23 An improved understanding of ovulation timing in two rhinoceros species (*Rhinoceros unicornis*, *Ceratotherium simum simum*): ultrasound examination of preovulatory follicles before and after ovulation induction

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Artificial insemination has proven to be a useful tool in the reproductive management of *ex situ* rhinoceros populations, the success of which is reliant on precise timing of ovulation for insemination. When conducted in the presence of a preovulatory follicle (POF) of the appropriate size and maturity, ovulation induction protocols paired with frequent ultrasound examinations allow visualisation of changes in the POF including follicular collapse, and in turn refine the window of timing for insemination and conception. In this study, serial ultrasound examinations were conducted on a greater one-horned (GOHR, *Rhinoceros unicornis*) and a white (WR, *Ceratotherium simum simum*) rhino to (1) monitor and characterise morphological changes of the POF through to ovulation, and (2) identify time of ovulation following the ovulation induction. Rhinos that were trained for transrectal ultrasound were examined 1–3 times per week before emergence of a dominant follicle, and then 3 times per week until a preovulatory size was reached. The POF (n = 2) from two consecutive oestrous cycles in a single GOHR, and a POF (n = 1) from a single WR were monitored. When the dominant follicle reached a preovulatory size (GOHR, 11.69 cm and 13.86 cm diameter; WR and 3.3 cm diameter) ovulation was induced using a sustained-release, synthetic gonadotropin-releasing hormone analogue suspension (GOHR, 3.6 mg deslorelin acetate, Sucromate® IM; WR 50 mg oestradiol cypionate IM, then 4.5 mg Sucromate IM 8 h later). In both species, females were examined 34 h after the Sucromate® injection, and then every 3 h until ovulation occurred. In a GOHR POF, an echogenic nodule, presumed to represent the cumulus/oocyte complex, was visualised. In both species, ~6 h before ovulation, the POF became pear-shaped with a decline in turgidity upon application of slight pressure from the transducer and a thinning of the wall at the follicle's apex. In both species, ovulation was characterised by visualisation of follicular fluid evacuation and initial collapse of the follicle, with ovulation confirmed 24–36 h later by visualisation of a corpus luteum or hemorrhagicum. Ovulation occurred 39 and 44 h after the ovulation induction in the GOHR first and second cycle respectively, and 48 h after the ovulation induction in the WR. This is the first study to document presence of the cumulus/oocyte complex in a GOHR, and follicular fluid evacuation and ovulation in both species. This study demonstrates homology of morphological changes of a developing and ovulating follicle between two rhinoceros species and indicates conserved mechanisms of ovulation in relation to another Perissodactyla species, the domestic horse (*Equus caballus*). These results on the temporal relationships between ovulation induction, POF morphology, and ovulation will be combined with data from additional females of each species to provide scientists and veterinarians with a tool to effectively time AI in rhinoceros species.

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