25 *In vivo* gene expression analysis of southern white rhinoceros (*Ceratotherium simum simum*) granulosa cells collected from growing, dominant, and preovulatory follicles after ovum pickup

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Analysis of granulosa cells (GC) can be a useful noninvasive tool to clarify aspects of ovarian physiology in the southern white rhinoceros (SWR). Gene expression profiles from different phases of follicle development may help improve ovum pickup (OPU) outcome and advance assisted reproductive technologies in rhinoceros species. The aim of this study was to evaluate the transcriptional effects of follicle growth synchronization and superovulation treatment before OPU on granulosa cells collected from growing (11–17 mm), dominant (25–29 mm), and preovulatory (30–34 mm) follicles. Three female SWR received synthetic chlormadinone acetate (CMA) at 3 mg/day for 33 days and a fourth female received no CMA. After CMA withdrawal, all animals received 1.8 mg of deslorelin every two days before OPU (3.6 mg total). On the day of OPU, cumulus–oocyte complexes and granulosa cells were collected separately from growing, dominant, and preovulatory follicles. Mural granulosa cells were stored at −80°C until analysed. Total RNA from GC was isolated and evaluated by quantitative polymerase chain reaction using primers designed to detect 20 SWR-specific transcripts involved in follicle development, oxidative stress, methylation, and acetylation. Three aliquots of GC were used for each follicle type and treatment group, and all samples were run in duplicate. Statistical analyses were performed with GraphPad Prism using an unpaired, two-tailed Student's *t*-test with the Benjamini-Hochberg method. For growing follicles between treatment groups, only one gene (*GDF9*) was differentially expressed and was more highly expressed in the GC from animals that received CMA. High *GDF9* is associated with nuclear maturation and embryo quality. More differentially expressed genes were identified from the dominant follicles. Five genes (follicle development: *LHR*, *PGR*, *TNF*, *TP53*; acetylation: *KAT8*) were differentially expressed and only *LHR* was more highly expressed in the no CMA group. *LHR* is associated with ovulation and high levels indicate follicular maturation. The absence of preovulatory follicles in the CMA treated animals prevented comparison with the no CMA animal. We compared gene expression across follicle types and found multiple differentially expressed genes (follicle development: *GDF9*, *LHR*, *PGR*, *PLA2G4A*, *TNF*, *TP53*; methylation: *CEBPB*; acetylation: *HDAC1*) and patterns inconsistent with gene expression patterns published in multiple domestic species for these genes, indicating a possible effect of CMA and deslorelin treatment. Overall, genes involved in follicle development showed the most significant differences if the rhino had been treated to synchronize and stimulate follicle growth. Interestingly, methylation and acetylation genes were differentially expressed independent of pre-OPU hormone treatment, indicating that other factors affect gene expression in these females. Although the study was limited by the sample number, this is the first preliminary study evaluating gene expression changes across follicular phases including epigenetic modifications, such as methylation and acetylation in this species.

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