Molecular Characterisation of the Marsupial Histotroph: a Dynamic Uterine Fluid Microenvironment

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Australian marsupials are facing a biodiversity crisis, requiring quick and decisive action to prevent further extinction. Assisted Reproduction Technologies are a promising frontier in species conservation: however, while embryo culture methodologies have been developed for eutherian species, marsupial embryo culture has not yet been optimized. Given marsupial reliance on histotrophic nutrition, close examination of uterine fluid contents is necessary for optimizing embryo culture. Using a dasyurid animal model, this research identifies key uterine morphological details, metabolites, and proteins of interest within the uterine fluid relevant to embryo culture medium composition, and has developed endometrial epithelial organoids as an in vitro model of the highly secretory marsupial uterus.

Uteri from fat-tailed dunnarts (*Sminthopsis crassicaudata*) were collected across gestation. Micro-CT defined the internal structure of the uterus, and cellular detail was resolved using routine histological techniques. Uterine fluid was collected and subjected to metabolomic and proteomic analysis. DAVID analysis identified secreted proteins and their relevant gene ontology pathways.

The dunnart uterus is highly glandular with a folded luminal surface, and contains a dynamic fluid microenvironment. Uterine fluid from different developmental stages had unique molecular signatures: >1800 peptides and 180 metabolites were detected. While significant overlap was detected between samples, each developmental stage had a small cohort of unique peptides, likely of significance to the key developmental pathways active in the developing embryo. Fluctuations in metabolic pathways, energy generation, and stress response are identified between developmental stages using gene ontology analysis. The metabolomic profile of the post-implantation uterine fluid was significantly different to pre-implantation uterine fluid, with enrichment of 57 metabolites observed.

The marsupial uterus is a dynamic microenvironment, with modulation of key proteins and metabolic pathways pertinent to the developing embryo. Insights from these molecular characterisations will be key to unlocking marsupial assisted reproductive technologies.