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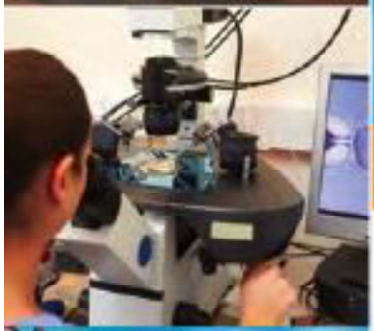

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ABSTRACT BOOK

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characterise human embryo implantation into the Ishikawa endometrial epithelial cell line by combining morphologic and gene expression analyses.

Methods: Live and fixed human embryo-Ishikawa cell co-cultures were examined by high resolution fluorescence microscopy using cell structure-specific dyes and antibody markers. Matched analysis of embryo gene expression was afforded through RT-qPCR.

Results: Hatched day 6 human blastocysts attach rapidly (15/20 attached after 6h), and go on to invade the Ishikawa cell layer (37/46 invading after 48h). Immunofluorescence revealed that all invasive embryos contained multinucleated syncytiotrophoblast (ST), and that most invading blastocysts exhibited a collapsed blastocoel ($p<0.01$). Moreover, we show for the first time that ST initiates the breaching of epithelial endometrial epithelial cells (n=7). Utilising fluorescent dyes for live imaging allowed us to morphologically characterise this process before purifying RNA for gene expression analysis. From a panel of 19 trophoblast-related genes, 8 were found to be specific to day 6-8 human blastocysts and not expressed in Ishikawa cells (n=12). Of these, GCM1, DLX3, HTRA4, GATA3, ERVW1 and PGF were consistently more highly expressed in embryos exhibiting invasive ST. Moreover, members of the highly homologous pregnancy specific β1-glcyoprotein family (PSG1, 2, 3, 5, 6, 7, 8, 11) were expressed in day 8 embryos implanting into Ishikawa cells, but not in control embryos maintained in culture without Ishikawa cells, and were elevated in ST-positive invasive embryos.

Conclusions: Data from our in vitro model therefore implicate ST formation as the key event initiating human embryo implantation, and highlight potential markers of early human embryo invasion which may lead to clinical applications as well as new biology.

Short papers session 3C: Sperm

SP3C.1 Rapid 'switching' of human sperm motility

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Motility of human sperm is typically quantified as a 'snapshot', estimating the proportion of cells showing each motility type. However, observation of cells for several seconds suggests that behaviour of individual sperm can change rapidly (2). Such behavioural switching may be adaptive, for instance during ascent of the female tract by 'hopping'. We captured behaviour of individual sperm over a period of 180 s (9000 frames at 50 Hz), using a motorized stage to centre the cell in the field of view when required.

For analysis 4 behaviours were defined. Types 1-3 resembled activated, transitional and hyperactivated behaviours. Type 3 cells occasionally arrested with the anterior flagellum in a 'J' shape (type 4). Each of 180, 1 s periods were assigned to one of these behaviours. A subset of cells was also analysed using Metamorph software to generate continuous 3 minute tracks. Fractal dimension analysis (1) confirmed that visual analysis reliably identified types and changes of behaviour. % hyperactivation under each incubation condition was separately assessed by CASA.

In control recordings (EBSS pH 7.4) 16/18 cells showed repeated, abrupt transitions in behaviour (mean=6.4±0.8 min-1, n=18). Under conditions that raise [Ca2+]i and stimulate hyperactivated motility (2 mM 4-aminopyridine at pH8.5, hyperactivation increased from 4±2% to 35±4 %), switching between behaviours persisted (9/20 cells switched within 180 s) but the duration of periods of type 3/ 4 (hyperactivated-like) behaviour from 5.9±0.5 seconds (control) to 82.3±11.2 seconds ($P<5*10-8$). Duration of type 1 (activated-like) behaviour was little affected (12.9±1.7 and 8.8±2.7 seconds respectively; $P=0.2$). We conclude that behavioural switching occurs continuously and that stimuli that induce hyperactivation greatly prolong periods of hyperactivated behaviour.

References:
1.Mortimer (1998) Reprod Fertil Dev. 10:465-9. 2.Pacey et al (1997) Hum Reprod Update 3:185--93.

SP3C.2 [Ca2+]i oscillations regulate behaviour of free-swimming human sperm

Elis Nitao [1]; Hector Guidobaldi [2]; Laura Giojalas [3]; Stephen Publicover [1]

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In immobilised human sperm, agonist-induced [Ca2+]i elevation induces the generation of large [Ca2+]i oscillations, which may play a role in regulating motility and AR (1,2). Whether such oscillations occur in free-swimming cells is unknown. We have used time-lapse, fluorescence imaging of free-swimming human sperm to investigate (a) whether [Ca2+]i oscillations occur under these conditions and (b) their effects on motility of the cell.

Time series (3 Hz or 10 Hz; 100-300 s) were collected from 92 progesterone-stimulated, free-swimming, fluo4-labelled human sperm (chamber depth 20 μm; 25 or 31oC). The cell was periodically re-centred in the field of view as required. Cells

were tracked using Metamorph software and analysed for fluorescence intensity ([Ca2+]i). Continuous sperm tracks were generated from X-Y coordinates so that sperm behaviour could be related to changes in [Ca2+]i. Large [Ca2+]i oscillations (≈150% fluorescence increase; ≈60-90 s period) were observed in 25-30% of cells analysed.

Oscillations typically had a symmetrical shape rather than the fast rise-slow decay seen in immobilised cells. The low frame acquisition rate precluded accurate CASA assessment of hyperactivation but average path velocity (VAP), straightness (STR) and fractal dimension (high in hyperactivated cells; 3), were calculated. Most cells clearly accelerated during periods of increased [Ca2+]i and several also showed reduced STR (increased turning). In two cells [Ca2+]i transients were clearly correlated with large increases in fractal dimension.

We conclude that [Ca2+]i elevation, including oscillations, occurs in free-swimming cells, though the kinetics of these oscillation differ from those observed in substrate-attached cells. We propose that this [Ca2+]i signalling activity regulates cell behaviour, potentially enabling escape of cells attached to the oviduct wall and/or generating periodic turning.

References:
1.Harper et al (2004) J Biol Chem 279, 46315-25. 2. Mata-Martinez et al (2018) BBRC 497, 146-52. 3. Mortimer (1998) Reprod Fertil Dev. 10:465-9.

SP3C.3 The intracellular actions of trequinsin improves sperm cell hyperactivation and viscous medium penetration

Rachel C McBrinn [1]; Joanna Fraser [1]; David W Gray [2]; Anthony G Hope [2]; Christopher LR Barratt [2]; Sarah J Martins Da Silva [3]; Sean G Brown [1]

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Background & purpose: Asthenozoospermia is a leading cause of male infertility. ICSI is used as the primary treatment for this due to a lack of knowledge regarding the complex signalling mechanism that regulate motility. However, CatSper is recognised as a key regulator of motility through its control of extracellular Ca2+ influx and represents a plausible target for the development of potential therapeutic compounds due to its confined expression in sperm. Thus, the aim of this study is to identify compounds that increase [Ca2+]i via CatSper in order to improve sperm cell function.

Experimental approach:
Phase 1 - Utilise High Throughput Screening (HTS) to identify compounds that are efficacious inducers of [Ca2+]i.
Phase 2 - Determine the effect on motility, viscous medium penetration and acrosome reaction.
Phase 3 - Determine the mechanism(s) of action through patch-clamp electrophysiology, HPLC and Fluorimetric assays.
Phases 2 & 3 conducted on human donor and patient cell populations.

Key results: HTS identified Trequinsin Hydrochloride, a putative PDE3 inhibitor. Examination of the pharmacological profile showed robust increases in [Ca2+]i via modulation of CatSper ion channel directly ($p = < 0.01$) and partially blocks KSper with no effect on pHi. Trequinsin also significantly increased cyclic- GMP ($p = < 0.05$). Functionally Trequinsin increased cell hyperactivation and penetration into viscous medium in all donors tested and did not induce premature acrosome reaction. Of the 29 patients assessed, 90% responded significantly to trequinsin treatment with boosts in cell hyperactivation.

Conclusion: Extensive examination of trequinsin hydrochloride has shown novel pharmacological actions that stimulate cell hyperactivation and viscous medium penetration. HTS is effective at identifying potential novel therapeutics that act by elevating [Ca2+]i. Utilising novel therapeutics that act on CatSper could provide insight into the intracellular regulation of normal and impaired sperm and aid treatment options for patients.

References:
1.Martins da Silva, S. J. et al. (2017) 'Drug discovery for male subfertility using high-throughput screening: a new approach to an unsolved problem', Human Reproduction, 32(5), pp. 974–984. 2.Tardif, S. et al. (2014) 'Clinically relevant enhancement of human sperm motility using compounds with reported phosphodiesterase inhibitor activity.', Human reproduction (Oxford, England). Oxford University Press, 29(10), pp. 2123–35. 3.Williams, H. L. et al. (2015) 'Specific loss of CatSper function is sufficient to compromise fertilizing capacity of human spermatozoa.', Human reproduction (Oxford, England). Oxford University Press, 30(12), pp. 2737–46.

SP3C.4 The presence of spermatozoa alters the physical characteristics and microRNA content of extracellular vesicles secreted by porcine oviductal epithelial cells in vitro

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It is widely accepted that extracellular vesicles (EVs) are potent vehicles for intercellular communication in the female reproductive tract, potentially via their transport of microRNAs (miRNAs) (Fazeli, 2016). We have demonstrated that EVs secreted by porcine oviductal epithelial cells (POECs) contain miRNAs (Jamaludin, 2016) and more recently, that spermatozoa contribute EVs, which interact with POECs in co-culture (Konstantinidi, 2017). This study investigated how the physical