Society for Reproduction and Fertility

SRF Early Career Researchers Symposium
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Abstract Book

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Oral Session - Early Career Symposium

Tuesday 10 January

Oral Presentations

ECR-1

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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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 more) cycles of IUI were calculated.

Result:
Among women aged <35 years, the LBR for IVF was 33.6% (CLB Â£20,669). The LBR for the 1st uIUI was 15.8% (TCLB Â£13,950), the 3rd uIUI was 13.2% (CLB Â£16,638) and the 6th uIUI (or above) was 11.2% (CLB Â£19,570).

For women aged 38-39 years, the LBR for IVF was 23.0% (CLB Â£30,277). The LBR for the 1st uIUI was 9.5% (CLB Â£23,228), and the â‰¥ 6th uIUI was 7.3% (CLB Â£30,250).

In contrast, for women aged 40-42 years, the LBR for IVF was 14.2% (CLB Â£48,858) and the LBR for the 1st uIUI was 2.9% (CLB Â£75,130). Therefore, IVF was cost-favourable compared with IUI in this age range.

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

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

ECR-2

TGF-β1 stimulates embryo attachment in an in vitro model system

Hanning Li, *Undergraduate Student, University of Edinburgh, UK*

Background:
Tubal ectopic pregnancy (tEP), when the embryo implants in the Fallopian tube, accounts for 1-2% of pregnancies[1]. tEP is a major cause of maternal morbidity and mortality[1] but the mechanism behind tEP remains unclear. Epithelial to mesenchymal transition (EMT) is a process whereby cells gain mesenchymal characteristics: cells start to migrate, have increased proliferation, and lose cell adherence and polarity[2]. Similar protein expression changes occur in cells undergoing EMT and in Fallopian tube epithelial cells in tEP, suggesting EMT in Fallopian tube epithelial cells may lead to tEP[3]. Our aim was to investigate the effect of the EMT-inducer transforming growth factor beta 1 (TGF-β1) on embryo attachment to Fallopian tube and endometrial epithelial cells.

Method:
This was explored using a co-culture model where murine embryos were cultured with a monolayer of OE-E6/E7 cells which are of human Fallopian tube epithelial origin to model tEP: a monolayer of Ishikawa cells which are of human endometrial epithelial origin was used to model normal intrauterine implantation. Epithelial monolayers were treated with TGF-β1 (20ng/ml) before the addition of murine blastocyst stage embryos (10 embryos/well, 2 wells/treatment). Co-cultures were incubated for 48hr, after which the embryo attachment level was determined and compared against embryo attachment on non-TGF-β1 treated monolayers.

Results:
In non-TGF-β1 treated control monolayers, embryo attachment to the endometrial epithelial cells (mean:69.8%) was significantly higher than the Fallopian tube cells (mean:23.0%) ($p<0.05$). TGF-β1 treatment significantly increased embryo attachment to the Fallopian tube cells (non-treated mean:23.0%, TGF-β1 treated mean:61.2%) ($p<0.005$) to similar levels seen with the endometrial epithelial cells (mean:69.8%). TGF-β1 treatment did not significantly change embryo attachment to the endometrial epithelial cells.

Conclusion:
Treatment with EMT-inducer TGF-β1 increases embryo attachment to Fallopian tube epithelial cells, suggesting that EMT in Fallopian tube epithelial cells may increase the risk of tEP.

References:
1. Sivalingam V, Duncan W, Kirk E, Shephard L, Horne A. Diagnosis and management of ectopic pregnancy. Journal of Family Planning and Reproductive Health Care. 2011;37(4):231-240.
2. Bilyk O, Coatham M, Jewer M, Postovit L. Epithelial-to-Mesenchymal Transition in the Female Reproductive Tract: From Normal Functioning to Disease Pathology. Frontiers in Oncology. 2017; 7:145.
3. Flanagan H, Lin C, Campbell L, Horner P, Horne A, Spears N. Ectopic pregnancy and epithelial to mesenchymal transition: is there a link? Reproduction. 2021;161(3):V11-V14.

ECR-3 *Sperm donation: A study on attitudes exploring motivations and barriers*

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Background:
There are currently not enough sperm donors coming forward to donate in the UK, which contributes to a lengthening waiting list of patients to access fertility treatment. To avert this crisis, understanding attitudes and motivations of non-donors as well as sperm donors is crucial in order to implement a targeted advertisement campaign, thereby increasing the availability of donated sperm for infertile patients.

Objective:
To study the attitudes while exploring motivations, barriers and awareness of prospective donors and current sperm donors.

Methodology:
An electronic anonymous survey (non-donors N=92; sperm donors N=3) was used to assess attitudes and motivations towards sperm donation among Scotland-resident men, categorised based on different demographic characteristics such as employment status, parental status, and previous body material donation status.

Results:
From our study population of non-donors, 31.5% would and 30.4% would maybe consider donating sperm and all sperm donors would continue donating sperm. Altruism appeared to be the most noteworthy motivation. About 55% non-donors and all sperm donors would donate sperm regardless of financial compensation, majorly because they empathise with couples suffering with infertility (66.7%). Lack of awareness regarding the donation process and donor recruitment eligibility criteria as well as the laws (ie. anonymity of the sperm donor, especially for men who had already fathered a child) that govern the sperm donation were the main barriers. An additional barrier includes commitment to donate sperm every 2-3 months. About 60.9% non-donors were unaware that they are eligible for implication counselling as part of the sperm donation process.

Conclusion:
The information received through this study will help in the recruitment campaign to understand, identify, and target specific groups that are most likely to donate sperm for altruistic reasons and be less likely to defer through the recruitment process or withdraw consent in the future.

ECR-4 *Correlations between equine breeding parameters and fertility - A retrospective study*

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[1] *Hartpury University, UK*

Rationale:
Although extensive literature investigating the correlation between equine breeding parameters and fertility exists, the methods of deducing this correlation and conclusions have varied. They lack the inclusion of the mares' breeding parameters, thereby introducing bias that may alter the interpretation of the finding (1,2).

Aim:
The primary purpose of this study was to investigate the correlations between stallion breeding parameters and mare breeding parameters to fertility and deduce any significant variables associated with reproductive efficiency through artificial insemination of fresh semen only, avoiding bias occurring from varying chilled and frozen semen processing protocols (3,4).

Methods:
Retrospective data was extracted from two stud farms for the successive breeding seasons in 2019, 2020 and 2021. A total of 218 breeding records of fresh semen collections and inseminations from 38 stallions and 141 mares were extrapolated and collated with pregnancy outcomes for analysis using SPSS (Version 26.0). Individual variables were tested prior with a univariate binary logistic regression test. Models fitted were evaluated using an Omnibus test, Nagelkerke's R2 and Hosmer–Lemeshow goodness of fit tests ($p<0.05$). The predictive abilities of the final models were investigated using ROC curve analysis.

Main Findings:
The administration of Deslorelin pre-insemination (P=0.083), Cervix score (P=0.065), Cycle number (P=0.048), Post insemination lavage (P=0.066) and Progressive Motility (P=0.012) had a significant impact on contributing towards a positive pregnancy outcome. Contrary to the literature, however, mare and stallion age were found to have shown no significance towards pregnancy outcomes.

Conclusion:
Based on the results of this study, evaluating stallion and mare breeding parameters for a certain outcome can only facilitate informed and accurate judgments about future breeding probabilities when both the stallions' and mares' intrinsic and extrinsic breeding parameters have been factored in equally. More research on this topic is warranted to investigate and create a malleable prediction model to assess the reproductive efficiency of an equine population.

References:
1. Amann RP. Weaknesses in reports of "fertility" for horses and other species. Theriogenology [Internet]. 2005 Feb 1 Available from: https://linkinghub.elsevier.com/retrieve/pii/S0093691X04001505
2. Amann RP. The fertility dilemma: perception vs. actuality.
3. Camargo K, Simioni Felicio LC, Chaves Macan R, Ernandes Kozicki L, Eduardo Camargo C, Macan R, et al. Effect of different types of artificial insemination and semen dose on reproductive efficiency in mares Equine Embryo and Fetus View project Efeito da dexametasona na fertilidade do touro. View project Effect of different types of artificial insemination.

Pferdeheilkunde-Equine Med 2018. Available from:
https://www.researchgate.net/publication/322271867
4. Kareskoski M, Venhoranta H, Virtala AM, Katila T. Analysis of factors affecting the pregnancy rate of mares after inseminations with cooled transported stallion semen. Theriogenology. 2019 Mar 15;127:7-14.

ECR-5 *A pilot study to examine ovarian hormones in women with long COVID across the menstrual cycle*

Cara Robb, *Undergraduate Student, The Queen's Medical Research Institute, University of Edinburgh, UK*

Background:
Long COVID occurs when symptoms of COVID-19 are present for >4 weeks. Women with long COVID have reported menstrual disturbance. The SARS-CoV-2 receptor (ACE2) has been detected in the ovary and endometrium(1).

This project examined (i) serum ovarian hormone levels and (ii) endometrial ovarian hormone receptors in women with long COVID compared to women who have never had COVID-19.

Methods:
With ethical approval and consent, women with long COVID provided peripheral blood samples during the proliferative (n=5), secretory (n=4) and menstrual (n=3) phases of the cycle. Three women also provided endometrial biopsies. All were aged 18-55, had regular menstrual cycles (24-38 days), with no exogenous hormone use. Controls were samples collected before December 2020, matched for age and parity.

Serum protein levels of oestradiol (E2), progesterone (P4) and testosterone (T) were measured by ELISA. Endometrial hormone receptor mRNA (PGR, PGR-B, ESR1, AR) was quantified by qRT-PCR. Differences were analysed using unpaired t-tests.

Results:
When comparing serum hormone levels in those with long COVID and controls, we found E2 and P4 protein were consistently lower in those with long COVID across all phases of the cycle, although differences were not statistically significant. T was significantly lower during the menstrual phase in those with long COVID versus controls (P=0.038).
Relative ovarian hormone receptor mRNA (ESR1, PGR, PGR-B and AR) concentrations in proliferative endometrium revealed no significant differences in those with long COVID versus controls.

Conclusions:
We reveal statistically lower menstrual serum T in women with long COVID. In addition, E2 and P4 levels were consistently lower in women with long COVID. Although not statistically significant, these findings may result in clinically significant endometrial effects that could contribute to menstrual disturbance. Future studies should examine a larger cohort and determine if long COVID affects endometrial function to result in abnormal uterine bleeding.

References:
1. Sharp GC, Fraser A, Sawyer G, Kountourides G, Easey KE, Ford G, et al. The COVID-19 pandemic and the menstrual cycle: research gaps and opportunities. Int J Epidemiol. 2022;51(3):691-700.

ECR-6 *The effect of cisplatin on the fetal mouse ovary in vitro*

Scarlett Green, *Undergraduate Student, University of Edinburgh, UK*

Introduction:
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 fetuses of pregnant mice at E13.5, with one ovary from each pair being cultured under control (saline) conditions, and the other with cisplatin (3 ÂµM) added to the media. Ovaries were cultured for either 8 h, 16 h or 24h. 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. Germ cell number and double-strand DNA damage were examined at the 24 h time-point, using immunohistochemistry for germ cell marker ddx4, and double-strand DNA breaks, yH2AX, respectively.

Results/Discussion:
Cisplatin 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 24 h (p<0.001). yH2AX expression was found to be significantly higher (p<0.001). These findings suggest that this loss of germ cells is likely due to increasing apoptic cell death, where cisplatin exposure increases the level of double stranded breaks in fetal ovaries, resulting in increased DNA damage within the germ cells.

ECR-7 *Investigating fibrosis in the ovary: a possible association with FSH/ testosterone in polycystic ovary syndrome (PCOS)*

Alexandra Rutherford, *Masters Student, St George's University London, UK*

Background:
PCOS is the most common endocrine system disorder affecting women of reproductive age. An important aspect of the condition is the altered ovarian morphology of enlarged stromal volume, and increased ovarian stiffness. Fibrosis is a normal process of aging, whereby synthesis and degradation of extracellular matrix, which is vital for normal ovarian functioning becomes dysregulated. However, this process can be pathological and is poorly understood in the context of PCOS. This study aimed to determine whether increased levels of fibrosis could be contributing to the altered structure of the polycystic ovary (PCO), and whether follicle stimulating hormone (FSH) and/or testosterone influenced fibrosis.

Methods:
To determine an association between PCO and an increase in fibrosis, Western blotting was performed using archived human ovarian tissue to evaluate the expression of fibrotic markers α-smooth muscle actin (α-SMA) and transforming growth factor β1 (TGF-β1) in normal and PCO. Additionally, lamb ovarian tissue treated with FSH or testosterone was subject to Masson's trichrome staining or immunohistochemistry to evaluate the expression of α-SMA and collagen. Shapiro-Wilk test was performed on all data and t-test and one-way ANOVA were used for normally distributed data, whereas Brown-Forsythe ANOVA tests were performed on not normally distributed data. Two-way ANOVA was also performed and post-hoc tests were conducted for all ANOVA tests.

Results:
No significant difference in expression of α-SMA ($p=0.65$, $n=8$) or TGF-β1 ($p=0.79$, $n=8$) between tissue compartments of normal and ovarian tissue was observed. However, expression of α-SMA was significantly reduced in FSH-treated tissue ($p=0.005$, $n=4$), and collagen was significantly reduced in FSH ($p=0.039$, $n=5$), and testosterone-treated ($p=0.021$, $n=10$) tissue.

Conclusion:
This study could not determine that increased levels of fibrosis were associated with PCOS, but showed that normal FSH or testosterone significantly reduced levels of fibrosis in the ovary.

ECR-8 *Use of human sperm functional tests to assess compounds with male contraceptive activity*

Rachel Myles, *Masters Student, University of Dundee, UK*

Background and rationale:
There is a crucial need to develop new methods of male contraceptive as there have been no effective forms established since the condom, with the only other method being a vasectomy. Globally, millions of people in developing countries do not have suitable choice of contraception which leads to health risks, unintended pregnancies, and a negative impact on the population's general wellbeing. The drug discovery process has potential to provide a plethora of options, but gaps in knowledge of basic human sperm biology has delayed progress of male contraceptive development. Whilst a phenotypic screening approach to drug discovery is effective, secondary assays are essential to ensure biological relevance.

Experimental approach:
Using sperm functional tests with semen samples from volunteer human donors, compounds which were deemed as hits from primary phenotypic screening were assessed in secondary assays. A modified viscous media penetration assay, using capillary tubes, involved testing the effects of compounds on human sperm penetration into methylcellulose, a known suitable substitute for cervical mucus. In vitro sperm penetration is known to provide valuable information regarding human spermatozoa function, particularly motility.

Key results:
Functionally, one particular compound series of interest significantly decreased sperm cell penetration into viscous medium in a dose-response fashion, compared to control compounds. This effect was observed across several individual donors (n=9 for each experiment). This compound series also significantly decreased overall sperm motility (total and progressive) in donor samples and performed well in cell viability assays; it did not cause cell death at concentrations ranging from 0.01µM - 30µM, indicating that the effects observed on penetration and motility were not a result of cell death.

Implications:
The compounds of interest may possess potential for development as novel male contraceptives, offering millions of people greater choice, aiming to address a crucial reproductive inequality burden that exists globally.

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