reassured the risk of mistakes were reduced (78%).

Conclusions: This research demonstrates laboratory staff find RI Witness intuitive and easy to use. Use of RI Witness was shown to positively impact overall work experience. It plays an important role in increasing confidence that procedures have been performed correctly while reducing job-related stress. There was agreement that the patient experience was positively impacted and it plays a role in clinic selection.

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