68: MOLECULAR PROFILING OF ENDOMETRIAL GLAND FORMATION USING SINGLE-CELL ANALYSIS

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Objective
The endometrium is comprised of luminal and glandular epithelia that develop from a single progenitor layer of fetal uterine epithelium that expresses Wnt7a. Gland morphogenesis occurs postnatally and depends on a number of genes including FoxA2. This study aims to systematically characterize the molecular mechanisms of luminal and glandular epithelial specification by single-cell sequencing.

Design
In vivo, descriptive study using a transgenic mouse model for isolation of primary endometrial cell populations during postnatal development.

Material and Methods
A transgenic mouse line expressing tdTomato fluorescent protein following Wnt7a-Cre-mediated recombination was used to trace the bifurcation of luminal and glandular fates from ancestral fetal epithelium. Uteri were dissected at several postnatal time points and analyzed for Wnt7a-reporter (tdTomato) expression and gland formation by immunofluorescence. E-Cadherin antibody was used to mark luminal/glandular epithelia; FoxA2 anitbody was used to identify glands.

Results
Wnt7a-reporter was undetectable in the P0 uterus. P7 uteri showed strong Wnt7a-reporter expression in the luminal epithelium, overlapping with E-Cadherin. Small epithelial invaginations were observed at this stage, but none were FoxA2 positive. P11 uteri showed considerable gland morphogenesis with Wnt7a-reporter expression in luminal and glandular epithelia. FoxA2+ cells were observed budding off from the luminal epithelium and in fully formed glands within the stroma. Gland formation was completed by P17, with all FoxA2+ cells contained within glands completely surrounded by stroma.

Conclusions
Combining a tissue-specific reporter mouse line with immunofluorescence allowed us to identify and track three distinct cell populations during postnatal endometrial development: luminal epithelia (tdTomato+, E-Cadherin+, FoxA2-), glandular epithelia (tdTomato+, E-Cadherin+, FoxA2+), and stroma (triple negative). We plan to use this as the basis for cell sorting and subsequent single-cell sequencing to establish distinct molecular signatures for luminal and glandular epithelia. These transcriptomic profiles will allow us to explore the independent and synergistic functions of these two cell types during implantation and decidualization.

Support
None