Utilising 3D Culture Systems to Investigate the Effect of Conceptus-Derived Factors Upon the Bovine Endometrium

Authors: Haidee Tinning[1], Jessica Edge[1], Gianluca Giovanardi[2], Dapeng Wang[3], Elton de Vasconcelos[4], Virginia Pensabene[2], Peter Ruane[5], Niamh Forde[1]
Author affiliations: 1. Discovery and Translational Sciences Department, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK; 2. School of Electronic and Electrical Engineering, University of Leeds, UK; 3. National Heart and Lung Institute, Imperial College London, UK; 4. Leeds Omics, University of Leeds, UK; 5. Division of Developmental Biology & Medicine, University of Manchester, UK.

Conceptus-endometrial bi-lateral communication is essential for successful early pregnancy in mammals including cattle. Interferon Tau (IFNT -the pregnancy recognition signal) is secreted by the bovine conceptus, to block luteolysis- thereby maintaining progesterone secretion from the corpus luteum, to sustain pregnancy. The bovine conceptus also secretes additional proteins, including macrophage capping protein (CAPG) and protein disulphide isomerase (PDI), which we have previously shown using 2D static *in vitro* culture system to alter the endometrial transcriptome. Our aim was to utilise novel 3D *in vitro* models (glandular organoids and a 3D microfluidic endometrium-on-a-chip) to better model the endometrial response to CAPG and PDI.

Using our 3D bovine endometrium-on-a-chip, we cultured culturing primary bovine stromal (bESCs) or epithelial cells (bEECs) on either side of a porous membrane (n=3). Conceptus-derived factors (recombinant bovine CAPG [rbCAPG] or PDI [rbPDI]) were added to the culture medium (RPMI 1640, 10% FBS, 1% antibiotic antimycotic) at 1μg/mL and flowed through the chip over the bEECs at 0.8μL/min flow rate for 24 hours, alongside PBS vehicle control samples (n=3). Unconditioned medium samples were also collected (n=2). Conditioned medium was collected and underwent tandem-mass-tag nano-LC mass spectrophotometry analysis, while recovered cells, were sequenced using Illumina NextSeq 500 with a single end 75bp length read. In tandem, bovine endometrial glandular organoids were produced and exposed to conceptus-derived factors (rbCAPG, rbPDI, and recombinant ovine IFNT [roIFNT]) for 24 hours at 1μg/mL (n=5). Organoids were collected and subjected to sequencing using the Illumina Novaseq 6000 with a paired end 150bp length read. All sequencing data underwent analysis at Leeds 'Omics at the University of Leeds to identify differentially expressed genes (DEGs), and downstream enrichment analysis was performed using Webgestalt. All data were also compared to DEGs previously identified in our 2D static culture systems.

The addition of rbCAPG in the 3D microfluidic endometrium-on-a-chip system flow through medium resulted in 228 DEGs (padj<0.05) in epithelial cells compared to controls. Enriched gene ontologies including signal transduction in the absence of ligand, cell adhesion mediated by integrin, and muscle cell migration, were significantly enriched (FDR<0.05). Ninety-one DEGs were also differentially expressed in response to rbCAPG in static 2D culture. The addition of rbPDI altered 314 DEGs (padj<0.05) in epithelial cells, within which signal transduction in absence of ligand, cell adhesion mediated by integrin, and inflammatory response were enriched gene ontologies (FDR<0.05). One hundred and twenty-five DEGs were also altered in a 2D static culture in response to rbPDI. Exposure to

rbCAPG altered abundance of 24 proteins in conditioned medium, and exposure to rbPDI altered abundance of 17 proteins when compared to vehicle control ($p<0.05$).
In bovine glandular organoids, roIFNT elicited 552 DEGs compared to vehicle control samples after 24 hours ($padj<0.05$) while 0 DEGs and 7 DEGs ($padj<0.05$) were identified in rbCAPG and rbPDI treated organoids respectively.

Exposure to conceptus-derived proteins PDI and CAPG altered pathways in the endometrial epithelium *in vitro* which may promote early pregnancy processes such as attachment during implantation, immune response to conceptus, and altering secretion of proteins into the uterine luminal fluid. In contrast, there was no effect on the deep glandular epithelial organoids, but IFNT elicited a transcriptional response, mostly altering immune-related pathways, in the endometrial glandular organoids. These data demonstrate that the effect of conceptus-derived proteins is cell-type specific.