Deriving Hormonally Responsive Bovine Endometrial Organoids

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In cattle, communication between endometrium and conceptus during the peri-implantation period is crucial for successful pregnancy. Understanding these interactions is vital as most early pregnancy loss occurs during this time. A major challenge in understanding uterine function and early pregnancy is lack of appropriate *in-vitro* models. Two-dimensional models are available, but do not recapitulate the endometrium's complex multicellular structure. Here, we describe a hormonally responsive organoid model of the bovine endometrium, developed as a tool for studying endometrial function and early pregnancy. Bovine glandular epithelial cells were isolated from reproductive tracts and cultured in Cultrex 2 extracellular matrix hydrogel at 37^{o}C, 5% CO_2 (n=3). RNA was extracted and qPCR confirmed the presence of gland markers: *Leukemia inhibitory factor, mucin-1, insulin-like growth factor binding protein-1, kruppel-like factor-5* and *forkhead box protein-A2*. Organoids were imaged at time points to monitor growth and passaged 3 times in 1:2 or 1:3 ratios after growing for a minimum of 10 days per passage. Morphologically, organoids were spherical and fast-growing at passages 0 and 1, but this declines following passage 2. Bovine endometrial organoids (n=3, passage 0) were treated with 10μg/ml progesterone (P4) for 24 hours and analysed by RNASeq to assess hormone responsiveness. Differential expression analysis by DeSeq2 negative binomial distribution model identified 373 transcripts significantly upregulated (padj<0.05 or log_2fold change >0.05) in response to P4 treatment, with downstream analysis showing significant overrepresentation (FDR<0.05) of genes associated with positive regulation of protein localisation to plasma membrane and cell periphery. Of the 240 genes significantly downregulated by P4 these were significantly overrepresented (FDR<0.05) in biological processes of cilium and cytoskeleton organisation. This model provides a tool to investigate bovine endometrial function and peri-implantation communication, subsequently allowing species comparison to understand diversity in reproductive strategies.