19 Granulosa cell gene expression and glucose consumption of *in vitro*-matured oocytes of the southern white rhino (*Ceratotherium simum simum*)

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With just two northern white rhinos left on the planet, it is imperative to study oocyte and embryo development of its subspecies, the southern white rhino (SWR). There are no published data about the metabolic requirements and competence acquisition of rhino cumulus-oocyte complexes (COC). This fundamental information is critical for the optimisation of *in vitro* oocyte maturation and the generation of viable embryos. The aims of this study were to (1) characterise gene expression in granulosa cells (GC) from SWR COCs before (Pre-IVM) and after IVM(Post-IVM), comparing two culture media and oocytes from donors with and without gonadotrophin treatment before ovum recovery; and (2) evaluate COC glucose uptake in spent medium Post-IVM. Twenty-two COCs were retrieved by transrectal ovum pick-up on four SWR. Non-atretic mural GC were selected Pre-IVM and cumulus cells were collected from COCs after ~36 h of IVM culture. COCs were matured in either M199 with 10% fetal bovine serum and 10% equine follicular fluid (SDZ) or Dulbecco's modified Eagle medium-F12 with 10% rhino oestrus serum (IZW). Post-IVM GC were stored at −80°C until analysed. Total RNA from GC was isolated and evaluated by quantitative PCR using primers designed to detect 19 SWR-specific transcripts involved in follicle development, oxidative stress, and metabolism. Statistical analysis was performed with GraphPad Prism (GraphPad Inc.) using an unpaired two-tailed Student's *t*-test with the Benjamini-Hochberg method. Spent medium was collected Post-IVM and D-glucose uptake was quantified using a colourimetric assay in which concentration was determined based on standard curve. Statistical analysis was performed with GraphPad Prism using an unpaired two-tailed Student's *t*-test for unequal variances. For all analyses, significance was determined at $P \leq 0.05$. Oocyte maturation for COCs cultured in SDZ medium was higher (45%) than that in IZW medium (9%). A comparison of gene expression of GC matured *in vitro* in each medium revealed only one gene differentially expressed (*TNFAIP6*). The expression of six genes associated with follicle development (*COX1*, *FLT1*, *FSHR*, *KCNJ8*, *LHR*, *SPP1*) was significantly increased in Pre-IVM GC from the gonadotrophin-stimulated donor compared with those from the unstimulated donor. Post-IVM GC from stimulated animals revealed 5 differentially expressed genes (*FSHR*, *KCNJ8*, *LDH1*, *PFKP*, *VNN1*) involved in follicle development, oxidative stress, and metabolism compared with unstimulated animals. COCs from stimulated animals consumed more glucose than those from unstimulated animals, regardless of culture medium.

Interestingly, 57% of the oocytes matured in SDZ medium consumed all available glucose, whereas no oocytes depleted the glucose in the IZW medium. This is the first study to characterise gene expression in GC matured in different media and to investigate the role of follicle stimulation on the metabolic requirements of rhinoceros oocytes. These preliminary studies are critical for optimizing IVM of rhinoceros oocytes, yielding valuable information for customising *in vitro* systems for assisted reproduction in this species.