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Detection of Reproductive Behavior in the Black Rhinoceros *(Diceros bicornis michaeli)*

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Detection of reproductive behavior in the black rhinoceros *(Diceros bicornis michaeli)* is critical for the successful natural breeding of captive rhinoceros. The urgency to breed these animals stems from the current inability to meet the breeding demands of the captive rhinoceros populations. The goal of breeding programs is to have birth rates surpass death rates (Smith and Read, 1992). Unfortunately, the rates barely equal each other. Most rhinoceros species are now endangered and face extinction.

There has been a 90% decline of wild rhinoceros populations since 1970 (Hodges and Hindle, 1988). As of 1996, 2,800 animals exist in the wild, while there are 1000 in captivity. Of these captive animals 210 are black rhinoceros. In the wild, the black rhinoceros have suffered an 85% decline in the last 10 years, with a 97% decline in the last 25 years (Fouraker and Wagener, 1996). Ideally, the zoological community would like to have a self-sustaining captive population.

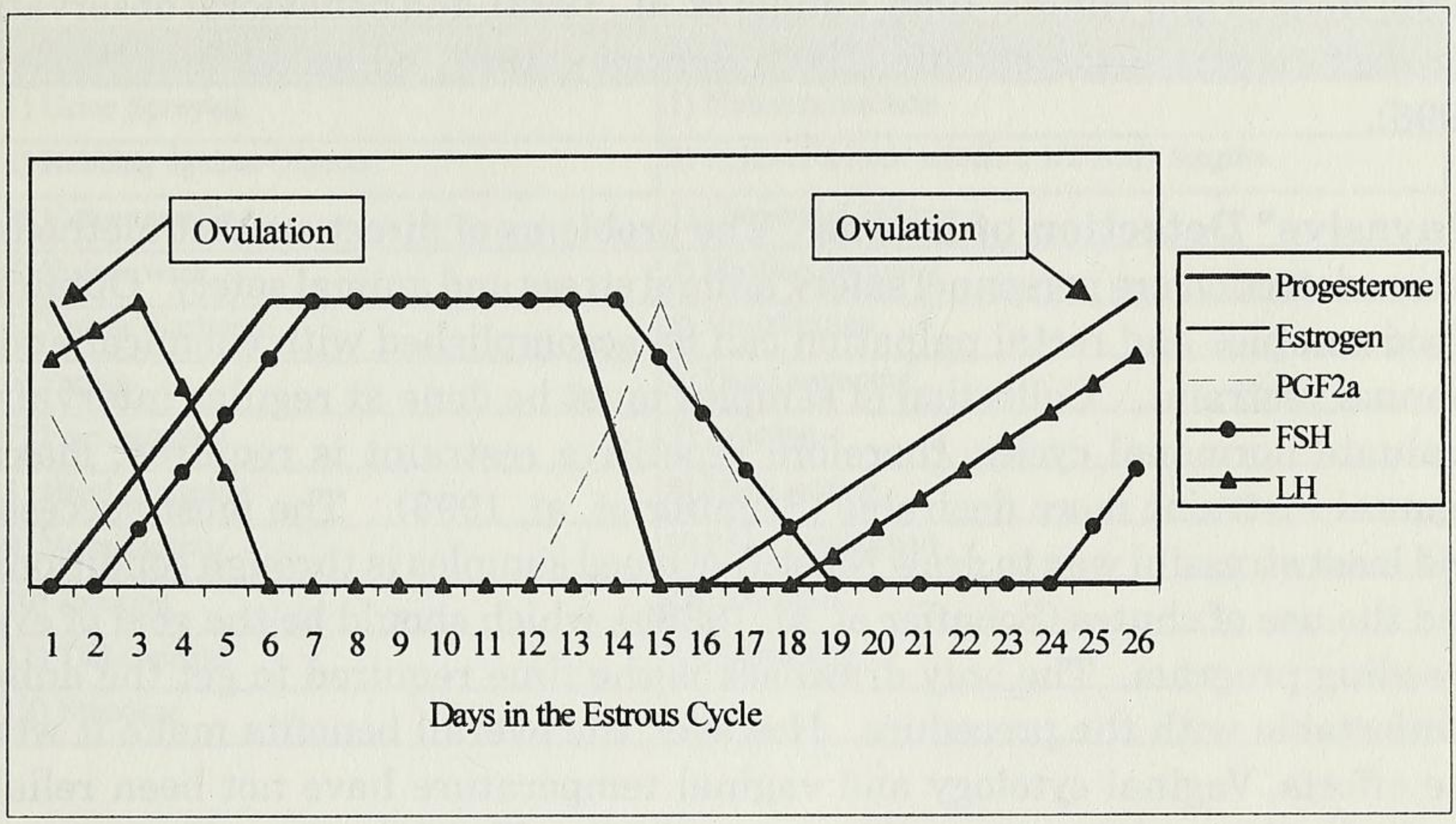
The Lincoln Park Zoo currently houses three black rhinoceroses *(Diceros bicornis michaeli)*, two females and one male between 17 and 18 years of age. In the last two years, zoo and hospital staff have been attempting to collect blood from the females to establish reproductive hormone levels to determine their normal estrous cycles. Samples have been successfully obtained repeatedly from only one of the females.

The objective of this paper is to outline normal mammalian estrus and estrous cycles, discuss rhinoceros reproductive behavior and to set up a system which keepers can use with serum hormone levels to establish the reproductive cycles of the animals in their collection. This would provide a great deal of insight concerning the times to introduce animals for breeding.

Reproduction

The General Mammalian Estrous Cycle. The estrous cycle is the period in which an ovarian follicle grows until the time it ruptures and releases an oocyte. It is driven by the normal fluctuations of various hormones and chemical mediators. Estrus is the period of time within the estrous cycle in which the female is most receptive to breeding with a male. This time period typically correlates with peak estrogen levels and ovulation.

Figure 1: Mammalian Estrous Cycle



The cycle begins with the growth of the ovarian follicle, which houses the oocyte. As the follicle grows, estrogen is produced. Estrogen stimulates an increase in gonadotropin releasing hormone (GnRH). The GnRH, in turn, stimulates increased secretion of leutinizing hormone (LH), which is responsible for ovulation (expulsion of the oocyte from the follicle). At peak estrogen levels, estrus behaviors are usually seen. Once ovulation occurs the emptied follicle becomes the corpus luteum. Progesterone begins to be produced by the corpus luteum and estrogen levels go down. Progesterone peaks about one week after ovulation. Post-ovulation, follicular stimulating hormone (FSH) levels rise, allowing the development of new follicles. When progesterone has been in circulation for 1-2 weeks, $PGF_2\alpha$, a prostaglandin, essentially destroys the corpus luteum. This causes a decrease of progesterone, and the new follicles begin secreting estrogen, starting the cycle over again. Should pregnancy occur, the corpus luteum is maintained and progesterone levels stay elevated (Youngquist, 1997).

Rhinoceros Reproductive Facts - In the rhinoceros, estrus is typically 3-6 days long with one day of standing estrus (when the female will allow the male to mount). The estrous cycle is about 26-46 days long (35 days average), is continuous and occurs year round. The average age reported to reach sexual maturity is 7 years for the female (Bertschinger, 1994). The last reported birth in captivity has been in a 28-year-old female (Smith and Read, 1992). The reproductive goal is one calf per female every two years (Bertschinger, 1994).

Detecting Estrus - The detection of estrus is accomplished by examining the fluctuations of the hormonal metabolites during the estrous cycle and the resulting behavioral estrus. This can be done by ìinvasiveî and ìnon-invasiveî methods. The term ìinvasiveî is used for techniques such as blood collection, rectal palpation and ultrasound (Radcliffe et. al, 1997). The term ìnon-invasiveî is reserved for methods not requiring direct contact such as collection of feces

(Radcliffe et. al., 1997; Berkeley et. al., 1997), urine and saliva (Read and Smith, 1992; Hodges and Hindle, 1988; Hindle et. al., 1993), and behavioral observation (Bertschinger, 1994; Radcliffe, 1997; Berkeley, 1997, Fouraker and Wagener, 1996).

"Invasive" Detection of Estrus - The problems of direct contact methods of estrus detection are personnel safety, animal stress and animal safety. Obtaining blood samples and rectal palpation can be accomplished with chemical and/or manual restraint. Collection of samples must be done at regular intervals to evaluate hormonal cycles, therefore repetitive restraint is required, making manual restraint more desirable (Schaffer et. al, 1993). The most successful and least stressful way to draw repetitive blood samples is through conditioning and the use of chutes (Schaffer et. al, 1998b), which should be the goal of every breeding program. The only drawback is the time required to get the animal comfortable with the procedure. However, the overall benefits make it worth the efforts. Vaginal cytology and vaginal temperature have not been reliable methods of assessing estrus (Pittenger, 1986). The detection of hormones in the serum is the best sampling method for assessing estrus hormones (Godfrey, 1993).

"Non-invasive" Detection of Estrus - Assaying hormone levels can also be done using feces, urine and saliva. Currently, saliva and urine are not being done on a routine basis at the Lincoln Park Zoo due to a lack of available testing techniques. Fecal hormone analysis has been shown to be useful in some animals. It is important to know that different hormones are excreted in different rhino species. For example, the Indian rhinoceros shows definite patterns of sex hormones in the feces, while these same hormone levels cannot be detected in the African species (Hodges and Hindle, 1988). Because of this, prior to choosing a test system, you must know the appropriate hormones to examine. Although fecal assessment of estrus is a valid tool, the Lincoln Park Zoo feels that serum and observation of behavioral changes are the best option for its breeding program.

Behavioral estrus is another valid method in assessing estrus and determining the estrous cycle. In conjunction with serum hormone levels, the animal staff can predict the appropriate times to introduce specific animals in the collection. The key is to understand and recognize the behavioral signs of estrus.

Behavioral Estrus - Correlating behavior and serum hormone levels may exponentially increase the success rate of natural breeding. Therefore, it is important for the keeper to notice behavioral changes. Table 1 is a list designed to help focus on these changes. It is not expected that any one animal will exhibit all of the signs, but it is a collation of various reported behaviors (Bertschinger, 1994; Radcliffe, 1997; Berkeley, 1997, Fouraker and Wagener, 1996). It is designed to be an aid in recognizing estrus.

Table 1: Differences and similarities between female and male behavioral responses to estrus

Female (Signs of estrus)	Male (Response to estrus)
1) Urine Spraying	1) Flehmen reaction
2) Rubbing against Objects	2) Follows female within 1 1/2 body lengths
3) Vulvar swelling	3) Urogenital exam
4) Vulvar wink	4) Hindleg dragging
5) Vulvar discharge	5) Vocalization
6) Pacing	6) Head sweeping
7) Foot scraping	7) Jousting
8) Head sweeping	8) Head resting
9) Vocalization	9) Penis unsheathed
10) Jousting	10) Erection
11) Tail erection	11) Mounting
12) Standing	

Definitions of terminology:

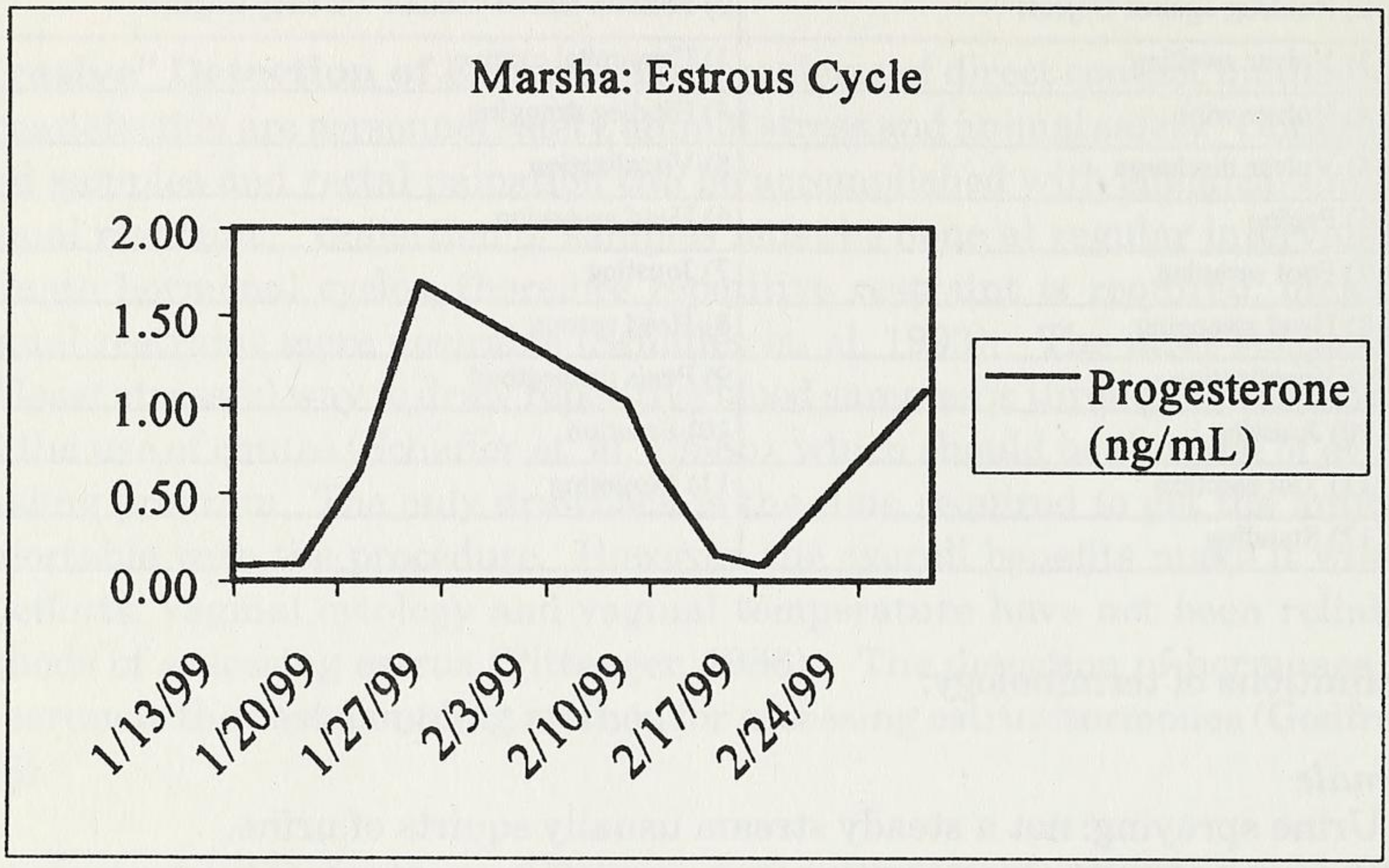
Female

1) Urine spraying: not a steady stream usually squirts of urine.
2) Rubbing against objects with horns
3) Vulvar swelling: the area of the vulva is noticeably swollen, there is usually a color change and there may be dilation.
4) Vulvar wink: the lips of vulva produce rapid contractions.
5) Vulvar discharge: there is a clear, wet discharge that can be associated with estrus, while post-estrus exhibits a thick, sticky and crusty discharge.
6) Pacing
7) Foot scraping: the animal stands in place, while the hindlegs scrape the ground.
8) Head sweeping: the head is to the ground, sweeping laterally, side to side.
9) Vocalization
10) Jousting: mock fighting with the intended male, may last up to an hour.
11) Tail erection: the female lifts her tail.
12) Standing: the female remains standing during a chin rest or mount.

Male

1) Flehmen reaction: the head raises and the upper lip curls up.
2) Follows female: usually next to the female or within one and a half body lengths behind the female.
3) Urogenital exam: the male sniffs the anal/genital region of the female.
4) Hindleg dragging: the hindlegs are stiff and straight behind.
5) Head sweeping: the head is to the ground, sweeping laterally, side to side.
6) Jousting: mock fighting with the intended female, may last up to an hour.
7) Head/chin resting: the male places his head on the femaleís back.
8) Penis unsheathed: a partial erection.
9) Erection
10) Mounting: the male stands on his hindlegs with his forelegs over the femaleís withers.

Cycles at the Zoo - Serum samples collected from a female rhino at Lincoln Park Zoo indicate that she is ovulating in the later middle portion of the month (P4 = progesterone).



However, it is not always possible to obtain serial samples in this animal. In some institutions it may not be possible to run any analyses. Therefore, monitoring the daily behavior of the females can improve our understanding of their breeding receptivity. Correlating female behavior with the responses of the male can indicate the appropriate times when the animals can be introduced. In order to help the animal staff coordinate their efforts, Charts 1 and 2 on the following pages were designed for daily record keeping.

Problems Other Than Inability in Recognizing Behavioral Estrus - Once it has been established that a proper program for detecting behavioral estrus has been implemented and breeding is not successful, it is then important to consider other factors.

1) Animal interaction

Two problems have been associated with breeding failure. The first is failure to introduce animals, which may result in serious trauma to either the male or the female. Proper introduction methods would include introduction of young females to proven bulls, use of bars or fences to introduce new animals, re-introducing the female to the male during the weaning period (decreases the calving intervals) and introducing the animals during periods of female receptivity (estrus).

The second is failure to terminate introduction. Since courtship and mounting in rhinoceros is already aggressive, it becomes critical for the keepers to understand when normal sexual behavior is occurring and when termination of the introduction is necessary to avoid trauma (Smith and Read, 1992).

Chart 1: Signs of Estrus in the Female Rhinoceros

House name:
Accession number:

Date	Time (am/pm)	Keeper initials	Observed behavior		Comments	Collected samples (Blood, feces, etc.)
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		
			☐ Urine spray ☐ Vulvar discharge ☐ Vocalization	☐ Vulvar swelling ☐ Vulvar winking ☐ Other (note in comments)		

Chart 2: Signs of Reproductive Behavior in the Male Rhinoceros

House name:
Accession number:

Date	Time (am/pm)	Keeper Initials	Observed behavior		Comments	Collected samples (Blood, feces, etc.)
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		
			☐ Flehmen reaction ☐ Erection	☐ Penis unsheathed ☐ Other (note in comments)		

2) Animal infertility
It may become necessary to evaluate the breeding potential of the animals in the collection. In females, it must be determined if they are cycling, if their anatomy is normal (ultrasound examination), if the females are being placed with the males at the appropriate times, and if they are healthy. Male reproductive potential can be determined by assessing semen quality (collection methods will be discussed), overall health, and the ability to mount.

3) Nutrition
The animals should be given a well balanced diet designed for adult rhinoceros.

4) Premature termination of pregnancy
Full physical and reproductive health evaluation (often requiring chemical immobilization) is necessary to assess spontaneous abortion.

5) Staff cooperation
It is important that all staff be involved in order to be able to assess daily changes and establish trends in a particular animal. This enables prediction of estrus and appropriate times to introduce animals.

Other Methods of Reproduction - Artificial insemination (AI) has been explored as an alternate method of reproduction to facilitate the maintenance of genetic balance in captive populations (Schaffer, 1993). To date, it has not been successfully accomplished. AI is difficult because detection of estrus is often not successful and the female cervix is highly convoluted (Godfrey 1993; Radcliffe, 1997). Since the cervix is arranged in this way, it represents a physical barrier to insemination and semen collection. Methods of semen collection include: 1) penile massage, 2) rectal massage, 3) artificial vagina, 4) rectal electroejaculation, and 5) postmortem collection (Schaffer 1993; Schaffer, 1998a). AI can be a useful technique only if you can adequately determine estrus, find methods of successful insemination, and adequately assess male fertility potential (sperm motility and normal sperm morphology).

Secondly, hormonal control may eventually allow manipulation of the estrus cycle and control of ovulation. This can possibly be done utilizing prostaglandins ($PGF_2\alpha$, gonadotropins (GnRH), and progesterone. Finally, embryo transfer should be considered (and is successful in equids) in females who can conceive, but not carry the fetus through gestation.

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