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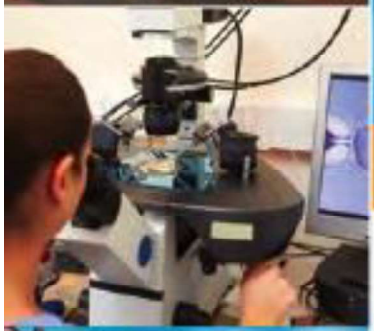

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ABSTRACT BOOK

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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

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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