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OESTROUS CYCLE INDUCTION IN A WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*) MONITORED BY FECAL PROGESTAGEN ANALYSIS

By F. Schwarzenberger and C. Walzer

Introduction

Captive breeding of the two species of the African rhinoceroses, the black (*Diceros bicornis*) and the white rhinoceros (*Ceratotherium simum simum* and *C. s. cottoni*), is becoming essential for their long term survival. Reproductive rate of white rhinoceroses in captivity is very poor. Most of the captive female white rhinoceroses were imported into zoos during the 1970s, but only about 20% of these animals had one or more offspring (KLÖS and FRESE, 1993). The reasons for this poor breeding performance in captivity are unclear. Management factors like the size of the enclosure, the group size, as well as the position of a given animal in its social hierarchy (MICKULICKA, 1993; MEISTER, 1994) are important influences.

Literature about the oestrous cycle of white rhinoceroses is controversial. Behavioural observations of captive white rhinoceroses indicated oestrous cycle lengths ranging from 30 to 90 days (LINDEMANN, 1982). Transrectal ultrasonography of the ovaries of a white rhinoceros detected only static and small follicles, but it failed to detect ovulation or a corpus luteum (ADAMS et al, 1991). WAGNER (1986) used rectal palpation, vaginal cytology and urinary steroid analysis and suggested an oestrous cycle length of 42 days. Urinary steroid analysis indicated oestrous cycle lengths of 25 and 32 days for the northern and southern subspecies, respectively (HINDLE et al., 1992). SCHWARZENBERGER et al. (1994a) investigated fecal progestagens in five white rhinoceroses for 14 - 24 months. They found (a) missing or erratic cyclicity as a considerable problem and (b) regular oestrous cycles ($n = 9$) of ≈ 10 weeks in only one of these five animals; the duration was 68.4 ± 3.4 days and follicular and luteal phases (LP) were 12.2 ± 0.8 and 56.5 ± 3.3 days, respectively. Progestagen levels in two other white rhinoceroses also indicated luteal activity, but intervals between LPs, and duration of LPs (9, 15, 19, 20 and 28 days, respectively) were irregular. Luteal activity was not detectable in the other two rhinoceroses studied by SCHWARZENBERGER et al. (1994a).

Since one of the two white rhinoceroses kept at Salzburg zoo had no luteal activity for > 2 years, we decided to try to induce cyclic activity in this animal. We applied a synthetic progestagen (CMA, Chlormadinone Acetate) for 1.5 months, and subsequently injected hCG. The dose and the treatment interval were calculated allometrically (SEDGWICK, 1993) using the horse mare as a model.

Materials and Methods

Faecal samples were collected 2 - 3 times / week for 28 months from the female white rhinoceros 'Baby'. The animal was born in 1971 and is kept together with a second white rhinoceros ('Kathy') at Salzburg zoo since 1991. Faeces (0.5 g) was extracted with methanol as described by SCHWARZENBERGER et al. (1993), except that 1.0 g powdered aluminium oxide was added prior to extraction. Methanol aliquots were analyzed with an enzyme-immunoassay (EIA), using an antibody against 5α -pregnane- 3β -ol-20-one 3HS:BSA. The assay is considered as group-specific and it quantifies total immunoreactive progestagens containing a 20-oxo group. Preliminary data using this assay were described by SCHWARZENBERGER et al. (1994a; 1994b; 1994c).

Since the animal 'Baby' showed no behavioral oestrous and since faecal progestagen analysis indicated missing luteal activity for > 2 years, we attempted to induce cyclic activity. Faecal progestagen analysis of the other animal from Salzburg zoo, 'Kathy', indicated an oestrous cycle of ≈ 10 weeks (follicular phase 12.2 ± 0.8 and luteal phase 56.5 ± 3.3 days). Therefore we supposed the normal oestrous cycle length of the white rhinoceros as 10 weeks. For the treatment protocol we used the horse mare as a model animal. The CMA

(Synchrosin[®], Firma Werfft-Syntex, Vienna) dose for a mare of 500 kg body weight is 10 mg (0.02 mg/kg body weight) and CMA is given in 24 h intervals; the hCG (Chorulon[®], Firma Intervet, Boxmeer) dose for a mare is 2,500 IE (5 IE / kg body weight). The dose for the white rhinoceros was scaled allometrically as described by SEDGWICK (1993). The body weight of the rhino was estimated to be 2,500 kg and the constant (K) for the calculation of the Minimum Energy Cost was 70. Using this assumptions, a CMA dose of 35 mg (0.014 mg/kg body weight) and a dose interval of 35 hours was computed. The hCG dose for the white rhinoceros was calculated as 8,400 IE. CMA (Synchrosin[®], Firma Werfft-Syntex, Vienna) was applied in 32 doses in 35 hour intervals (45 days); hCG (Chorulon[®], Firma Intervet, Boxmeer) was given in a single intra muscular injection five days after the CMA application.

Results and Discussion

Faecal progestagens in the white rhinoceros 'Baby' were analyzed for > 2 years; the results of the progestagen evaluations during 1994 are shown in figure 1. Progestagens were low before and during the application of CMA; CMA metabolites did not affect faecal progestagen measurements. Ten days after the hCG application the faecal progestagen concentrations increased and thus indicated luteal activity. The length of this induced luteal phase was about 20 days. Oestrous behavior for two days was seen 70 days after the hCG application, and thus for the first time since the animal is kept at Salzburg zoo. Mating was not observed, may be due to the inexperienced male confined with the rhino 'Baby'.

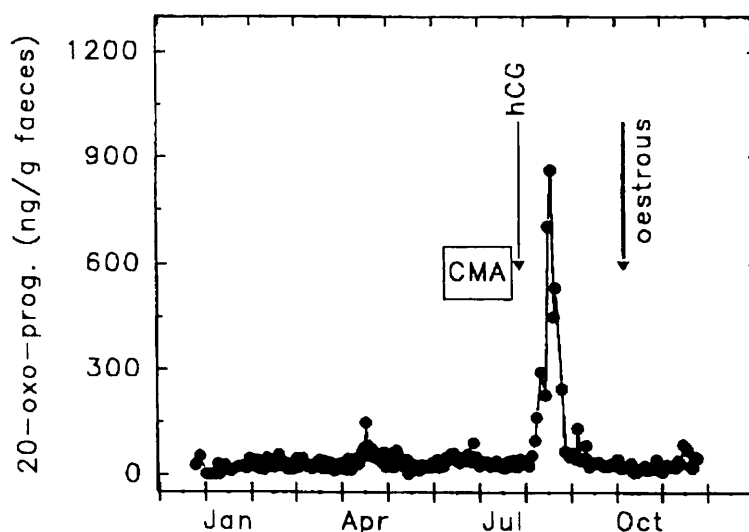


Fig. 1: Concentrations of immunoreactive 20-oxo-progestagens in faecal samples of the white rhinoceros 'Baby' before, during and after the application of the synthetic progesterone derivative CMA (Synchrosin[®]; Chlor Madinon Acetate), and hCG (Chorulon[®]; human Chorion Gonadotrophin). Samples were analyzed with a group-specific enzymeimmuno-assay for 20-oxo-progestagens, using an antibody against 5 α -pregnane-3 β -ol-20-one 3HS:BSA.

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 injection and thus within the range of the follicular phase (12.2 ± 0.8 days) described by SCHWARZENBERGER et al., (1994a). Our results contrast a study by GODFREY et al. (1990), which attempted to superovulate a white rhinoceros prior to its euthanization. Their study failed to induce ovulation and thus subsequent luteal activity. Further studies including more animals from different zoos will be necessary to find an optimal treatment protocol. These treatments should also include GnRH, FSH or PMSG. To increase efficiency, treatments should be applied in conjunction with parallel non-invasive monitoring of corpus luteum function.

The rhino 'Baby' showed oestrous behavior 70 days after the hCG injection; this was the range of what we suggest to be the 'normal' duration of the oestrous cycle in the white rhinoceros (SCHWARZENBERGER et al., 1994a). Induction of behavioral oestrous was also achieved with treatments done at zoo Dvur Kralove using altrenogest (Regumate[®]) and hCG (VAHALA, pers. comm.). However, similar to our study none of the treatments at Dvur Kralove resulted in 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 can be monitored using faecal steroid analysis. Since one of the reasons for the poor breeding performance of captive white rhinoceroses is the missing or erratic luteal activity, it is suggested to continue attempts to induce cyclicity in the white rhinoceros. In order to find optimal treatments protocols, faecal monitoring will be necessary.

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Summary

Oestrous cycle induction in a white rhinoceros (*Ceratotherium simum simum*) monitored by faecal progestagen analysis.

Reproductive rate of white rhinoceroses (*Ceratotherium simum simum* and *C. s. cottoni*) in captivity is very poor. A recent study using faecal progestagen evaluations indicated missing or erratic luteal activity as a possible reason. Faecal progestagen evaluations in one white rhinoceros kept at Salzburg zoo failed to detect luteal activity for a period of > 2 years. Therefore we attempted to induce cyclic activity in this animal using CMA (Chlor Madinone Acetate, Synchronin®) and hCG (Chorulon®). CMA was applied orally in 32 doses (35 mg each) in 35 hour intervals (45 days); hCG (8,400 IE) was given in a single intra muscular injection 5 days after the CMA application. Faecal progestagens were low before and during the application of CMA. They increased 10 days after hCG application and the duration of the induced luteal phase was about 20 days. Oestrous behavior was seen 70 days after the hCG application, but cyclic activity did not continue. In conclusion, monitoring of faecal progestagens demonstrated that luteal activity can be induced using a synthetic progesterone derivative, followed by an injection of hCG. Attempts to induce cyclicity in the white rhinoceros should be continued and monitored by faecal progestagen analysis in order to find optimal treatment protocols.

Zusammenfassung

Überwachung der Ovarialzyklus-Induktion bei einem Breitmaulnashorn (*Ceratotherium simum simum*) mittels Gestagenanalysen im Kot

Breitmaulnashörner (*Ceratotherium simum simum* und *C. s. cottoni*) vermehren sich nur sehr schlecht in Gefangenschaft und eine kürzlich veröffentlichte Studie zeigte fehlende oder unregelmäßige Corpus luteum-Aktivität als eine mögliche Ursache. Untersuchungen von Gestagenen im Kot einer Breitmaulnashornkuh aus dem Zoo Salzburg wurden > 2 Jahre durchgeführt. Dabei konnte keine Gelbkörperaktivität festgestellt werden. Deshalb wurde versucht mittels CMA (Chlormadinon-Acetat, Synchronin®) and hCG (Chorulon®) einen Zyklus zu induzieren. CMA wurde oral in 32 Dosen zu je 35 mg, in 35 h Intervallen (= 45 Tage) verabreicht; fünf Tage danach wurden intramuskulär 8.400 IE hCG appliziert. Die Gestagenanalysen der Kotproben zeigten niedrige Konzentrationen vor und während der CMA-Applikation. Zehn Tage nach hCG Injektion stiegen die Gestagenkonzentrationen an und die Dauer der induzierten Lutealphase betrug 20 Tage. Brunstverhalten wurde 70 Tage nach hCG Injektion beobachtet, jedoch trat keine nachfolgende Gelbkörperaktivität auf. Zusammenfassend wurde durch Gestagenanalysen von Kotproben gezeigt, daß die erfolgreiche Induktion der Gelbkörperaktivität bei Breitmaulnashörnern mittels synthetischer Progesteronderivate und nachfolgender hCG Injektion möglich ist. Versuche zur Zyklusinduktion bei Breitmaulnashörnern sollten fortgeführt werden. Um die Behandlungsschemata zu optimieren, sollte deren Effizienz mittels Gestagenanalysen von Kotproben überprüft werden.

Résumé

Contrôle de l'induction du cycle ovarien chez un rhinocéros blanc (*Ceratotherium simum simum*) au moyen d'analyses de gestagène dans les fèces.

Les rhinocéros blancs gardés en captivité (*Ceratotherium simum simum* et *C.s. cottoni*) se reproduisent mal, et une étude publiée récemment à ce sujet montre qu'une des raisons de cette défaillance pourrait être l'absence d'une activité du Corpus luteum ou l'irrégularité de celui-ci.

Les analyses de gestagène dans les fèces d'un rhinocéros blanc femelle du Jardin zoologique de Salzbourg ont été effectuées >2 ans durant, une activité des corps jaunes n'ayant cependant pas été constatée. Voilà pourquoi nous avons essayé d'induire un cycle au moyen du produit CMA (acétate de chlore madinone, Synchronin®) et hCG (Chorulon®). Le CMA a été administré en 32 doses perorales à 35 mg chacune, par intervalles de 35 heures (=45 jours). 5 jours après, 8400 UI de hCG ont été appliquées par la voie intramusculaire. Les analyses de gestagène des prélèvements coprologiques ont révélé de faibles concentrations avant et pendant l'application de CMA. Dix jours après les injections à hCG, les concentrations de gestagène ont augmenté, et la durée de la phase lutéale induite a duré 20 jours. Un comportement de chaleur a été observé 70 jours après l'injection hCG mais aucune activité des corps jaunes ne s'est manifestée par la suite. En résumant, on peut dire que les analyses de gestagène dans les prélèvements coprologiques ont montré qu'il est possible de réussir l'induction de l'activité des corps jaunes chez le rhinocéros blanc en appliquant des dérivés synthétiques de progestérone et en procédant par la suite à l'injection de hCG. Il faudrait poursuivre les essais visant à une induction du cycle chez des rhinocéros blancs, et pour optimiser les schémas de traitement, il conviendrait de vérifier leur efficacité au moyen d'analyses de gestagène dans les prélèvements coprologiques.

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