

# ESTROUS CYCLE INDUCTION IN A WHITE RHINOCEROS (*Ceratotherium simum simum*) AND CONCOMITANT EIA FECAL PROGESTAGEN ANALYSIS

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## Introduction

Captive breeding of the African rhinoceroses, the black (*Diceros bicomis*) and the white (*Ceratotherium simum simum* and *C.s.cottoni*) could become essential for their long-term survival. At present the reproductive rate in captivity is very poor. Sixty seven percent of captive rhino are essentially in a non-breeding situation.<sup>4</sup> Only 8.10 animals in 7 European facilities are breeding regularly.<sup>5</sup> Aggravating this situation is the fact that 66 percent of these European animals are wild-caught and over 19 years of age.<sup>4,5</sup> If these potentially valuable animals are to make a contribution to the captive rhinoceros population, intensive management procedures must be initiated immediately. The reasons for the poor breeding performance are unclear. Management factors such as the size of the enclosure, the group size and composition, and the position of a given animal in the social hierarchy are important influences<sup>6,7,8</sup>.

Literature concerning the estrous cycle in white rhinoceroses is controversial. Behavioral observations of captive white rhinoceroses have indicated various estrous cycle lengths, ranging from 30 to 90 days.<sup>6</sup> Using rectal palpation, vaginal cytology and urinary steroid analysis a cycle length of 42 days has been suggested.<sup>15</sup> Hindle et al. using urinary steroid analysis have suggested an estrous cycle length of 25 and 32 days for the northern and southern subspecies.<sup>3</sup> While investigating fecal progestagens in 5 white rhinoceroses over a period of 14-24 months Schwarzenberger et al. described missing or erratic cyclicity as a considerable problem, and regular estrous cycles (9) of approximately 10 weeks duration in only 1 of these 5 animals. The cycle duration was  $68,4 \pm 3,4$  days, follicular and luteal phases (LP) were  $12,2 \pm 0,8$  and  $56,5 \pm 3,3$  days, respectively.<sup>12</sup> Progestagen levels in 2 of the 5 white rhinoceroses also indicated luteal activity, but the intervals between LPs and duration of LPs were irregular.<sup>12</sup>

Salzburg Zoo Hellbrunn started holding white rhinoceroses (2.2) in 1991. The animals are held in a very large, natural outdoor facility (15,000 m<sup>2</sup>) together with oryx (*Oryx gazella gazella*). Since fecal analysis in one of the two female rhinoceroses indicated missing luteal activity over a period of >2 years, it was decided to attempt cyclic activity induction in this animal. A synthetic progestagen (Chlor Madinon Acetate, CMA) was applied for 1.5 months, and subsequently hCG was injected. The dose and treatment interval were calculated allometrically<sup>13</sup> using the horse mare as a model.

## Materials and Methods

Fecal samples were collected 2-3 times/week for 28 months from the female white rhinoceros "Baby". The animal was wild-caught in 1971 and has been kept with a second white rhinoceros "Kathy" and two alternating males at the Salzburg Zoo since 1991. Feces (0.5 g) were extracted with methanol as described by Schwarzenberger et al.<sup>9</sup>, an additional 1.0 g of powdered aluminum oxide was added prior to extraction. Methanol aliquots were analyzed with an enzyme-immunoassay (EIA), using an antibody against 5 alpha-

pregnane-3B- $\alpha$ -20-one 3HS:BSA. The assay is considered as group-specific and quantifies total immunoreactive progestagens containing a 20-oxo group. Preliminary data using this assay were described.<sup>10,11,12</sup>

Since the animal "Baby" showed no behavioral estrous and fecal progestagen analysis indicated missing luteal activity for >2 years, induction of cyclic activity was attempted. Long-term fecal progestagen analysis of the second female from the Salzburg Zoo, "Kathy", indicated a regular estrous cycle length of ~ 10 weeks (follicular phase  $12.2 \pm 0.8$  and luteal phase  $56.5 \pm 3.3$  days). Due to this long-term data we considered the normal estrous cycle length in the white rhinoceros to be 10 weeks.

The treatment protocol was based on the horse mare as a model animal. The CMA (Synchrosin\*, Fa. Werfft-Syntex, Vienna) dose for the mare is 0.02 mg/kg body weight, and the application interval is 24 hours. The hCG (Chorulon®, Intervet, Boxmeer) dose for a mare is 2500 IU (5 IU/kg body weight). The dose was scaled allometrically as described by Sedgwick.<sup>13</sup> The body weight was estimated to be 2500 kg and the constant (K) for the calculation of the Minimum Energy Cost was 70. Using these factors, a CMA dose of 35 mg (0.014 mg/kg) and a dose interval of 35 hours was computed. The hCG dose for the white rhinoceros was calculated as 8400 IU (3.36 IU/kg). CMA was applied perorally in 32 doses in 35 hour intervals (a period of 45 days); hCG was given as a single intramuscular injection at the base of the ear by means of a Jab Stick (Dan-Inject Denmark) 5 days after the last CMA application.

## Results and Discussion

Fecal progestagens in the white rhinoceros "Baby" were analyzed for >2 years; the results of the progestagen evaluations during 1994 are shown in Fig. 1. Progestagens were low before and during the application of CMA. CMA metabolites did not affect the fecal progestagen measurements. Ten days after the hCG application the fecal progestagen concentrations increased and thus indicated luteal activity. The length of this induced luteal phase was about 20 days. Estrous behavior lasting 48 hours was observed 70 days after the hCG application, and thus for the first time since the animal was at the Salzburg Zoo (from 1974-1991 the animal was kept at the Munich Zoo where estrous behavior was also not observed<sup>2</sup>). Mating was not observed, which may be due to the inexperience of the male placed with the female at the time.

The present study indicates that induction of luteal activity in white rhinoceroses is possible. Although the described treatment protocol failed to induce continuous cyclicity, results are encouraging. Luteal activity was seen 10 days after the hCG application and thus within range of the follicular phase ( $12.2 \pm 0.8$ ) described in a previous study.<sup>12</sup> These results differ from those obtained by Godfrey et al.,<sup>1</sup> who attempted to superovulate a white rhinoceros prior to euthanasia. Their study failed to induce ovulation and subsequent luteal activity. Estrous behavior 70 days after hCG injection is within the range we suggest to be the "normal" duration of the estrous cycle in the southern white rhinoceros.<sup>12</sup> Induction of behavioral estrous was also achieved at the Dvur Kralove Zoo using altrenogest (Regumate\*) and hCG.<sup>14</sup> Similar to our study none of the treatments at the Dvur Kralove Zoo resulted in a subsequent pregnancy.

In conclusion our results demonstrate that luteal activity can be induced using a synthetic progesterone derivative followed by an injection of hCG. Treatment efficiency and results can be monitored using EIA fecal steroid analysis. Since one of the reasons for poor breeding results in captive white rhinoceroses is missing or erratic luteal activity, it is suggested to continue attempts to induce cyclicity. Further treatment protocols could also include GnRH, FSH or PMSG. In order to find optimal treatment protocols, fecal monitoring will be necessary. It should, however, not be overlooked that the various breeding technologies can only be considered as an adjunct to the implementation of adequate management and holding structures.<sup>5,6</sup>

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