22 Transcriptomic analysis of granulosa cells in growing, dominant, and preovulatory follicles in the southern white rhinoceros (*Ceratotherium simum simum*)

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Granulosa cells (GC) provide key information regarding folliculogenesis and the oocyte's competence via bidirectional communication during follicle and oocyte maturation. The GC gene expression differences across follicle stages may elucidate the pathways necessary for the oocyte to reach developmental competence and ultimately help improve assisted reproductive technologies in the southern white rhinoceros (SWR). Understanding *in vivo* gene expression patterns can also support the replication of physiological conditions *in vitro*. The aim of this study was to evaluate global gene expression from GC obtained from growing, dominant, and preovulatory follicles, and identify transcripts present exclusively in each follicle stage. For this study, ovum pickup was performed on three female SWR. Cumulus–oocyte complexes and granulosa cells were collected from growing (GF), dominant (DF), and preovulatory (POF) follicles separately (n = 2 per follicle type). Mural granulosa cells were stored at −80°C until analysed. Total RNA from GC was isolated, cDNA libraries were prepared with the TruSeq Stranded Total RNA Library preparation kit, and then sequenced on the NovaSEqn 6000. After sequencing, all bioinformatics were performed utilising the web platform Galaxy. FastQC and Trimmomatic were performed to ensure all low-quality reads were removed from analysis. Reads were aligned to CerSimCot1.0 using HISAT2. Stringtie was used for transcript assembly and counts for both annotated and unannotated transcripts. Additional sequence alignment was done with EquCab3.0 to increase annotation counts. Transcripts present exclusively in one follicular stage were used for this analysis and protein analysis through evolutionary relationships (PANTHER) classification system was used for biological pathway identification, focusing on reproductive processes. Overall, 39 455 transcripts were identified in the GC across all follicle stages. Further analysis identified 427 transcripts (385 genes; 35 (8%) transcripts unannotated) present only in GF, 812 transcripts (677 genes; 104 (13%) transcripts unannotated) present only in DF, and 945 transcripts (433 genes; 484 (51%) transcripts unannotated) present only in POF. In GF, the mostly highly expressed gene was *AFF1*. *EIF4G1* was the most highly expressed gene in DF, and an unannotated gene was the most highly expressed gene in POF. Although each follicle group had its own unique set of genes, the top two reproductive processes for each were meiotic cell cycle and multicellular organismal. This is the first report of transcriptomic differences in granulosa cells across follicle stages in the SWR. Identification of the functional role of these transcripts will inform the optimization of culture conditions and enhance *in vitro* oocyte maturation in the SWR.

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