97 Ovulation induction in anovulatory southern white rhinoceros (*Ceratotherium simum simum*)

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Abstract

The extant *Rhinocerotidae* family is experiencing threats in the wild, making captive populations important genetic reservoirs for species survival. Because each species faces distinct challenges in captivity, populations are not self-sustaining. Therefore, assisted reproductive technologies (ART) such as AI will be necessary to maintain or increase captive genetic variation. Captive-born female white rhinoceros (*Ceratotherium simum simum*) have low reproductive rates and apparent acyclicity is a common issue. Although females fail to ovulate and progesterone remains at baseline levels, follicle growth may occur and ovulation can be induced with exogenous hormones. Female southern white rhinos ($n = 6$) housed as a bachelorette group were determined to be ovulatory ($n = 1$) or anovulatory ($n = 5$) by serial ultrasound and fecal progesterone (P4; ng g^{-1}) analysis. However, all anovulatory females grew follicles beyond preovulatory size, which then regressed. At the time of study, when follicles were preovulatory size (35.4 ± 1.2 mm; mean ± SD), anovulatory females ($n = 4$) were induced to ovulate ($n = 11$) with a gonadotropin releasing hormone (GnRH) agonist (4.5 mg, SucroMate™; Bioniche Animal Health, Bogart, GA, USA) in a single intramuscular injection. Nine treatments resulted in ovulation (81.8%), all between 36 and 48 h post-treatment, while 2 hemorrhagic anovulatory follicles (18.2%; HAF) formed, both in the same female. Ovulations were confirmed by disappearance of the follicle by 48 h and P4 elevation above baseline was coincident with corpus luteum (CL) and HAF formation. All resulting luteal structures were included in analysis. Follicle growth was permitted to proceed without GnRH treatment between inductions ($n = 6$); dominant follicles grew beyond preovulatory size (43.8 ± 6.1 mm) followed by regression and growth of another preovulatory follicle that was subjected to GnRH treatment. Data were analyzed by R studio (ver. 1.1.383). Luteal phases were characterized as “short” (<50 days) or “long” (>50 days). Only P4 above baseline (days) was significantly different ($P < 0.05$) between long and short cycles. Other cycle parameters, such as CL visibility (days), time from ovulation to P4 above baseline (days), maximum P4 (ng g^{-1}), and maximum luteal size (mm), were not significantly different between cycle types. Both cycle types were observed following both spontaneous (short, $n = 5$; long, $n = 3$) and induced ovulations (short, $n = 8$; long, $n = 3$). These data provide additional insight into the differences between long and short cycles and that GnRH is a reliable and effective method to overcome anovulation in SWR. We also prove that long cycle lengths are not necessarily indicative of early pregnancy loss, as has been previously suggested. Taken together, this information can enhance captive breeding efforts and the genetic diversity of the ex situ, SWR insurance population.