Follicle-Stroma Interplay Unravel the Mechanisms Involved in Bovine Early Folliculogenesis

Noemi Monferini[1]; Pritha Dey[1]; Federica Franciosi[1]; Filippo Zambelli[2]; Valentina Lodde[1]; Alberto Maria Luciano[1]

[1]Reproductive and Developmental Biology Laboratory (ReDBioLab), Department of Veterinary Medicine and Animal Sciences, University of Milan, Milan, Italy

[2]Eugin Group, Barcelona, Spain

Exploiting primordial follicle reserve to improve assisted reproductive technologies is still ineffective. So far, no defined culture system has been able to replicate the folliculogenesis in vitro due to the lack of knowledge of the mechanisms involved. During folliculogenesis, diverse and unique pathways control the fate of primordial follicles, from remaining dormant to undergoing cell death or activation. Morphological and functional changes occur within the follicles and interact with the ovarian stromal environment, particularly the ovarian cortex. The ovarian cortex surrounds the preantral follicles and mediates dialogue through paracrine factors. It is reported that the ovarian cortical matrix can influence the action of local paracrine biochemical signaling pathways to sustain the development of primordial, primary, and secondary follicles.
The present study aims to delineate the signaling networks involved in preantral follicle differentiation and the role of the ovarian cortex in guiding early folliculogenesis from the primordial to the secondary follicle stage. A bovine follicle dataset was generated in-house by mechanically isolating pools of primordial (n=3), primary (n=3), and secondary (n=3) follicles and subjecting them to 50bp paired-end bulk RNA sequencing on Illumina NextSeq2000. Sequenced data were trimmed with TrimGalore, mapped to the bovine transcriptome assembly ARS UCD 1.3, and quantified with Salmon. Differential gene expression analyses were performed with DESeq2 on R. Functional enrichment analyses were then conducted to identify overrepresented pathways. The transcriptome analysis of primordial, primary, and secondary follicles reveals an evident clustering of the samples via principal component analysis. Pairwise comparisons of the subsequent follicle stages, i.e., primordial-primary and primary-secondary, identified 1083 and 4596 significant differentially expressed genes (padj<0.1), respectively. Collectively, overrepresentation analyses of the differentially expressed genes revealed signaling pathways, such as PI3K-Akt, Wnt, mTOR, and cAMP, guiding the transitional phases. To explore preantral follicle gene expression dynamics, we simultaneously compared all three follicle categories using the Likelihood Ratio Test of R(DESeq2). Significant genes (padj<0.1) were subjected to hierarchical clustering. Four clusters were generated that contained 2030, 1527, 536, and 147 genes in clusters 1-4, respectively, showing distinct expression trends.
To better understand the interplay between preantral follicles and the ovarian cortex, a meta-analysis was then performed using the publicly available data (GSE147176), downloaded from GEO, processed and analyzed with the same pipeline described above. The metanalysis between the follicles and the ovarian cortex transcriptomic profiles shortlisted 2479 differentially expressed genes (padj<0.01) with substantial differences in expression levels (log2FoldChange <-4 and >4). Our preliminary data report key overrepresented pathways between follicles and cortex, such as focal adhesion. Further studies are needed to narrow down key regulators involved in follicle-stroma communication. However, the definition of pathways involved in follicle-cortex crosstalk may translate into effective systems for supporting early folliculogenesis in vitro. These analyses uncover the networks governing the development of follicles, from the primordial to the secondary stage, also by including the signal transduction from the ovarian cortical matrix. Indeed, understanding the support provided by the ovarian cortex during early folliculogenesis is essential

for reconstructing 3D culture systems to sustain the growth of follicles in vitro. The in vitro development of preantral follicles can represent a significant achievement in mammalian fertility preservation plans to maximize the exploitation of the ovarian reserve, adding a step to assisted reproductive technologies.
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