A transcriptomic approach towards the improvement of physiological systems for the *in vitro* culture of isolated bovine primordial follicles

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Application: Advancement of culture strategies targeting pre-antral follicle development to pursue female fertility preservation.

Introduction: The ability to grow undifferentiated oocytes in vitro from primordial follicles would increase the supply of fully grown oocytes destined for downstream applications in the livestock industry and fertility preservation programs. To date, the production of living offspring using in vitro follicle growth from the primordial follicle reserve has only been achieved in mice (Eppig 1996), proving the principle of the potential value of follicle culture as a source of fully grown oocytes. While in large mammals, in vitro follicle growth systems to produce mature oocytes from primordial follicles are still experimental (Araújo 2014) due to high follicle mortality following isolation from the surrounding tissue. Herein we study the transcriptome profiles of isolated bovine primordial follicles to identify programmed cell death mechanisms triggered after a short period in culture.

Methods: Bovine ovaries were collected from the abattoir and transported on ice to the laboratory in saline. Primordial follicles were mechanically isolated and cultured in a defined system (Dey 2023, Manuscript submitted). Follicle viability was assessed at the collection and after 16 and 24 hours of culture. Freshly isolated and 16-hour cultured primordial follicles were pooled for RNA extraction and library preparation. RNA sequencing was then performed on Illumina NextSeq2000, generating 50bp paired-end reads. Raw data were trimmed with TrimGalore to remove artificial constructs and low-quality bases. Trimmed data were mapped to the Bos taurus ARS-UCD1.3 transcriptome, and reads were quantified with Salmon. Differentially expressed genes were then obtained with DESeq2.

Results: After 16 hours of culture, a significant reduction in follicle viability was observed, while no significant differences were observed between 16 and 24 hours ($p<0.0001$ and $p=0.9753$, respectively, two-way ANOVA followed by Tukey's test).

PCA plot of global transcriptomic results showed evident clustering of the samples. A total of 1430 genes were differentially expressed with FDR<0.05.

Conclusion: Here, we report for the first time the transcriptome profiling of isolated bovine primordial follicles at the time of collection and after a short period of culture following the triggering of cell death. We hypothesize that by contrasting the transcriptome profiles of cultured primordial follicles against freshly isolated ones, activated cell death signaling networks could be delineated and subsequently inhibited to improve current culture systems.

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