24 Cross-species transcriptomic analysis of mural granulosa cells between the southern white rhinoceros, human, and cattle

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Granulosa cells are vital to various fundamental processes in follicle development. Modulating granulosa cell gene expression is necessary for extracellular signalling and ovulation, linked with follicle growth, oocyte development, and steroidogenesis. Assisted reproductive techniques have been developed for the southern white rhinoceros (SWR) to help the survival of the functionally extinct northern white rhinoceros (NWR). Ovum pickup (OPU) has been performed in the SWR, providing oocytes to test *in vitro* culture protocols and granulosa cells (GC) to study. To help understand follicle development, oocyte and follicle metabolism, differentiation, and cellular signalling and help develop *in vitro* culture systems in these species, we performed transcriptomic analysis of mural GC collected from follicles after OPU in the SWR. This study aimed to: (1) evaluate the transcriptome of SWR mural granulosa cells, and (2) compare the SWR transcripts to other species. GC were collected from two SWR (one stimulated, one during natural cycle) following OPU. For each animal, mural GC were pooled from all follicles and stored in multiple aliquots. Total RNA was isolated from different aliquots of GC, and libraries were prepared using the NEBNext® Ultra™ II RNA Library Prep Kit (New England Biolabs Inc.). Sequencing was performed on an Illumina NextSEq 500 (Illumina Inc.). Bioinformatic analyses were performed with the Galaxy web platform (galaxyproject.org). Reads were aligned to CerSimCot1.0, an unpublished genome assembly for the NWR, using HISAT2. This assembly was used as the analysis reference because it has a more complete annotation than CerSimSim1.0 for the SWR. The Stringtie algorithm was used for the assembly and quantification of transcripts. Manual curation of the CerSimCot1.0 gene annotation improved transcript identification by more than 50%. Our results are the first report on transcriptomic data of GC in the SWR. We identified 37,407 transcripts present in mural granulosa cells of SWR. The PANTHER gene ontology database (pantherdb.org) was used to perform biological pathways analysis. The top biological pathways associated with the identified transcripts were the centrosome cycle, mitochondrial translation, DNA geometric

change, and nuclear-transcribed mRNA catabolic processes. Human and cattle expression data in mural GC were the most available and obtained from the European Bioinformatics Institute Expression Atlas (ebi.ac.uk/gxa) and other published sources for comparison with the SWR results. On average, 45% of the transcripts identified in the SWR data showed similarity with transcripts from human (48%) or cattle (43%). Interestingly, there were 6,935 transcripts identified in common between all three species. The top biological processes associated with these transcripts were Golgi to plasma membrane transport, nuclear-transcribed mRNA catabolic processes, and protein localisation to the endoplasmic reticulum. This study was the first to evaluate the transcriptome of granulosa cells from the southern white rhinoceros and represents a crucial step in developing *in vitro* systems for embryo development.