Investigating the Role of Extracellular Vesicles in Paracrine Communication Between Epithelial Cells from Different Regions of the Domestic Cat Epididymis

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Sperm maturation is dependent upon exposure to the proper luminal environment within the epididymis, but the mechanisms that control how this environment is created and maintained are poorly understood. Epididymal extracellular vesicles (EVs) have been hypothesized to play a role in this process by acting as paracrine communicators between epithelial cells from different regions of the epididymis. The objectives of this study were to (1) describe the development and validation of an *in vitro* culture system for domestic cat epididymal tissue and (2) investigate the effect of caput-derived EVs on the gene expression profiles of cultured corpus explants.

Fresh domestic cat epididymal tissue was separated into caput, corpus, and cauda. EVs were isolated from the caput from pools of tissue from 5 individuals (n= 13 pools of 5 individuals each) using previously published methods. Explants were obtained from separate individuals (n=5, 1 individual per experimental replicate) by cutting 1 mm^3 pieces of corpus tissue. Explant culture was performed on agarose inserts for a period of 3 days and was supplemented with fresh culture medium and EVs every 24 hours. Controls were explants from the same individual that were treated identically but without EV supplementation. Validation that culture conditions were not differentially affecting the explants was performed by assessing tissue morphology (H&E staining), apoptosis (*In Situ* Cell Death Detection Kit, Fluorescein, Roche), and transcription (Click-iT RNA AlexaFluor 594 Imaging Kit, Invitrogen) in treatment and control groups. Using optimal culture conditions, RNA sequencing at a depth of 30M stranded reads then was performed to compare transcriptional profiles of explants supplemented with EVs and control explants.

Preliminary culture experiments determined that supplemental fetal bovine serum (FBS) and androgens were necessary to maintain intact tubule epithelium and stereocilia in the cultured explants. Final culture conditions with FBS and androgens also led to levels of apoptosis and transcription that were similar to fresh tissue controls. Analysis of RNA sequencing results using DEseq2 revealed that EV supplementation caused 384 protein coding genes to be differentially expressed with a p-adjusted value <0.05 and a fold change of 1.5 or greater. Of these genes, 260 were upregulated and 124 were down regulated. Functional analysis of the upregulated genes using the STRING network revealed hub genes that encode for cytokines, including IL6, IL1A, IL1B, and CSF3. This indicates that EVs may play a role in maintaining production of cytokines in the epididymis, which are also produced by non-immune cells in the testis and are important for normal testis function. Additionally, we identified upregulation of the genes WNT9B, SUSD2, TMEM100, and JAG1 which are involved in the Gene Ontology biological process of regulation of epithelial cell differentiation. These results suggested that EVs produced by the caput epididymis can control gene expression in downstream epithelial cells from the corpus epididymis. These findings lay a foundation for further studies on the role of EVs in paracrine communication within the epididymis.