

# Hormonal Manipulations of Rhinoceros

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Reproductive efficiency of domestic animals has been considered essential for maximum production of offspring in minimum time. To this end, several decades of research have resulted in reliable procedures to induce estrus and ovulation (Britt, 1987). Attempts to stimulate these reproductive events in rhinoceros have, in general followed protocols developed for domestic animals.

Limited data on the gonadotropins of the Rhinocerotidae exist. McFarlane *et al.*, 1991, reported on the purification of hormones from a single pituitary from a black rhinoceros and a single pituitary from a white rhinoceros. Based on LH bioassays, radioreceptor assays and radioimmunoassays the authors concluded that rhino gonadotropins were similar to those of other mammals. Recent molecular studies have shown that white rhinoceros LHb subunit differ from equine LHb subunit in lacking a C-terminal peptide extension and is thus more like the LHb subunit of other mammalian species (Sherman *et al.*, 1997). The southern white rhinoceros but not Sumatran and black rhinoceros, however, appears to have a duplication of the LHb subunit gene (Lund and Sherman, 1998). The significance of this finding to rhino reproduction is unknown. There is no information on rhino chorionic gonadotropin. The objective of this report is to review attempts to induce estrus and ovulation in rhinoceros.

## I. Attempts to induce estrus and ovulation using a synthetic progestagen, Regu-Mate<sup>®</sup> (altrenogest), sometimes preceded by PGF<sub>2</sub> $\alpha$ , Estrumate<sup>®</sup> (cloprostenol sodium)

### 1. Southern White Rhinoceros (*Ceratotherium simum simum*)

#### a. Schaffer, Nan (1995) (unpublished)

Animal: Southern White Rhinoceros (SB 696) 27 yr. old wild caught, nulliparous

Day	Treatment
0-23	Regu-Mate <sup>®</sup> (altrenogest, 50 ml /day) orally

#### Results

- Ultrasonographic exams revealed no changes
- No rebound response in pregnane values 30 days post Regu-Mate<sup>®</sup>

b. Durrant, Barbara (1996) (unpublished)

Animal: Southern White Rhinoceros (SB 188) wild caught in 1968/69, nulliparous

Day	Treatment
0	Estrumate® (cloprostenol sodium, 75 mg) IM
12	Estrumate® (cloprostenol sodium, 75 mg) IM
53 - 66	Regu-Mate® (altrenogest, 42 ml/day) orally

Results

- Pregnanes were not changed by treatment
- No signs of estrus

2. Northern White Rhinoceros (*Ceratotherium simum cottoni*)

a. Durrant, Barbara (1994 -1998) (unpublished)

Animals: Northern White Rhino "Nadi" (SB 376) wild caught, birth: est. 1972, nulliparous (no. 1-3). Northern White Rhino "Nola" (SB 374) wild caught, birth: est. 1974, nulliparous (no. 4-5).

No.	Treatment	Estrumate® (75 mg/day)	Regu-Mate® (42 ml/day)
1	Day	0, 13	51 - 65
2	Day	0 - 3*	39 - 45
3	Day	0 - 2	4 - 17
4	Day	0 - 2	3 - 16
5	Day	0, 13	51 - 65

\* Estrous behavior (chin rest) following Estrumate® (30 mg/day)

Results

- No conclusive signs of estrous behavior were noted post Regu-Mate®
- Pregnanes did not indicate a functional CL post-treatment

3. Eastern Black Rhinoceros (*Diceros bicornis michaeli*)

a. Kristina Tomasova (1993) "An example of continued efforts to breed with a potential founder rhino", Europäisches Erhaltungszucht-Programm (EEP) Annual Conference, Salzburg, Austria

April - December, 1987

- A 17 yr. old black rhinoceros which had never bred and was considered to have aberrant behavior was introduced to a male. There were severe conflicts between the two. She was subsequently given Alestrum and PMSG to induce estrus, but no behavioral signs were observed November, 1989

- the female was treated with Regu-Mate® (altrenogest, 37.5 ml/12 days) at "theoretical estrus" she was introduced to the same male and again no breeding occurred December, 1989

- a new male was introduced in an adjacent pen  
March, 1990

- rhino was treated with Regu-Mate® (altrenogest, 50 ml/12 days)

April 22, 1990

- first mating for this female at age 20, with the new male

December 1992

-calf born

II. Attempts to induce estrus and ovulation using synthetic progestins (Regu-Mate<sup>®</sup> (altrenogest) or Synchronin<sup>®</sup> (Chlormadinon acetate), sometimes preceded by PGF<sub>2</sub> $\alpha$  (Lutalyse<sup>®</sup> (dinoprost tromethamine), Estrumate<sup>®</sup> (cloprostenol sodium) and followed by various combinations of FSH, PMSG, hCG, and GnRH.

1. Southern White Rhinoceros (*Ceratotherium simum simum*)

a. Godfrey, R.R.W., Pope, C.E., Dresser, B.L., Bavister, D.D., Andrews, J.C. and Olsen, J.H. 1990. An Attempt to Superovulate A Southern White Rhinoceros (*Ceratotherium simum simum*) Theriogenology Vol 323 :1 p 231.

Animal: SB45 (Maude) Tampa, 27 year old scheduled to be euthanized due to infirmities.

Day	Treatment
0	Estrumate <sup>®</sup> (cloprostenol sodium, 500 $\mu$ g) IM
1 - 21	Regu-Mate <sup>®</sup> (altrenogest, 2.2 mg/50 kg of body weight/day) orally
19	5000 IU PMSG (Calbiochem) IM
22	2500 IU PMSG (Calbiochem) IM
23	Estrumate <sup>®</sup> (cloprostenol sodium, 500 $\mu$ g) IM
26	Cystorelin <sup>®</sup> (gonadorelin, 500 ug) IM
28	euthanasia

Results

- no hormone data available
- day 25, male interested in female
- day 27 and 28, mucus from vagina had characteristic ferning pattern of estrual female
- antral follicles were found
- ovaries did not appear to be super stimulated
- no evidence of ovulation

Conclusion: Results suggest follicular growth can be stimulated in southern white rhinoceros

b. Ranglack, G.S., Gross, T., Schaffer, N., Mikota, S., Martin, G., Foley, G., Denniston, R. and Godke, R.A. (1992) The use of FSH To induce ovarian stimulation in a Southern White Rhinoceros (*Ceratotherium simum simum*). (unpublished)

Animal: Southern White Rhinoceros, 21 yr old, wild caught, nulliparous, no luteal activity, scheduled to be euthanized

Day	Treatment
0	Lutalyse® (dinoprost tromethamine, 50 mg) IM
15 - 30	Regu-Mate® (altrenogest, 0.044 mg/kg/day) orally
29 - 33	20 Armour Units FSH-P® (follicle stimulating hormone-pituitary) IM
32	Lutalyse® (dinoprost tromethamine, 50 mg) IM
38	5,000 units hCG IM
39	euthanasia

#### Results

- Increase in ovarian steroids in urine
- Multiple large follicles
- No ovulation

c. Schwarzenberger, F., Walzer, C., Tomasova, K., Vahala, J., Meister, J., Goodrowe, K., Zima, J., Straub, G., and Lynch, M. (1998) Fecal Progesterone metabolite analysis for non-invasive monitoring of reproductive function in the white rhinoceros (*Ceratotherium simum*). *Animal Reproduction Science, Wildlife Reproduction Issue* Ed. J.L. Brown and D. Wildt (in Press)

Animal: "Baby" SB 361, DOB est 1971, no luteal activity in 2 years

Day	Treatment
0 - 45	Synchrosin® (Chlormadinon acetate, 32 doses, 35 mg; 0.014 mg/kg fed at 36 h intervals for 45 days)
50	Chorulon® (hCG; 8,400 IU, 3.36 IU/kg) IM at ear base

This regimen was applied twice in 9 months, however during the second bout the CMA was given for 35 days.

#### Results

- CMA did not change metabolite measurements
- Pregnane concentrations rose 10 days after hCG resulting in luteal phase of 18 and 17 days, respectively
- Estrous behavior was observed 70 days post hCG

Conclusion: Ovulation and CL formation can be induced in the southern white rhinoceros

## 2. Northern White Rhinoceros (*Ceratotherium simum cottoni*)

After a review of the literature and consulting with the following: Jack Britt, R.A. Godke, Janine Brown, Steven Monfort, and Earl Pope, the following protocol was tried (1998).

Animals: Northern White Rhinos "Nola" (SB:374) age 24, nulliparous, and "Nadi" (SB:376) age 26, nulliparous.

Day	Treatment
0-21	Regu-Mate® (altrenogest, 60 ml/day) orally
20	40 Armour Units of FSH - P (Sioux Biochemical, 5% LH) IM
21	30 Armour Units of FSH - P (Sioux Biochemical, 5% LH) IM
22	20 Armour Units of FSH - P (Sioux Biochemical, 5% LH) IM
23	10 Armour Units of FSH - P (Sioux Biochemical, 5% LH) IM
25	8,000 IU hCG (Parlow, National Hormone & Pituitary Program, lot CR-127) IM

Results:

"Nadi"

- Pregnanes did not indicate an ovulation
- Day 24: mounted at least 9 times
- Day 27 - 30: mountings were observed throughout these days

"Nola"

- Pregnanes did not indicate an ovulation
- No estrous behavior

Conclusions:

- I. Attempts to induce estrus and ovulation using a synthetic progestin sometimes preceded by PGF2 $\alpha$ , suggest more extensive treatment regimes may be necessary to induce estrus and ovulation in rhinoceros.
  - II. Attempts to induce estrus and ovulation using synthetic progestin, sometimes preceded by PGF2 $\alpha$  and followed by various combinations of FSH, PMSG, hCG, GnRH suggest follicular growth and ovulation can be stimulated in rhinoceros.
3. Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

Terri Roth (1998) GnRH trial on old female Sumatran rhino

This female was subjected to ultrasound examinations 3 to 7 times/week for 4 months. Her ovaries were small and relatively inactive, containing only a few small, persistent cysts. She also had a large uterine mass that did not appear to be growing. Although reproductive senescence was suspected, the female was treated with gonadotropin releasing hormone (Cystorelin; 500  $\mu$ g, i.m.). This hormone should stimulate pituitary release of endogenous gonadotropins (particularly LH) that, in turn, should induce ovulation and/or CL development if the ovaries are capable of responding. Therefore, the treatment allows evaluating both pituitary and ovarian responsiveness. The female was subjected to daily ultrasound examinations for several days before GnRH administration, for 10 consecutive days following treatment and then 3 weeks later. Frequent urine, fecal and blood samples also were collected throughout the trial to monitor pituitary and ovarian endocrine responses to the treatment. A significant change in ovarian activity was noted on Day 35 post-GnRH. Upon palpation, the left ovary felt enlarged and lobular. The ultrasound examination revealed two large, fluid-filled structures on the left ovary in addition to the small cysts observed months before treatment. The structures remained for about 1 week before rapidly disappearing. Although the fluid-filled structures did not ovulate, the data suggested that GnRH was effective in stimulating rhino pituitary and ovarian function. The former was later confirmed by measuring serum LH. Blood was collected just prior to Cystorelin treatment and then ~40 min after the i.m injection of Cystorelin. LH was baseline (1.7 ng/ml) at time 0 and rose to >20 ng/ml 30 min after Cystorelin.

Summary:

- 1) A single GnRH injection (500  $\mu$ g Cystorelin, im) induced a rapid (1 h) increase in serum LH;
- 2) ovarian activity (development of follicles and/or cysts) followed ~30 days later; and
- 3) ovaries returned to their quiescent state by about 50 days without ovulating.

Note: No ovarian activity has been observed in the year following GnRH treatment suggesting that the activity noted, although later than expected, was a result of the Cystorelin treatment.

## Drug Glossary

Alestrum - ?

Chorulon<sup>®</sup> (human chorion gonadotropin, Intervet, Boxmeer)

hCG - human chorionic gonadotropin

Synchrosin<sup>®</sup> (Chlormadinone acetate, Werfft-Syntex, Vienna) 10 mg/ml, synthetic progestin

Cystorelin<sup>®</sup> (gonadorelin; CEVA Laboratories, Overland Park KS), contains 50 µg/ml GnRH diacetate tetrahydrate

Estrumate<sup>®</sup> (cloprostenol sodium; Haver, Shawnee KS) - synthetic prostaglandin analogue related to PGF<sub>2</sub>α, 250 µg/ml cloprostenol

FSH-P<sup>®</sup> (Follicle stimulating hormone - pituitary, Schering, Union N.J.)

FSH -P (Follicle stimulating hormone - porcine, Sioux Biochemical, Sioux City, IA)

Lutalyse<sup>®</sup> (dinoprost tromethamine, The Upjohn Co., Kalamazoo Michigan) - naturally occurring PGF<sub>2</sub>α as the tromethamine salt, 5 mg/ml dinoprost

PMSG - Pregnant mare serum gonadotropin (Calbiochem)

Regu-Mate<sup>®</sup> (altrenogest; Hoechst-Roussel, Somerville, N.J.) - oral synthetic progestin, 2.2 mg/ml altrenogest in an oil solution

## References

Britt JH (1987) Induction and synchronization of ovulation. In, *Reproduction in Farm Animals*, 5th ed pp 507-516 Ed ES.E Hafez. Lea & Febiger

Lund LA, Sherman GB. (1982) Duplication of the southern white rhinoceros (*Ceratotherium simum simum*) luteinizing hormone b subunit gene. *Journal of Molecular Endocrinology* 21:19-30.

McFarlane JR, Cabrera CM, Coulson SA, Papkoff H (1991) Partial purification and characterization of rhinoceros gonadotropins, growth hormone, and prolactin: comparison with the sheep and horse. *Biology of Reproduction*. 22: 94-101.

Sherman GB, Lund LA, Bunick D, Winn RJ. (1997) Characterization and phylogenetic significance of rhinoceros luteinizing hormone beta (LHbeta) subunit messenger RNA structure, complementary DNA sequence and gene copy number. *Gene* 195(2):131-139.