Optimising a Multi-omics Approach for Characterising the Progenitor to Theca Cell Transition in Mice

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The theca cell layer of the ovarian follicle plays a key role during the development and ovulation of a mature oocyte. Until recently, the stromal origins and identity of theca cell progenitors were unknown. In the mammalian ovary, at any given time, each follicle is at a unique stage of development. Thus, theca cells will be present at all stages of development, from progenitors to differentiated theca cells, but will be differentially localised. We exploited this feature of follicular development and a 'multi-omic' approach to identify theca cell progenitors, characterising the transition to differentiated theca cells.

Ovaries were collected from two week-old mice and were either dissociated for single-cell RNAseq (scRNAseq) or frozen in optimal cutting temperature compound for spatial transcriptomics. For scRNAseq, dissociated samples with high cell viability (80 ± 5 %; mean ± SEM) were fixed and prepared as per manufacturer's instructions for the Chromium Fixed RNA Mouse Transcriptome kit (10X Genomics), targetting 10,000 cells per reaction (n=3). Libraries were sequenced at a minimum coverage of ~60,000 reads per cell. For spatial transcriptomics, RNA quality was evaluated by Bioanalyzer and tissue blocks with RIN >7 were systematically sectioned and the ovarian morphology evaluated. Once an appropriate section was identified, the next section was mounted onto the Stereo-seq Chip T Slide (STOmics). Slides (n=2) were imaged by confocal microscopy to generate 20X fluorescent images and permeabilised to prepare libraries for sequencing on a DNBSEQ-G400 sequencer (MGI).

Herein, the optimised experimental approach is described including preliminary data characterising the progenitor to theca cell transition. Given that some infertility associated with maternal aging and obesity is caused by ovulation failure, understanding the development of this cell type may provide novel insights into ovulation failure-related infertility.

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