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Reproductive Cycle Length and Pregnancy in the Southern White Rhinoceros (*Ceratotherium simum simum*) as Determined by Fecal Pregnane Analysis and Observations of Mating Behavior

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Pregnancy and the reproductive cycle were monitored in 13 captive southern white rhinoceros (*Ceratotherium simum simum*) by measuring progesterone metabolites in fecal extracts and by observing behavior patterns. Fecal hormones were measured using a monoclonal antibody produced against 4-pregnen-11-o1-3,20-dione hemisuccinate:BSA. Several subjects exhibited flat or erratic endocrine profiles, but we found evidence for 17 reproductive cycles in five females. Mating behavior coincided with nadirs in pregnane concentrations. These cycles appeared to fall into two general categories: those of approximately 1 month in duration (Type I: $\bar{X} \pm$ SEM = 35.4 ± 2.2 days; n = 10) and those lasting approximately 2 months (Type II: 65.9 ± 2.4 days; n = 7). Interluteal phase lengths were similar for the two cycle types, but Type II cycles were characterized by extended luteal phases lasting more than twice as long as Type I luteal phases. Because Type I cycles predominated in our data and because evidence suggests that some Type II cycles may be aberrant, we argue that these approximately monthly cycles represent the typical reproductive cycle for this species. Three females became pregnant during the course of the study. We were able to detect pregnancy by approximately 3 months post-breeding, as indicated by sustained pregnane concentrations markedly higher than nonpregnant luteal phase concentrations. These data help to characterize important reproductive events of this species and should be useful for captive breeding efforts for this threatened species. Zoo Biol 18:111–127, 1999.

Key words: radioimmunoassay; captive breeding; estrus; rhinoceros

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INTRODUCTION

Although the current southern white rhinoceros population of 8,400 [Foose, 1999] appears to be viable, the commercial demand for rhinoceros horn continues unabated, and changes in the political climate in African countries could lead to the rapid decimation of the in situ population comparable to that observed in other rhinoceros species. Facing such an uncertain future in the wild, it is incumbent upon those working in zoological institutions to maintain a self-sustaining captive population and to learn as much about this species' biology as possible. The ex situ population may be needed as a reservoir for reintroduction, and research may prove invaluable for the management of the wild as well as the captive population. Although a few institutions have experienced remarkable success in breeding white rhinoceroses in captivity, a large proportion of the founding population has yet to produce offspring, and, moreover, captive-born rhinoceroses appear to have an alarmingly low birth rate [Foose, 1997; Swaisgood et al., 1998]. Due to these factors affecting the viability of the captive population, rhinoceros managers predict a crisis in the coming years.

Our understanding of the reproductive biology of the southern white rhinoceros is at best fragmentary. There is still disagreement, for example, on the length of the reproductive cycle. Hindle and coworkers [1992] report a 32-day cycle based on urinary hormone analysis of total estrogen and 20-dihydroxy-progesterone from one animal. Combined serial ultrasonographic evaluations and fecal pregnane analysis of a single female showed two non-conceptive cycles of 31 and 35 days [Radcliffe et al., 1997]. In contrast, a recent study of 16 southern white rhinoceroses led researchers to suggest a 10-week cycle based on fecal pregnanes [Schwarzenberger et al., 1998], whereas Wagner [1986] suggests that cycle lengths vary from 38 to 58 days based on urinary hormone analysis, vaginal cytology, and rectal examination. Behavioral observations in the wild [Owen-Smith, 1973, 1975] indicate a cycle period of approximately 30 days, whereas a multi-institutional survey [Lindemann, 1982] provided evidence that cycle length varies by multiples of approximately 30 days.

Similarly, the hormonal profile of pregnancy in the southern white rhinoceros has yet to be definitively characterized. Existing data from urinary hormone analysis reveals high concentrations of pregnanediol during the last half of gestation [Hodges and Green, 1989], and data acquired through fecal hormone analyses suggest that pregnane values are elevated above luteal values by the fourth and fifth months post-breeding [Schwarzenberger et al., 1998]. Gestation length is estimated to be 16–19 months [Owen-Smith, 1975; Jones, 1979; Rawlins, 1979; Pienaar, 1994]; this variability may be attributed in part to difficulty in accurately identifying the time of conception.

There are several reasons for the paucity of endocrine data for southern white rhinoceros. Sample collection in group-housed animals is difficult because defecation and urination must be observed or marked to insure accurate sample identification, a task made more difficult by the rhinoceroses' habit of defecating in communal dung heaps. Another problem is that many of the reproductively active females are pregnant most of the time and do not often exhibit non-conceptive cycles, whereas the remaining animals available for study are often non-reproductive and exhibit erratic cycles or none at all [Schwarzenberger et al., 1998]. In consequence, research

in this area suffers from small sample size and lack of data representative of normal reproductive patterns. The goal of our research was to address this lack of data by undertaking a long-term study combining non-invasive monitoring of progesterone metabolites and behavioral observations to infer reproductive cyclicity and pregnancy.

MATERIALS AND METHODS

Animals and Study Sites

The situation at the San Diego Wild Animal Park (SDWAP) affords an excellent opportunity to study the reproductive biology of southern white rhinoceroses because these animals live in a large naturalistic enclosure and in a social arrangement that approximates that of the wild. With 86 live births, SDWAP has achieved the highest captive breeding success for southern white rhinoceroses of any institution [Killmar, 1997], providing a successfully reproducing group for the study of what should be normal reproductive events of the species.

The primary study animals were kept together in a 36-hectare mixed-species exhibit at the SDWAP 24 hr/day. The animals were fed daily with high-fiber herbivore (1/2") pellets, Sudan grass hay, and coastal Bermuda grass hay and given ad libitum access to live grass and water. During the course of the research, the SDWAP population was comprised of two breeding-age males, eight breeding-age females, and four juveniles, but there was never more than one adult male present simultaneously (Table 1). To increase our sample size, we enlisted the cooperation of several other zoological institutions, obtaining sufficient fecal samples and behavioral data from five individuals residing in four institutions. Husbandry practices varied in these institutions, but all animals were housed in smaller enclosures than the SDWAP animals.

TABLE 1. History of individuals included in the study

Name	Studbook number	Date of birth	Birth origin	Number of offspring		Social situation		Institution	Data collection (years)
				Male	Female	Male	Female		
Dumisha	819	24 Jun 84	CB	0	0	1	8	SDWAP	1.9
Komaas	157	1963	WB	7	7	1	8	SDWAP	1.7
Mjuba	154	1963	WB	2	6	1	8	SDWAP	1.3
Mudder	188	1968	WB	0	0	1	8	SDWAP	1.2
Nthombi	277	1966	WB	8	1	1	8	SDWAP	1.2
Sinyaa	822	28 Aug 84	CB	0	0	1	8	SDWAP	1.1
Ujima	1051	26 Feb 95	CB	1	0	1	8	SDWAP	1.9
Umfolozi	159	1963	WB	7	8	1	8	SDWAP	1.6
Michelin	1114	1 Sep 91	CB	0	0	1	7	SFGASP	0.2
Marci	192	1967	WB	0	0	1	1	HZG	0.3
Ybonga	238	15 Apr 73	CB	0	0	1	1	RPZ	0.4
Milley	619	1966	WB	0	0	1	2	RHR	0.6
Wagasa	467	1972	WB	0	0	1	2	RHR	0.6

WB, wild born; CB, captive born. Social situation refers to numbers of reproductively mature animals housed together at the institution. Facility names: SDWAP, San Diego Wild Animal Park, San Diego, CA; SFGASP, Six Flags Great Adventure Safari Park, Jackson, NJ; HZG, Houston Zoological Gardens, Houston, TX; RPZ, Reid Park Zoo, Tucson, AZ; RHR, Rolling Hills Refuge, Salina, KS.

Fecal and Behavioral Data Collection

The rhinoceroses were monitored intensively for fecal collection and behavioral indices of estrus/courtship. All fecal samples were collected fresh (within approximately 1 hr after defecation) from the ground into plastic sample cups (Starstedt Inc., Newton, NC) and stored at –20°C. Keepers working in the area throughout the day (06:30–15:30) collected fecal samples opportunistically and noted any signs of reproductive behavior. As part of an ongoing research project at the SDWAP, systematic behavioral observations were made each day during the last 3 hr of daylight, which also extended the time for fecal collection. This intensive effort provided nearly complete daylight coverage of obvious behavioral signs of courtship and estrus and yielded an average of 2.8 fecal samples per animal each week. Staff at other institutions opportunistically recorded behavioral signs of estrus and courtship using a checksheet protocol. The study was carried out from February 1996 to November 1998.

Extraction and Processing

Fecal samples were lyophilized for 72 hr in a Flexi-Dry microprocessor manifold lyophilizer (FTS Systems, Inc., Stone Ridge, NY) to reduce variability in water content. Vegetation was removed from the lyophilized samples by sifting through a mesh screen (2 × 1.5 mm). A 0.2-g sample of the sifted feces was added to a 16 × 150-mm borosilicate culture tube, wetted with distilled water (2 mL), and vortexed (2 min). Five milliliters of diethyl ether anhydrous (Mallinckrodt, Paris, KY) was added to each tube, vortexed (2 min), and flash frozen in a methanol/dry ice bath. The supernatant was poured into 12 × 75-mm culture tubes and allowed to evaporate in a water bath (37°C). The ether extract was resolubilized in 1 mL absolute ethanol.

Radioimmunoassay

To analyze fecal extracts, we used a monoclonal progesterone antibody produced against 4-pregnen-11-o1-3,20-dione hemisuccinate:BSA [Grieger et al., 1990], which cross reacts 100% with progesterone, 96% with allopregnanolone, and to a lesser degree with a number of other progesterone metabolites [Grieger et al., 1990; Wasser et al., 1994], as well as 5β-pregnane-3β,20 α diol (0.42%), 5β-pregnane-3,20-dione (4.5%), and 5α-pregnane-3,20-dione (90%). Allopregnanolone is the most abundant pregnane in the feces of black rhinoceros [Patton et al., 1996] and is the principal pregnane in the feces of white rhinoceros [Patton et al., unpublished data]. Tritiated progesterone (10,000 cpm/0.1 mL, Dupont, NEN, Boston, MA) was used to compete against standard progesterone (7–1000 pg, Sigma, St. Louis, MO).

Ten microliters of the ethanolic fecal extract was diluted 1:100 in phosphate buffered saline 0.1 mol pH 7.0 (PBS) and 250 μL of this diluent was assayed in duplicate from samples of non-pregnant animals. Ethanolic extracts from samples of pregnant animals were diluted 1:1,000 in PBS and 10 μL of this diluent was taken to the assay. After an overnight incubation at 4°C, the competitive reaction was terminated by the addition of 0.25 mL of charcoal dextran solution to separate bound from free hormone. A further 30-min incubation period at 4°C was followed by sample centrifugation at 4°C for 15 min at 1,500 *g*. The supernatant was decanted into scintillation vials and scintillation fluid (5 mL, Ultima Gold, Packard Instrument, Meriden, CT) was added and the vials counted for 2 min in a Beckman liquid scintillation spectrometer (LS 7000).

Validation of the assay was tested by comparing parallelism in a serial dilution of fecal extract with the progesterone standard curve (r = 0.997). Extraction efficiency of added tritiated progesterone was 50 ± 2.4% (± SD, n = 7). Assay sensitivity was 8.35 pg/tube (calculated as mean pg/tube at 90% B/BO, n = 10). Buffer blanks were below the assay sensitivity. Accuracy was determined as 99 ± 7.7% (± SD) by recovery of seven known quantities of standard that were equivalent to the quantities used in the standard curve added to a pool of feces prior to extraction. A diluted fecal sample from a study female was used for this pool that contained an immunoreactive content just above the sensitivity of the assay. Interassay coefficients of variation (% SD/, n = 10) were 14.35% based on duplicates of a rhinoceros fecal pool with an immunoreactive content that yielded a %B/BO >60%, and 9.67% based on duplicates of rhinoceros feces pool with an immunoreactive content that yielded a %B/BO >30%. Intra-assay variation estimates (10 replicates of the same pools in a single assay) were 8.7% for the high pool and 7% for the low pool. Each assay generally contained 56 fecal samples from various rhinoceroses as they were collected. Results are presented as nanogram per gram (equal to nanogram per gram of dry fecal weight).

Data Analysis

To characterize the reproductive cycle, we applied a set of behavioral and endocrine criteria to estimate cycle length for each individual cycle. First, we determined whether the pattern of fecal pregnane levels indicated a cycle. A cycle was identified by a pattern of pregnane levels in which two consecutive values of <150 ng/g feces were followed by a peak that contains at least three values of >250 ng/g feces before falling again to a nadir of two or more consecutive values of <150 ng/g. Thus, behavioral observations did not play a role in determining the presence of a cycle. Once the presence of a cycle was identified, we applied a separate set of criteria to estimate the length of cycles and phases. For cycles preceded by observations of mating behavior (copulation, mount), we considered the onset of the luteal phase to occur on the day of mating (n = 13). For those cycles that were not preceded by mating, we estimated the onset of the luteal phase to occur 6 days before the first pregnane value rose to a level above 150 ng/g feces (n = 4). This estimate of 6 days is based on the average delay observed in this study for the 13 cycles preceded by mating. Termination of the luteal phase was defined as the point at which pregnane values decreased to less than half of the average of the three highest luteal phase pregnane concentrations for two consecutive values. When fecal samples were not collected on consecutive days during the transition from the luteal to interluteal phase, half of the missing days were assigned to the luteal and half to the interluteal phase. The interluteal phase began at the end of the luteal phase and continued until the day of the next mating episode (n = 13). In cases in which an identified cycle was not followed by observations of mating (n = 4), we used the average interluteal phase length (9.7 days for Type I and 10.5 days for Type II cycles). Total cycle length was calculated as the sum of the luteal and interluteal phases. For 11 of 17 cycles, our total cycle length estimates correspond to the interval between matings.

Matched-pairs *t*-tests were performed to determine when pregnane concentrations in fecal extracts from pregnant rhinoceroses rose significantly above nonpregnant luteal values.

RESULTS

Endocrine Cycles

We identified 17 cycles in five females (Table 2). These cycles appeared to fall into two general categories: those of approximately 1 month in duration (Type I: ± SEM = 35.4 ± 2.2 days; n = 10) and those of approximately 2 months (Type II: 65.9 ± 2.4 days; n = 7). Because mean interluteal phase lengths did not differ between cycle types, the difference in overall cycle length can be attributed to variation in luteal phase length. Thus, Type II cycles are characterized by extended luteal phases. Cycle lengths appeared to be bimodally distributed, suggesting that they reflect two cycle types with different biological significance, rather than continuous variation or noise. Type II cycles were found in only two females, both of which also exhibited Type I cycles. There were no apparent differences in management or social environment between cycle types.

A sustained rise in pregnane concentrations above 150 ng/g feces was not observed until approximately 6.4 ± 1.9 days post-mating, in part attributable to the 2–3 days it takes for progesterone metabolites to be excreted in feces [Hindle and Hodges, 1990]. A delay of approximately 5 days was reported as the interval from mating to increasing pregnane values in black rhinoceros, the smaller of these two rhinoceros genera [Schwarzenberger et al., 1993]. For Type I cycles, average pregnane concentrations during the luteal phase following day 6 post-mating were 390.0 ± 14.4 ng/g feces, whereas the interluteal phase was characterized by average pregnane levels of 103.9 ± 7.8 ng/g feces. Pregnane values for Type II cycles were similar, with an average of 391.7 ± 13.7 and 107.1 ± 7.1 ng/g feces for the luteal and interluteal phases, respectively.

Corroborating evidence for the biological validity of the 17 endocrine cycles identified in this study is found in the observation that mating behavior was clearly associated with a well-defined nadir in pregnane values after luteal phase concentrations of pregnane. To confirm this impression, we analyzed the relationship between all observations of mating behavior (including those occurring outside of identified cycles) and pregnane concentrations. This test compared samples collected the day of, the day before, and the day after mating with samples collected during the nonmating cycle midpoint 16–18 days later, the presumed luteal phase. To avoid the

TABLE 2. Individual cycle lengths (days)

	Type I cycles				Type II cycles		
Animal	Luteal	Interluteal	Cycle	Animal	Luteal	Interluteal	Cycle
Dumisha	21[a]	16	37	Dumisha	48.5[a]	10.5[a]	59
Dumisha	34[a]	9.7[a]	43.7	Dumisha	56	8	64
Michelin	25	8	33	Dumisha	44.5	17.5	62
Michelin	26	9.7[a]	35.7	Sinyaa	66.5	2.5	69
Mjuba	32.5	9.7[a]	42.2	Sinyaa	61	14	75
Sinyaa	25	7	32	Sinyaa	60.5	10.5	71
Sinyaa	27	4	31	Sinyaa	50.5	10.5	61
Ujima	19.5[a]	18.5	38				
Ujima	23	9	32				
Ujima	23.5	5.5	29				
Average	25.7	9.7	35.4		55.4	10.5	65.9
SEM	2.1	2.3	2.2		2.8	2.2	2.4

[a]Indicates that values were estimated using criteria described in methods section.

problem of data pooling [Machlis et al., 1985], values were averaged so that each individual contributed only two observations for the matched-pairs *t*-test. The results show that pregnane values were significantly lower near the time of mating (± SEM = 86.1 ± 14.3) than during the non-mating period (263.9 ± 70.3; $t_{1,5} = 2.73$, $P = 0.04$). Moreover, only four identified cycles were not immediately preceded by mating behavior and only four were not followed by mating. In one case, however, no male was present at the time and in another instance, it was before the female's first post-pubertal cycle. A single female accounted for the remaining endocrine cycles unaccompanied by mating, perhaps as a result of being paired with a non-preferred male (see discussion of silent estrus below).

Of the 13 females included in this study, only five exhibited cyclic profiles. Pregnane profiles remained low and acyclic for five wild-caught females and one captive-born that exhibited no behavioral signs of estrus during the course of the study. Fecal samples from these females rarely yielded any pregnane values above 150 ng/g feces (data not shown). One female exhibited a period of erratic acyclic peaks and nadirs in her pregnane profile after introduction of a new male (unfortunately, we have no hormonal data before the arrival of this male), followed by periods of little luteal activity (Fig. 1). Interestingly, mating behavior in this female was seen only during periods of erratic ovarian activity, and it coincided with nadirs in

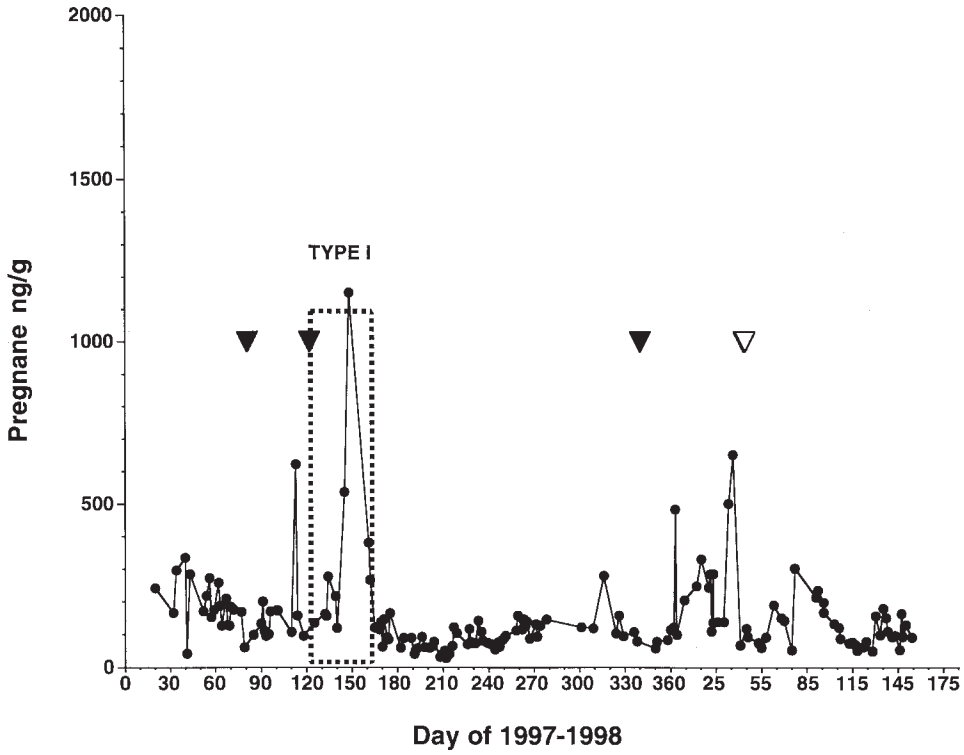

Fig. 1. Concentrations of fecal pregnane in southern white rhinoceros Mjuba. One Type I cycle is enclosed within the dotted line. Darkened triangle indicates copulation, open triangle indicates mounting. Note that no male was present from days 134 to 231, precluding the possibility of mating at the end of the identified cycle.

pregnane concentrations. However, this pattern of pregnane fluctuation yielded only one cycle according to our criteria.

Two nulliparous captive-born females exhibited periods of erratic pregnane fluctuation, interspersed with apparently cyclic luteal activity (Fig. 2a–c). Some of these cycles occur at approximately monthly intervals (Type I), whereas others are of much longer duration (Type II). Neither of these females exhibited periods without luteal activity, as observed in some of the older females. The two remaining subjects included in this study exhibited two and three Type I cycles (Fig. 3a and b, respectively). The female whose data are presented in Fig. 3b is a young nulliparous female who entered puberty during this study. Samples collected during the first half of 1997 indicate little ovarian activity, followed by three Type I cycles accompanied by mating behavior coinciding with low pregnane levels.

Finally, we found no evidence for a seasonal effect on reproductive cycles. Because seasonality could be affected by climatic or photoperiodic factors, we only included animals from the SDWAP in this analysis. The results show that mating occurred 11 times during winter, 13 in spring, 10 in summer, and nine in autumn. Thus, although several females exhibited both cyclic and acyclic periods, mating did not appear to be associated with any particular season.

Pregnancy

Two wild-caught lactating females became pregnant on the first post-partum mating. Pregnane levels for these females did not indicate a luteal phase before observed breeding behavior (Fig. 4a and b). In addition, a young captive-born nulliparous female became pregnant soon after undergoing puberty, apparently at the end of her third reproductive cycle (Fig. 4c). [Note added in proofs: Ujima (Fig. 4c) delivered after 509 days (16.9 months).] For these three females, a noticeable rise in pregnane levels is evident between 1 and 3 months post-breeding. To determine when this difference becomes significant, we randomly selected 10 pregnane values from non-pregnant luteal phases and compared them with the first 10 pregnane values collected post-breeding, the next 10 values post-breeding, etc. Separate analyses for each female revealed that pregnane levels during pregnancy first surpassed non-pregnant luteal phase concentrations approximately 98 days ($t_{1,9} = 3.97$, $P = 0.002$; Fig. 4a), 145 days ($t_{1,9} = 2.96$, $P = 0.008$; Fig. 4b), and 101 days ($t_{1,9} = 3.91$, $P = 0.002$; Fig. 4c) post-breeding.

Pregnane levels continued to rise until approximately the seventh month of gestation and then remained at similar levels through the remainder of gestation. Pregnane values for this period averaged >40,000 ng/g feces, >100 times that observed during the nonpregnant luteal phase. One female (Fig. 4a) gave birth after 490 days (16.3 months) and another (Fig. 4b) after 525 days (17.5 months). (The first female bred 62 days post-partum, and our endocrine data indicate that she is pregnant again.) Pregnane values remained elevated until parturition. In one female (Fig. 4b), pregnane values 1 day post-partum, had fallen to 2.4% of those observed at the end of pregnancy. On the next sample, collected 4 days post-partum, pregnane values were at baseline values.

DISCUSSION

Our results demonstrate that our method of analysis of progesterone metabolites in fecal extracts in southern white rhinoceroses can be used to characterize the

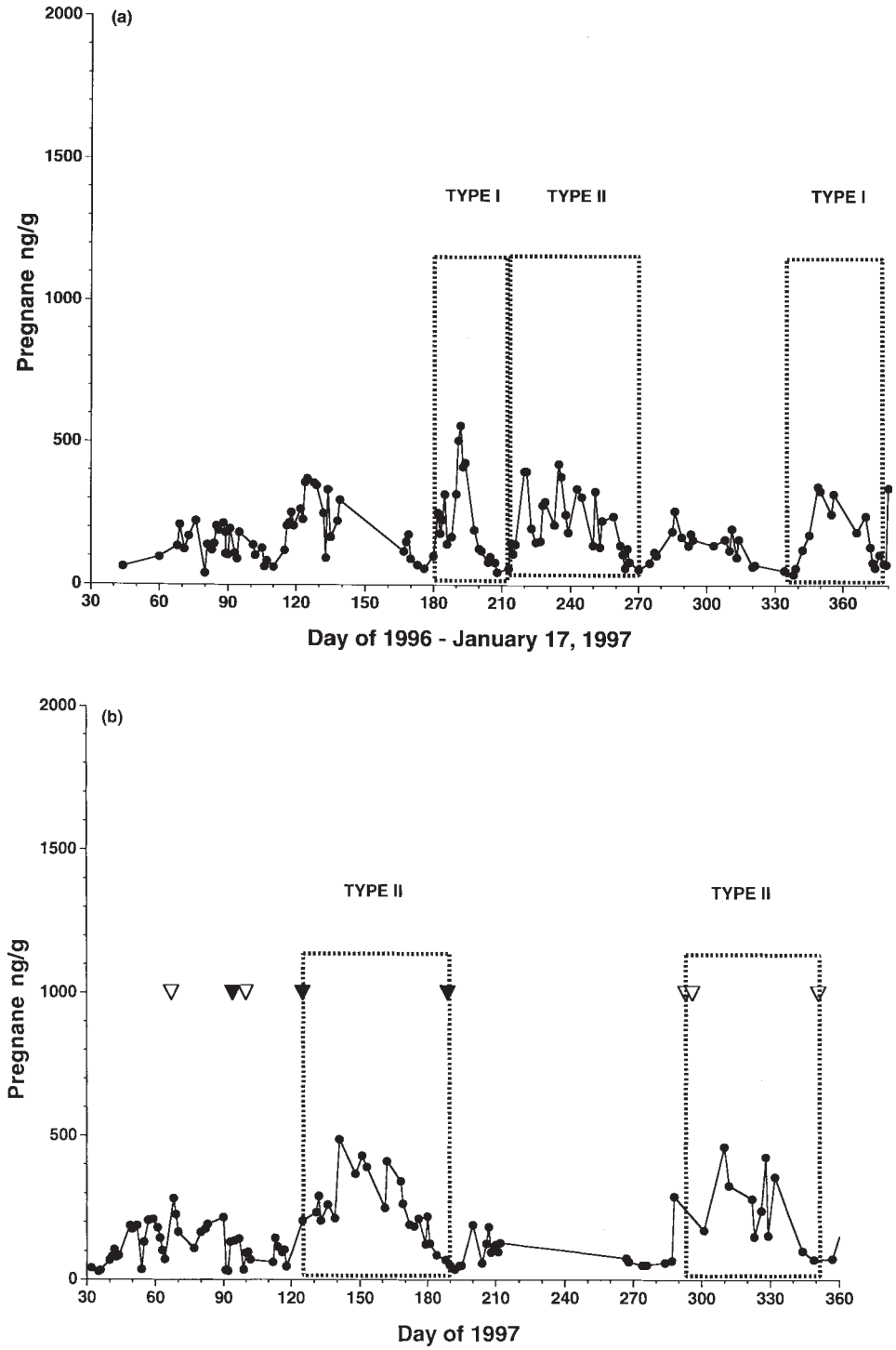

Fig. 2. Concentrations of fecal pregnane in southern white rhinoceroses illustrating Type I and II cycles. **a:** Dumisha, February 1996–January 1997. **b:** Dumisha, 1997. **c:** Sinyaa, February 1996–April 1997. Cycles are enclosed within the dotted line. Darkened triangle indicates copulation, open triangle indicates mounting.

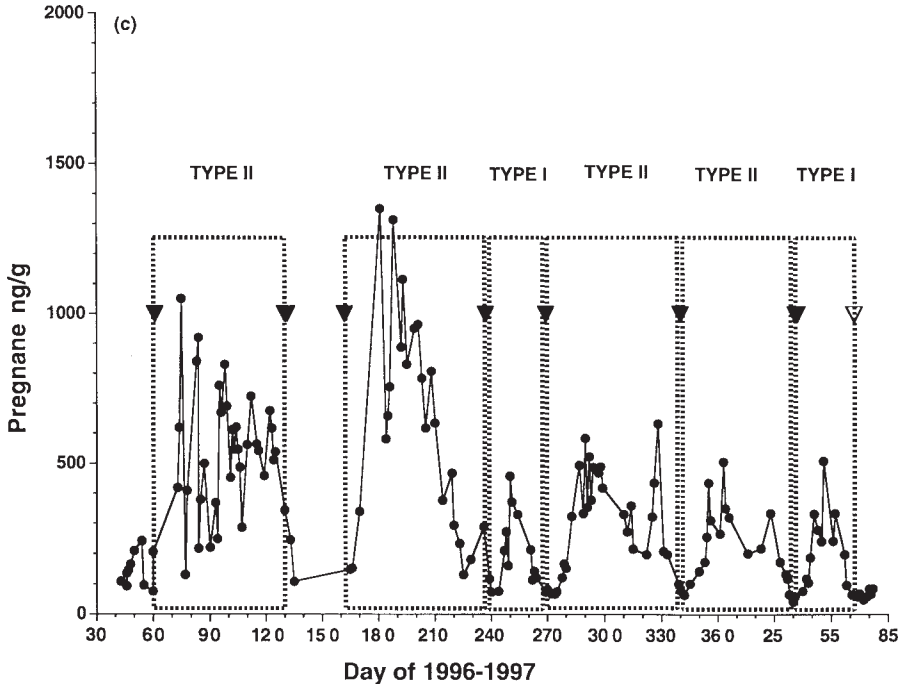

Fig. 2. (*continued*).

major reproductive events in this species. We showed that application of this technique can be used to detect pregnancy, monitor reproductive cyclicity, and reveal aberrant patterns of reproductive malfunction. Behavioral support for our assay's ability to profile accurately the reproductive cycle is found in the observation that pregnane values around the time of mating and mounting were significantly lower than luteal values approximately 17 days post-mating. Moreover, most observations of mating behavior occurred at the nadir of pregnane concentrations of a reproductive cycle identified by our endocrine criteria. Although a decline in progesterone documents only the regression of the corpus luteum, the fact that nadirs in progesterone levels coincided with mating behavior suggests that it often reflects the fertile period. Nadirs in progesterone concentrations have been shown to coincide with ovulation for many mammalian species [Pineda, 1989], including the southern white rhinoceros [Radcliffe et al., 1997]. Thus, we cautiously suggest that progesterone nadirs may correlate with ovulation in the present study.

Despite periods of erratic and acyclic ovarian activity, a clear pattern of approximately monthly cycles (Type I cycles) emerges during many periods that bear evidence of cyclic luteal activity. Our data suggest a typical reproductive cycle length of 35.4 ± 2.2 days, with 59% of cycles falling between 29 and 41 days. An additional female not included in this study exhibited two behavioral cycles of 31 and 28 days shortly after attaining puberty [Swaisgood, unpublished data], but she died before the endocrine study commenced. These findings are consistent with previous physiological and behavioral research supporting a cycle length of approximately 1 month [Owen-Smith, 1973, 1975; Lindemann, 1982; Hindle et al., 1992; Radcliffe et al., 1997].

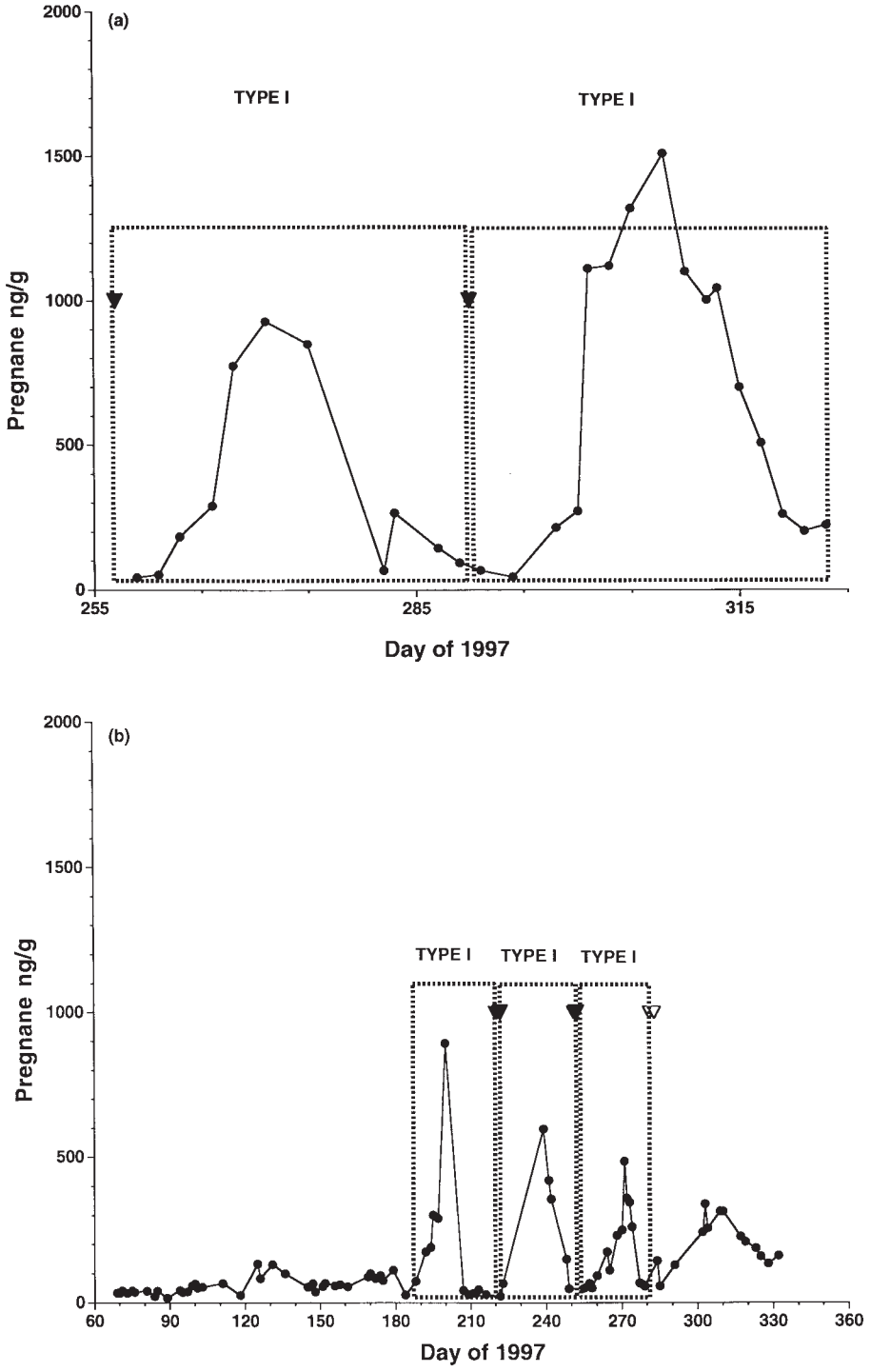

Fig. 3. Concentrations of fecal pregnane in southern white rhinoceroses illustrating Type I cycles. **a:** Michelin. **b:** Ujima. Cycles are enclosed within the dotted line. Darkened triangle indicates copulation, open triangle indicates mounting.

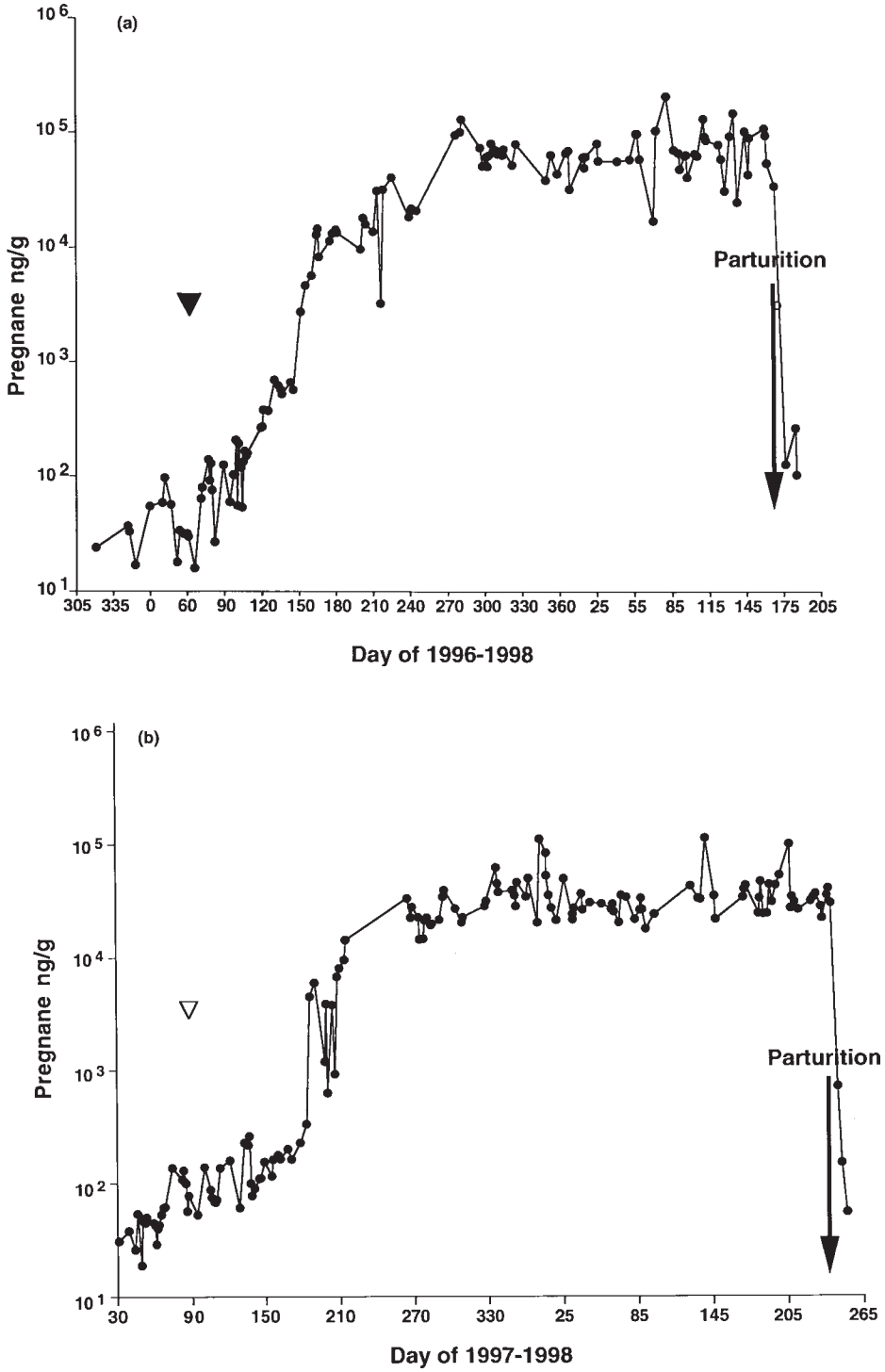

Fig. 4. Fecal pregnane pregnancy profiles on a $\log_{10}$ scale in southern white rhinoceroses. **a:** Komaas. **b:** Umfolozi. **c:** Ujima. Darkened triangle indicates copulation, open triangle indicates mounting.

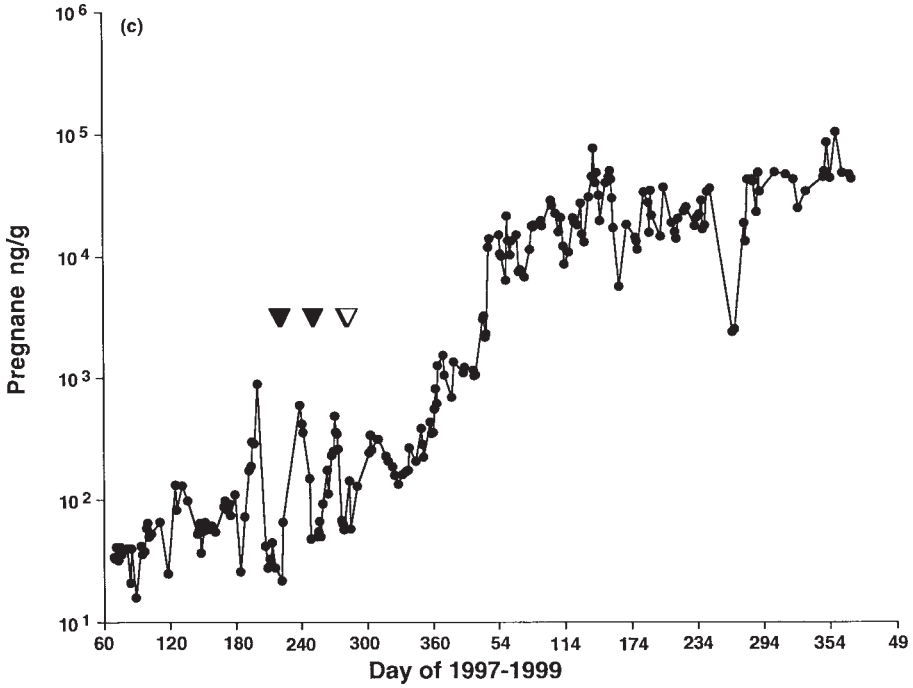

Fig. 4. (*continued*).

Further corroboration of our argument for a typical cycle of approximately 1 month is found in the observation that one female conceived at the end of 3 cycles, each approximately 1 month in duration. (Unfortunately, the other two females conceived on the first post-partum mating without exhibiting a luteal phase, so cycle length could not be calculated for these individuals.) In addition, a recent study of a single southern white rhinoceros female identified two approximately 30-day ovulatory non-conceptive cycles, documented with combined ultrasonography and fecal hormone assay [Radcliffe et al., 1997]. Conception followed the second cycle. Clearly, cycles followed by confirmed ovulation and/or conception are more likely to represent "normal" (i.e., fertile) reproductive cycles.

Although more than half of our data indicates cycles of approximately 1 month, we found evidence for a second category of endocrine cycle (Type II), characterized by an extended luteal phase. Type II cycles were 65.9 ± 2.4 days in length. Because most of our endocrine cycles were bounded on either side by observations of mating behavior and no mating behavior was observed during periods of elevated pregnanes, we can be more certain that our endocrine analysis accurately identified one extended cycle, not two shorter cycles with missing or flawed data. Other authors argued that these approximately 2-month cycles represent the typical cycle length for the southern white rhinoceros, despite the fact that only a small fraction of cycles they identified fell into this category [Schwarzenberger et al., 1998].

The frequent occurrence of extended luteal phases found in both of these studies may be attributed to several factors. For example, pyometra and endometritis are known to cause irregular and extended luteal phases in the horse and are a major

cause of infertility in this relative of the rhinoceros [Hughs et al., 1979; Daels and Hughs, 1993]. Pyometra has been diagnosed using ultrasonography in both of the rhinoceroses at the SDWAP that exhibited extended luteal phases (Fig. 2a–c), and uterine inflammation, as identified by intrauterine fluid collections, was also diagnosed in an earlier study of a single southern white rhinoceros that exhibited extended luteal phases [Radcliffe et al., 1997]. These authors used ultrasonography to document two conceptions followed by embryonic loss approximately 1 month post-conception in this female. In both cases, pregnane values remained elevated for 70–80 days post-conception, producing endocrine profiles remarkably similar to those observed in our study and the study by Schwarzenberger and colleagues [1998]. Recently, ultrasonographic examination also confirmed fetal resorption for one of the SDWAP females with extended luteal phases. It is interesting to note that this female had two Type II cycles with markedly higher pregnane values, suggesting the possibility of pregnancy and embryonic loss during these cycles (Fig. 2c). Thus, we hypothesize that some extended luteal phases are the result of uterine pathology and/or early embryonic death (which may be caused by uterine inflammation). Extended luteal phases can also be caused by early embryonic death in horses [Niswender and Nett, 1993]. Finally, as in the horse, diestrous ovulation may cause luteal phase prolongation [Daels and Hughs, 1993]. Taken together, these observations suggest that Type II cycles do not represent the "normal" reproductive cycle and may be a causal factor contributing to infertility in captive the southern white rhinoceros.

Pregnancy was determined for three females on the basis of a sustained, markedly high elevation of pregnane, which was easily differentiated from the short-term moderate rise in pregnane found in the luteal phase of the reproductive cycle. Pregnane values during pregnancy rise to significantly higher levels than non-pregnant luteal concentrations by 3–4 months post-conception, providing an important diagnostic tool for pregnancy detection and allowing informed management decisions for pregnant cows. The timing of gestational increases in fecal pregnane concentrations is similar to those found in black [Schwarzenberger et al., 1993; Berkeley et al., 1997] and Indian rhinoceroses [Kasman et al., 1986]. However, we did not find a decline in pregnane values prior to parturition, as has been observed in the black [Schwarzenberger et al., 1993] and Indian rhinoceroses [Kasman et al., 1986]. Our results are more consistent with the findings of Berkeley and coworkers [1997] for black rhinoceroses, which do not reveal a decline in pregnane values until after parturition. These differences may result from differences in antibody cross-reactivities.

We also found evidence for completely acyclic luteal activity in six females showing low or flat pregnane profiles. Schwarzenberger and coworkers [1998] monitored pregnane levels in several females and found endocrine patterns strikingly similar to ours, with the majority of females demonstrating acyclic or irregular cycles. These observations beg the question of why so few females exhibit regular, cyclic ovarian activity and consequently fail to reproduce. Apparently, even "cycling" females fail to cycle consistently throughout the year. It is possible that periods of reproductive quiescence, interspersed with ovulatory cycles, is the norm for this species even in the wild. Owen-Smith [1973, 1988] found some evidence for a seasonal effect on births, suggesting periods of infertility, and horses undergo extended seasonal anestrous periods associated with erratic ovarian activity [Sharp and Davis, 1993]. Although our data do not suggest a seasonal effect for southern white rhinoceroses, it is rea-

sonable to hypothesize that rhinoceroses may undergo anovulatory periods that are not strictly governed by season, as is seen in wild elephants [Poole, 1989].

Although this scenario may explain in part the lack of consistent year-round cyclic activity in many rhinoceroses, it fails to address the pattern seen in those females that rarely exhibit any cyclic luteal activity. Some of our observations at the SDWAP may shed light on this phenomenon. Two of the females with acyclic luteal activity in our study are primiparous but have not bred for several years. It is of interest to note that reproductive behavior ceased in both females after one breeding male was replaced, and resumed in one of these females upon the arrival of a new male. The other reproductively quiescent female at the SDWAP spent the first two decades of her life with a single male rhinoceros, but has not responded to transfer to the SDWAP nor to access to a new male. It is possible that some female rhinoceroses undergo irreversible reproductive atrophy if they fail to reproduce by a certain age, a phenomenon known as the maiden mare syndrome in horses [Douglas, 1982]. The remaining four acyclic females in our study are also held with one male and at most one other female.

These observations highlight the potential importance of allowing mate selection opportunities. We also have anecdotal evidence suggesting that mate preference may play a role in the behavioral manifestation of estrus. One female did not mate nor show any signs of estrous behavior while housed with a specific male in 1996 [Swaisgood, unpublished data]. Pregnane concentrations in 1996 and early 1997 indicate at least three cycles for this female, one Type II and two Type I cycles (Fig. 2a). With the introduction of a new male in January 1997, this female showed a marked increase in reproductive behavior and mated six times, although she showed little substantive change in her pregnane profile (Fig. 2b). Thus, this may be a case of "silent estrus," a phenomenon known to occur in several domestic species [Jainudeen and Hafez, 1987]. Additional evidence for a role of mate preferences is found in the observation that southern white rhinoceroses kept as male-female pairs rarely reproduce [Lindemann, 1982; Reece, 1991; Bertschinger, 1994], suggesting that access to a single opposite-sex individual may be insufficient. Examination of the southern white rhinoceros studbook reveals that non-breeding individuals sometimes become breeders when moved to a new facility or when a new opposite-sex animal is introduced [Ochs, 1997].

Female white rhinoceros home ranges in the wild encompass several male territories [Owen-Smith, 1975; Pienaar, 1994], providing ample time away from males and exposing them to several different males. When a female comes into estrus, she may select a male simply by entering his territory, as males will not encroach on other males' territories even to breed [Owen-Smith, 1975]. It is reasonable to hypothesize that the female may require a period of isolation from the male to maintain sexual responsiveness and perhaps even ovarian function, as has been documented in sheep [Martin and Scaramuzzi, 1983]. The success of the rhinoceros breeding program at SDWAP and similar institutions may be attributed in part to the large enclosure size and multiple-female arrangement, which prevents the male from "shadowing" a single female. Coupled with individual idiosyncrasies influencing mate preferences, such observations underscore the importance of paying attention to the details of social arrangements. Clearly, more research on the effect of the social environment on reproduction is needed to fully understand the chain of causal mechanisms underlying reproductive failure in this species.

CONCLUSIONS

1. The majority of female rhinoceroses in this study appeared to be reproductively compromised by abnormal ovarian activity characterized by acyclicity, erratic luteal activity, or a combination of cycles of varying lengths.

2. Among cycling females, two cycle types were indicated: monthly and bimonthly. The two cycle types differ with regard to luteal phase length, but the interluteal phases are equal in duration. We argue that the approximately monthly cycles are typical, whereas the longer cycles indicate a pathologic lengthening of the luteal phase.

3. Mating behavior coincided with nadirs in pregnane levels for both types of cycles, providing behavioral validation of our endocrine assay.

4. Pregnancy determination was possible by approximately 3–4 months postbreeding via endocrine profiles exhibiting a sustained elevation of pregnane values.

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