

AN ATTEMPT TO SUPEROVULATE A
SOUTHERN WHITE RHINOCEROS (*Ceratotherium simum simum*)

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All five species of rhinoceros are endangered, and captive breeding programs may be the last chance to save these species from extinction. In order to maximally utilize the captive population, it will be desirable to develop techniques to increase the reproductive capacity of captive animals. One such technique that will be beneficial is embryo transfer in conjunction with superovulation. This study was designed to evaluate the response of a female southern white rhinoceros (*Ceratotherium simum simum*) to a superovulation hormone regimen. The animal was 27 years old and was scheduled to be euthanized due to infirmities. On the first day of treatment (day 0), 500 ug of cloprostenol (Estrumate, Haver) was given I.M. On days 1 through 21, altrenogest (Regu-Mate, Hoechst-Roussel) was administered orally at a dose of 2.2 mg/50 kg of body weight/day. Regu-Mate was mixed with the daily feeding of grain. On day 19, 5000 IU of pregnant mare's serum gonadotropin (PMSG, Calbiochem) was given I.M., and on day 22 an I.M. injection of 2500 IU of PMSG was given. A cloprostenol injection (500 ug, I.M.) was given on day 23. On day 26, 500 ug of gonadotropin releasing hormone (Cystorellin, CEVA) was administered I.M. On day 28, frozen white rhinoceros semen was thawed at 35°C for 90 sec, and diluted 1:2 with Test-yolk buffer to a final volume of 1.5 ml. The sperm was maintained at ambient temperature for 45 to 60 min prior to insemination. A swine AI catheter was used to deposit 260.4×10^6 total sperm in the posterior third of the cervix. Twenty-five minutes after sperm deposition, 360 ml of T-61 Euthanasia Solution (Hoechst-Roussel) was administered I.V. Approximately 1.5 hr after euthanasia the reproductive tract was removed and transported to the lab. The uterine body and horns were flushed with sterile phosphate buffered saline, and the flushings were examined for the presence of oocytes or sperm. Antral follicles on one ovary were aspirated in an attempt to recover oocytes. No oocytes or sperm were found in the uterine flushings, and no oocytes were recovered by aspirating follicles. Five oocytes were recovered from the remaining ovary after the follicles were cut open and the wall of the follicle was scraped. After *in vitro* maturation of oocytes for 32 h and incubation for 24 h with rhinoceros sperm ($4-8 \times 10^5$ /ml) there were no visible indications of fertilization. Mucus collected from the vagina on days 27 and 28 had the characteristic ferning pattern of estrual mucus. Although the male rhinoceros was not in the same enclosure as the female, there was an increase in the interest level of the male towards the female on day 25. These results suggest that follicular growth can be stimulated in the white rhinoceros. The timing and/or dose of hormone treatments may need to be adjusted since there was no evidence of ovulation. Supported in part by a grant from the Institute of Museum Services (IC-70171-87).