PROGRESS IN REPRODUCTIVE PHYSIOLOGY RESEARCH IN RHINOCEROS

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In order to more effectively manage the captive population of rhinoceros it will be necessary to gain more knowledge about the reproductive physiology of these animals. The ability to manipulate and/or control the estrous cycle of rhinoceros in captivity will be a useful tool when developing captive breeding programs. To accomplish this, endocrine profiles during the estrous cycle (both normal and abnormal) need to be characterized. It will also be necessary to determine if exogenous hormone treatments (prostaglandins, progestins, gonadotropins) can be used to manipulate the estrous cycle of rhinoceros. The potential to use assisted reproductive technologies such as artificial insemination (AI) and embryo transfer (ET) in rhinoceros species will be increased only once a broader knowledge of the anatomy and endocrinology of the rhinoceros is established.

ENDOCRINOLOGY OF THE ESTROUS CYCLE

Previous researchers have developed procedures to monitor hormone metabolites in both urine and saliva collected from female white, black, greater one-horned Asian and Sumatran rhinoceros (see Hindle, *et al.*, this volume). Although these samples are relatively easy to collect from the animal, the optimal situation would be to collect blood samples to measure the concentration of the native hormone in peripheral circulation. By measuring both blood hormone and urine/saliva hormone metabolite concentrations, a correlation between the two can be determined. If the profiles are similar enough, it will then be possible to concentrate on collecting urine/saliva samples for analysis (which is much easier than blood sample collection in most instances).

A 21-year old, multiparous female black rhinoceros (*Diceros bicornis michaeli*) at the Cincinnati Zoo was conditioned to stand in a restraint chute for blood sample collection. Samples (15 ml) were collected three times a week into evacuated, heparinized tubes by venipuncture of the radial vein (Miller, *et al.*,1989). Blood samples were centrifuged to yield plasma which was stored at -20°C until analyzed by radioimmunoassay (RIA) for progesterone (P4) and estradiol (E2) concentrations. The female was placed with a mature male rhinoceros for breeding and was observed to stand for mating (Day 0). Blood samples were collected for approximately 90 days after an observed mating. The male and female were separated for management reasons shortly after the mating was observed so there was no opportunity to determine if the female returned to estrus in 30 days, which was noted to be the length of her cycle based on keeper observations of previous cycles.

The concentration of E2 was elevated on the day of observed mating (Figure 1), and declined within 3 days. The concentration of E2 remained low for 14 days and then began to rise and reached a peak concentration 26 days after mating. Concentration of E2 declined rapidly after this and stayed low for a period of 32 days, at which time it rose again. The concentration of P4 was low on day 0 and remained low until after the E2 peak at day 26. There is no explanation available as to why the P4 concentration remained low after the observed mating. Within 2 days after the E2 peak (day 26), P4 began rising and reached a maximum 9 days after the E2 peak. Concentration of P4 then declined to follicular phase levels within 14 days. Progesterone concentration began to rise 10 days after the second E2 peak (day 58) and stayed elevated until day 79. Since the male and female were separated shortly after the observed mating, there was no opportunity to observe behavioral estrus at the subsequent cycles. The hormone profiles would indicate that the female did come into estrus again at approximately 26 and 58 days after the observed mating. This length of the hormonal estrous cycle (26 and 32 days) corresponds well with the observations of the keepers regarding previous behavioral estrous cycles of this animal. The keepers indicated that this animal came into estrus approximately every 30 days during previous breeding periods. The hormone profiles also indicate that ovulation occurred and was followed by formation of a functional corpus luteum during the second and third cycles.

ANATOMY OF THE FEMALE REPRODUCTIVE TRACT

Before the techniques of AI and ET can be used successfully in rhinoceros, it will be necessary to gain information about the anatomy of the female reproductive tract. The ability to deposit semen or embryos at the proper site within the female tract will be critical to the success of AI and ET. Another consideration involves developing methods to collect embryos or oocytes nonsurgically.

The gross anatomy of the female reproductive tract of white and black rhinoceros has been described previously in a report by Godfrey, *et al.*, (1991). Briefly, reproductive tracts were collected from three black rhinoceros and two white rhinoceros at necropsy. All females were nulliparous, except for one primiparous white rhinoceros. All nulliparous animals had a constriction in the vaginal canal which appeared to be a hymen. The total length of the adult tracts averaged 102 cm, and the distance from the vulva to the external cervical os averaged 40 cm. The endometrium of a 28 year old nulliparous black rhinoceros and a 27 year old nulliparous white rhinoceros exhibited signs of hyperplasia. It is not known if this condition was related to the fertility of these animals. The cervix of all tracts examined was firm and the lumen followed a very tortuous path through eccentric rings of tissue. Visual examination of the ovaries revealed the presence of surface follicles on the ovaries in both species. The overall size of the reproductive tract, especially the vagina and uterine horns, and the extremely tortuous cervical lumen will present obstacles that must be overcome when developing artificial insemination and embryo transfer procedures for use in rhinoceros.

A second method of evaluating the anatomy of the reproductive tract involves the use of ultrasound. It has been shown that the sections of the reproductive tract of several large exotic species, including rhinoceros, can be imaged using rectally guided ultrasound equipment (Adams, *et al.*, 1991). The cervix, uterine body and uterine horns of white and black rhinoceros were imaged using this technique. It was also possible to image the ovaries of these animals by using a flexible rubber extension attached to the transducer. In one instance an embryo with a heartbeat was imaged in a black rhinoceros 27 days after an observed breeding.

PHYSICAL RESTRAINT OF RHINOCEROS

Both of the previously described projects (endocrinology and ultrasound) were possible, in part, due to the fact that the animals were conditioned to stand in a restraint chute. Several zoos in the United States have constructed chutes for rhinoceros and are able to collect various types of samples. There is a large amount of time and money that must be invested in order to construct a chute and condition the animals to use the chute, but the potential for data collection from these animals is greatly enhanced by using a restraining chute. The risks of using chemical immobilization are avoided and there is potentially less stress on the animal which could influence the quality of samples collected, specifically in the case of endocrine samples. More information on the design and use of restraint chutes can be found in the paper by Dr. Schaffer elsewhere in these proceedings.

RHINOCEROS BIOACOUSTICS

Vocalization by rhinoceros occurs in both the wild and captive populations. Recent projects have attempted to correlate rhinoceros vocalizations with specific behavioral events. One study (T.J. Spellmire, Dept. of Zoology, Ohio State University, Columbus, OH 43202, USA) reported several sounds with fundamental frequencies in the 300-600 hz range in captive black rhinoceros, with males having calls on the upper end of the range and females on the lower end. There was some indication of sex and age-specific vocalizations in these rhinoceros.

A second study by von Muggenthaler, *et al.*, (this volume) reported the detection of infrasonic sounds produced by white rhinoceros. These sounds are below the range of human hearing in the 5-75 hz range. It was noted that some animals may possess individual infrasonic signatures, and that there may be sex differences in the signals. Preliminary results indicated that black, Sumatran and greater one-horned Asian rhinoceros also produce infrasonic sounds. It is postulated that these sounds may be a form of inter-animal conversation. The potential to use previously recorded acoustics to stimulate specific behaviors in captive rhinoceros will be explored.

SUMMARY

Currently the technology is available to monitor the endocrinology of the estrous cycle of female rhinoceros by several methods. By comparing the urinary/salivary metabolite and native steroid hormone concentrations it will be possible to determine if the more readily accessible urine/saliva samples will be adequate for monitoring the estrous cycle in these animals. The ability to image the reproductive tract with ultrasound will aid in the development of AI and ET procedures in the future. Both endocrine and ultrasound studies can be enhanced by the use of manual restraint chutes for the rhinoceros being studied. Bioacoustics and related behaviors are being studied to determine if they may be beneficial to the management and breeding of captive rhinoceros.

ACKNOWLEDGMENTS

The authors would like to thank Paul Reinhart, Tom Tenhundfeld and Steve Romo for assisting with collection of blood samples.

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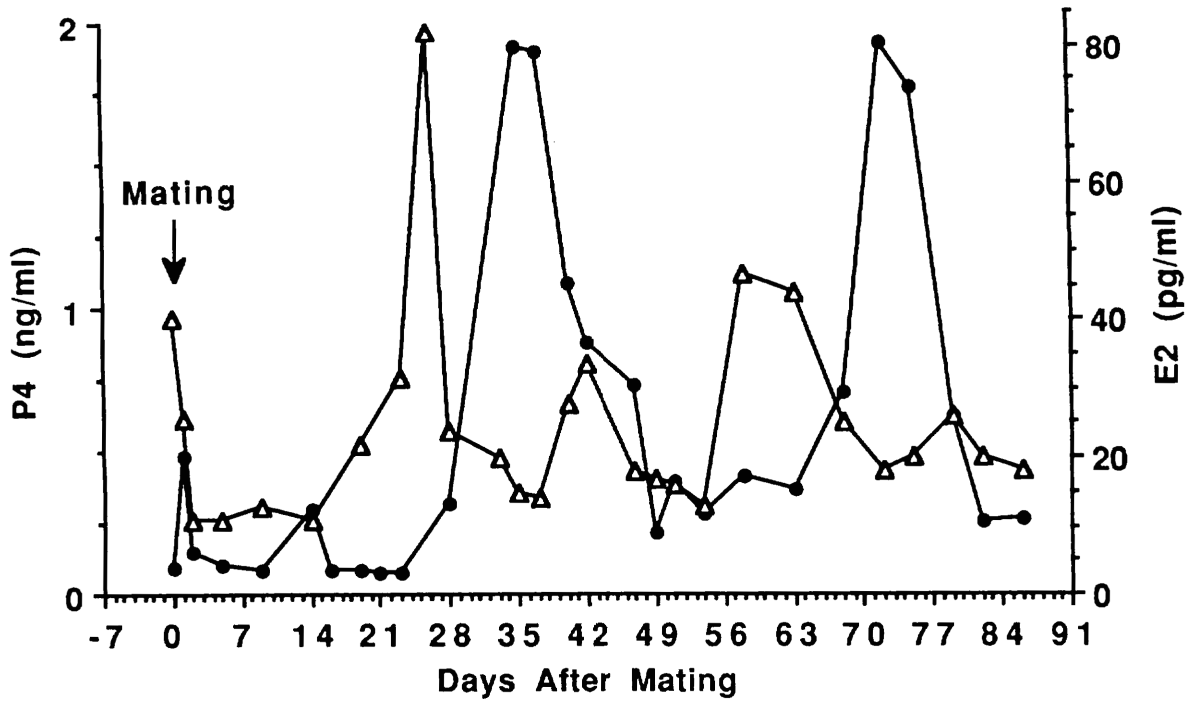

Figure 1. Plasma concentrations of progesterone (P4; •) and estradiol (E2; Δ) in a 21 year old, multiparous female black rhinoceros (*D.b. michaeli*). Samples were collected after the animal was observed to be in standing estrus (Day 0) by venipuncture of the radial vein. The animal was placed in a restraint chute three times a week for sample collection.