Gene expression profiling by Next Generation Sequencing of primordial, primary and secondary bovine follicles

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Application: Improve the exploitation of the ovarian reserve with the development of a culture system from primordial to secondary follicles.

Introduction: Folliculogenesis is a highly regulated process and, from the primordial follicle's formation, several pathways intervene to control different fates of the follicle: it can remain dormant, undergo cell death or activation (Dri 2021). Two of the most studied activation pathways are PI3K/AKT/mTOR and Hippo. From the pre-granulosa cells, various stimuli can regulate the PI3K/AKT signaling and interact with the oocyte for the activation and subsequent growth of the follicle (De Felici 2021, Zhang 2014, Zhao 2018). However, most mechanisms guiding the primordial to secondary follicle stage, are still not well-identified and deciphering the intricate networks involved in the earlier stages of folliculogenesis is paramount to orchestrating the follicular growth in a culture system. The present study aims to delineate the mechanisms involved in follicle differentiation from the primordial to the secondary stage through analysis of the transcription profile of isolated primordial, primary, and secondary follicles.

Materials and method: Heifer ovaries were collected at the abattoir, kept on ice and processed to mechanically isolate follicles at different stages of preantral development (Dey 2023). An average of 177 primordial follicles (N=3), 47 primary follicles (N=4), and 53 secondary follicles (N=3) were isolated, RNA extracted, libraries prepared and sequenced on Illumina NextSeq2000 generating 50 bp paired-end reads. TrimGalore was used to trim artificial constructs and low-quality bases. Trimmed data were mapped against Bos taurus ARS-UCD 1.3 transcriptome with Salmon, and differentially expressed genes were obtained with DESeq2.

Results: PCA analysis showed clear clustering of the samples. Sixty-eight genes were differentially expressed on comparing primary versus primordial follicles and 1301 genes between secondary and primary follicles with FDR<0.05.

Conclusions: Our preliminary data report the transcriptome profiles of isolated bovine primordial, primary, and secondary follicles for the first time. Identifying key regulators of follicular differentiation will help ameliorate current *in vitro* culture systems.

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