Of the five rhinoceros species in existence, all but the South African white rhinoceros are considered endangered (IUCN, 1996). The decimation of this taxon can be attributed to previous uncontrolled hunting and current illegal poaching for the horn of the animal, which in some Asian cultures is believed to possess medicinal properties. The Sumatran rhinoceros (Dicerorhinus sumatrensis) is the most primitive of surviving rhino species and descended from the woolly rhinoceros that lived during the last ice age. The Sumatran rhinoceros is also the most endangered: there are fewer than 400 animals worldwide and populations continue to dwindle (Foose and Reece, 1998). A captive breeding programme was initiated for this species and from 1984 to 1996, 40 animals were placed in zoos and reserves worldwide after being rescued from snares or forests destroyed by logging (Foos and van Strien, 1997).

Longitudinal ultrasound and endocrine evaluations were conducted in two adult female Sumatran rhinoceroses (Dicerorhinus sumatrensis) over a period of 12–22 months to learn more about their reproductive physiology. Rectal ultrasonography was conducted to monitor ovarian activity. Blood samples were collected and analysed for progesterone and LH, and faecal samples were analysed for progestin metabolites. One female showed cyclic ovarian activity during the study period, whereas the other female showed no evidence of ovarian activity. The cyclic Sumatran rhinoceros appeared to be an induced ovulator, the first of its kind reported within the Perrisodactyla. Ultrasound examinations of the ovaries revealed the formation of anovulatory haemorrhagic follicles when the animal was not mated. These follicles appeared to undergo varied degrees of luteinization that resulted in irregular faecal progestin profiles. When allowed to mate, the female showed a 21 day reproductive cycle that was reflected in both faecal progestin and serum progesterone profiles. The concentration of serum LH was baseline before mating, increased approximately 30-fold within 1–2 h of intromission and returned to baseline within 22 h. Ovulation occurred within 46 h of copulation. The female conceived three times during the study. Pregnancy was detected using ultrasonography 14–16 days after mating, and the concentration of both serum progesterone and faecal progestins remained high. Early embryogenesis appeared to be similar to that in horses. However, each pregnancy terminated unexpectedly within the first 3 months of gestation. This study demonstrates the important role that basic research and reproductive technology can play in developing a natural breeding programme for an endangered animal in captivity.

Introduction

Of the five rhinoceros species in existence, all but the South African white rhinoceros are considered endangered (IUCN, 1996). The decimation of this taxon can be attributed to previous uncontrolled hunting and current illegal poaching for the horn of the animal, which in some Asian cultures is believed to possess medicinal properties. The Sumatran rhinoceros (Dicerorhinus sumatrensis) is the most primitive of surviving rhino species and descended from the woolly rhinoceros that lived during the last ice age. The Sumatran rhinoceros is also the most endangered: there are fewer than 400 animals worldwide and populations continue to dwindle (Foos and Reece, 1998). A captive breeding programme was initiated for this species and from 1984 to 1996, 40 animals were placed in zoos and reserves worldwide after being rescued from snares or forests destroyed by logging (Foos and van Strien, 1997).

When allowed to mate, the female showed a 21 day reproductive cycle that was reflected in both faecal progestin and serum progesterone profiles. The concentration of serum LH was baseline before mating, increased approximately 30-fold within 1–2 h of intromission and returned to baseline within 22 h. Ovulation occurred within 46 h of copulation. The female conceived three times during the study. Pregnancy was detected using ultrasonography 14–16 days after mating, and the concentration of both serum progesterone and faecal progestins remained high. Early embryogenesis appeared to be similar to that in horses. However, each pregnancy terminated unexpectedly within the first 3 months of gestation. This study demonstrates the important role that basic research and reproductive technology can play in developing a natural breeding programme for an endangered animal in captivity.

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metabolites to characterize reproductive cyclicity and detect pregnancy (Schwarzenberger et al., 1993, 1998; Berkeley et al., 1997; Radcliffe et al., 1997; Patton et al., 1999). Data indicate a mean duration of the reproductive cycle of 25 days for the black rhinoceros, whereas data for the white rhinoceros are more difficult to interpret. According to studies involving faecal hormone analyses and behavioural data, both 30 day and 70 day reproductive cycles have been reported (Radcliffe et al., 1997; Schwarzenberger et al., 1998; Patton et al., 1999; Roth and Brown, 1999). In one case in which ultrasonography was used to assess ovarian activity directly while monitoring faecal progesterone metabolites, a cycle duration of 33 days was determined (Radcliffe et al., 1997). Reproductive cyclicity in the Indian rhinoceros has been studied by monitoring urinary hormone metabolites and matching data to behavioural oestrus (Kasman and Lasley, 1981; Kasman et al., 1986; Hodges and Green, 1989). In this Asian rhinoceros species, the duration of the ovarian cycle appears to vary significantly, ranging from 39 to 64 days (Kasman and Lasley, 1981; Roth and Brown, 1999). A recent report by Heistermann et al. (1998) determined that nearly all progesterone is metabolized and excreted in the faeces of Sumatran rhinoceroses. A 25 day reproductive cycle was described in a single female based on the number of days between two progestin peaks detected by faecal metabolite analysis (Heistermann et al., 1998). Together, these studies demonstrate species diversity with regard to reproductive cyclicity among Rhinocerotidae.

The present study was conducted at the Cincinnati Zoo and Botanical Garden on a group of three Sumatran rhinoceroses, the only animals of this species in captivity outside Asia. The overall aim was to characterize aspects of female reproductive physiology that would help in developing a successful natural breeding programme. Specific objectives were to: (i) characterize ovarian activity by ultrasonography; (ii) evaluate serum progesterone and LH concentrations during the oestrous cycle, mating and pregnancy; (iii) validate a faecal progestin metabolite assay for non-invasive monitoring of ovarian activity; (iv) develop a successful strategy for breeding the females naturally; and (v) document early pregnancy.

Materials and Methods

Animals

Three Sumatran rhinoceroses (two females, one male) were used in this 22 month study. Animals were on loan to the United States from the Indonesian Government and represent the entire population outside Southeast Asia. The male (SB no. 28) was caught in the wild as an adult and was estimated to be > 20 years old. One female (SB no. 27) from the Bronx Zoo was caught in the wild as an adult and was also estimated to be > 20 years old. The second female (SB no. 29) was caught in the wild as a juvenile and raised at the Los Angeles Zoo. Both females were transferred to the Cincinnati Zoo and Botanical Garden on breeding loan and were maintained there throughout the study.

Each Sumatran rhinoceros was provided each day with approximately 30–50 kg of fresh browse comprising up to ten types of Ficus and occasionally Kaffir plum, 2–3 flakes of hay (40% alfalfa, 60% orchard grass) and 1.8 kg of grain (ADF16; Mazuri, St Louis, MO). Diets were supplemented with fresh fruits and vegetables, and all animals had unlimited access to a mineral block and water. Each morning animals received 6 ml vitamin E supplement (Emcelle Tocopherol; 500 iu ml−1; Stuart Products Inc., Bedford, TX) orally in a banana. Animals were walked across a floor scale each day to monitor body weight. The average body weights for female no. 27, female no. 29 and the male were 600, 775 and 705 kg, respectively.

Animals were maintained individually in adjacent enclosures, except during mating introductions. Each animal had access to a stall within a heated barn. During the summer months, animals were maintained on display in an outdoor exhibit, but were allowed access to the barn at night. During the winter months, animals were allowed access to their yards (2000–2500 m2) for at least 1 h each day except during inclement weather (for example, a heavy snow fall, temperatures ≤ 4°C or severe wind chill). During the winter, the barn was heated (21°C) and animals were exposed to artificial lighting from 07:00 h to 20:00 h each day.

Animal introductions and mating

No behavioural or external signs of oestrus were observed in either female rhinoceroses. Introductions for mating were based on the presence of a preovulatory follicle identified during an ultrasound examination. After several months of conditioning, animals tolerated blood collection from the ear veins with a 23 g butterfly catheter attached to a 6 ml syringe. Thereafter, the progesterone enzyme immunoassay provided an additional tool for predicting the appropriate day of mating as serum progesterone concentrations could be determined within 8 h of blood collection. Animals were introduced when progesterone was baseline (usually < 0.05 ng ml−1) and a preovulatory follicle was present. After several matings, cyclic ovarian and endocrine patterns were established and could be used to predict the next mating after a non-conceptive copulation.

Ultrasonography

Female rhinoceroses were conditioned for several weeks to allow rectal ultrasound examinations. An Aloka 500 machine (Aloka, Wallingford, CT) connected to a video cassette recorder and a 5 MHz linear array probe (standard equine rectal probe) was used for all examinations. A Sony thermal printer (Aloka) was used to print the images. The examination followed a standard protocol in which the bladder, cervix, uterine body, uterine horns and both ovaries were examined. Ovarian follicles and
luteinizing follicles ≥ 10 mm in diameter were measured, and the presence of corpora lutea was recorded. Measurements were also taken of all embryonic vesicles observed. The entire examination typically lasted ≤ 10 min.

Each female was examined three to seven times each week for 6 months. After the first 6 months, female no. 27 was examined once a month for another 6 months, whereas female no. 29 was subjected to more intensive monitoring that continued throughout the 22 month study period (from day 1 to day 647; Fig. 1). Female no. 29 conceived on three occasions; however, none of the pregnancies developed to term. The first pregnancy lasted about 6 weeks and the female was examined three times each week. During the second pregnancy, the female was examined every 9–18 days for 12 weeks. After the third pregnancy was detected using ultrasonography, the female was not examined again until day 30 of gestation, when pregnancy loss was determined.

**Collection of blood and faecal samples**

For female no. 29, freshly defecated faecal samples were collected each day from day 1 to day 375 of the 22 month study period (Fig. 1). Samples were placed in plastic tubes and stored frozen (−20°C) until processed for progestin metabolite analysis. Blood samples were collected two to three times each week from August 1997 to June 1998 (from day 151 to day 480) and then each week until December 1998 (day 647; Fig. 1). For additional LH data, samples were collected each day from 5 February to 10 February 1998, and samples were collected twice (before and after mating) on two occasions when mating occurred (16 January and 8 August 1998).

For the older female (no. 27), blood sample collection was conducted two to three times each week for 5 months (from August 1997 to December 1997). Serum was obtained after centrifugation at 1315 g for 10 min and stored in 1 ml aliquots at −80°C until analysis.

**Serum progesterone enzyme immunoassay**

Chemicals and hormones were obtained from Sigma Chemical Company (St Louis, MO). Serum progesterone was extracted and concentrations determined by enzyme immunoassay following the protocol described by Munro and Stabenfeldt (1984) and using a monoclonal antibody produced against 4-pregnen-11-ol-3,20-dione hemisuccinate: BSA (provided by J. Roser, University of California, Davis, CA). Intra- and interassay coefficients of variation were 5.9 and 14.9%, respectively. Parallelism with the standard curve was confirmed by comparing dilutions (1:1, 1:2 and 1:4) of peak luteal phase serum samples with standard dilutions. Assay sensitivity was 0.05 ng ml⁻¹.

**Faecal progestin metabolite radioimmunoassay**

Faecal progestin metabolites were quantified using a radioimmunoassay as described by Brown et al. (1994). Faecal extracts were diluted (1:800 in PBS) and assayed (100 µl) in duplicate. Parallel displacement curves were obtained by comparing serial dilutions of pooled Sumatran rhino faecal extracts with progesterone standard preparations. Interassay coefficients of variation for two separate internal controls were 11.9% (n = 8) and 9.6% (n = 8). Intra-assay coefficients of variation were < 10% and assay sensitivity was 3.75 pg ml⁻¹.

**Serum LH radioimmunoassay**

Serum LH was quantified according to Brown et al. (1999) using a monoclonal anti-bovine LH antibody (no. 518-B7; provided by J. Roser, University of California, Davis, CA). The antibody bound 30–40% of the iodinated tracer with approximately 4% non-specific binding. The assay was validated by demonstrating parallelism between dilutions (neat; 1:16) of pooled rhinoceros serum and the standard curve, and significant recovery (> 98%) of exogenous ovine LH added to rhinoceros serum. Assay sensitivity was 0.3 ng ml⁻¹.

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**Fig. 1.** Schematic representation of the sampling schedule for female Sumatran rhinoceros (*Dicerorhinus sumatrensis*) no. 29 throughout the 22 month study (from day 1 to day 647). The study period encompassed 11 matings (●) and three pregnancies (□).
**Statistical analysis**

Both the size of the follicle and LH concentrations were analysed by one-way ANOVA and means within each data set were compared using a least significant difference test (Statview 5.0.1 Macintosh statistical software package) (Table 1). Faecal progestin metabolite and serum progesterone profiles were compared using Pearson’s correlation coefficient test. Data are presented as means ± SD.

**Results**

**Animal introductions and mating**

When introduced to the female, the male showed one of three types of behaviour within 30 min: (i) ignoring the female; (ii) following the female at a slow and steady pace; or (iii) chasing the female. On several occasions, the male followed the female and mounted her when she appeared to be receptive, only to dismount and immediately pursue her aggressively. On these occasions, the animals were separated until the next day when introductions typically resulted in slow steady following behaviour, mounting and copulation.

Copulation typically lasted 30–50 min and the animals were separated immediately afterwards. On two occasions, a second introduction was attempted on the same day but resulted in an aggressive chase. Therefore, in subsequent cycles animals were not reintroduced after a successful mating.

**Ovarian activity and progesterone**

In female no. 27, a large (8 cm × 10 cm) uterine mass was observed within the uterine body and the ovaries appeared small (approximately 2.5 cm × 4.0 cm) and inactive. Ovarian structures were not observed to be developing during the 12 months of monitoring. Serum progesterone concentrations in samples collected from August to December were consistently below 0.160 ng ml⁻¹ and often undetectable (< 0.05 ng ml⁻¹).

In contrast, in female no. 29, the ovaries were larger (approximately 4.5 cm × 7.0 cm) and were active throughout the study. Large follicles (> 50 mm circumference or > 15 mm diameter) were observed on both ovaries and were more abundant (P < 0.05) during periods of the study when mating was not occurring compared with periods during which the female was either mating at regular intervals or was pregnant (Table 1).

Corpora lutea were not observed during periods of the study when mating did not take place (days 1–188, 350–393 and 611–646). Instead, follicles grew to preovulatory size or greater (79.5 ± 11.0 mm circumference; n = 11). Upon ovarian ballottement (Ginther, 1995), echogenic specks were visible within the follicular fluid, and fibrinous quivering bands formed shortly thereafter. The follicle appeared web-like (Fig. 2a) for an average of 15.7 ± 4.6 days (ranging from day 11 to day 23) before it was no longer discernible on the ovary. During the initial period of the study when mating did not occur (March–August 1997; from day 1 to day 188) faecal progestin concentrations increased to > 10 µg g⁻¹ on several occasions. These high concentrations of progestins were similar to those associated with the luteal phase of the oestrous cycle after mating and ovulation. However, the changes in progestin concentrations during this period were more erratic and did not show a regular cyclical pattern that could be interpreted as the reproductive cycle of this female (Fig. 3a). Increased serum progesterone concentrations during intervals when mating did not take place were associated with the formation of anovulatory haemorrhagic follicles, but concentrations varied considerably and did not always reach the peak concentrations (> 1.0 ng ml⁻¹) observed during confirmed luteal phases. For example, progesterone concentrations increased only slightly to about 0.25 ng ml⁻¹ after the formation of anovulatory follicles from February to March 1998 (from day 350 to day 393) when the female was not paired with the male for mating. In contrast, the first large increase in progesterone (Fig. 3b) was associated with the formation of a large anovulatory follicle (Fig. 2a) and did not differ in

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Number of follicles (&gt; 15 mm)*</th>
<th>Number of days examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-mating and non-pregnantᵃ</td>
<td>2.40 ± 0.99d</td>
<td>134</td>
</tr>
<tr>
<td>Mating and non-pregnantᵇ</td>
<td>0.75 ± 0.63e</td>
<td>59</td>
</tr>
<tr>
<td>Pregnantᶜ</td>
<td>0.51 ± 0.67e</td>
<td>51</td>
</tr>
</tbody>
</table>

ᵃMean ± SD number of follicles on both ovaries.
ᵇIncludes ultrasound examinations before any mating activity and during intervals when animals were not placed together for mating due to inclement weather.
ᶜIncludes ultrasound examinations from the day of a conceptive mating until embryo loss was detected.
ᵈValues that have different superscripts within the columns are significantly different (P < 0.05).
concentration or duration from that produced later in the study after mating and ovulation. On the basis of the increase in progesterone associated with the presence of these structures and their close resemblance to the haemorrhagic and luteinizing follicles described in horses (Ginther, 1995), these follicles are referred to as luteinized follicles throughout the remainder of this paper.

On the day of mating, the largest (preovulatory) follicle was typically 20–25 mm in diameter (Fig. 2b), and serum progesterone concentrations were consistently low, often below the level of assay sensitivity (< 0.05 ng ml⁻¹; Fig. 3b). There were 11 successful or attempted matings during the study and all were associated with cyclical patterns of progesterone. Progesterone concentrations began to increase 3–5 days after mating, peaked at approximately 1.0 ng ml⁻¹ (1.25 ± 0.41 ng ml⁻¹) 11.6 ± 2.2 days after mating and decreased to undetectable amounts (< 0.05 ng ml⁻¹) by 19–21 days after mating if the female was not pregnant.

Although the faecal and serum progesterone profiles were positively correlated (P < 0.05) during the study period when the two types of sample were evaluated concurrently (Fig. 3c), the relationship was weak (r = 0.594). In general, there was a lag between the changes in serum progesterone concentrations and those of faecal progestins, which on occasion exceeded 48 h. Nevertheless, faecal progestin concentrations were consistently low at the time of mating and high during the luteal phase.

During two mating encounters, the male failed to achieve full penile intromission and ejaculation despite hours of mounting and attempted penetration. Nevertheless, ovulation was confirmed after all 11 mating encounters, whereas ovulation failed to occur on all three occasions when oestrus was predicted but animals were not paired for mating. The day of ovulation was determined after nine of the mating encounters and occurred by 22 (n = 1) or 46 (n = 8) h after copulation. Typically, only one ovary developed a preovulatory sized follicle, although on one occasion both ovaries developed a large follicle, which ovulated within 46 h after mating. Within 2–4 days after ovulation, a corpus luteum was discernible and often remained visible into the next follicular phase (Fig. 2c). Formation of a corpus haemorrhagicum by the ovulating follicle was not observed.

**Pregnancy detection and evaluation**

Early pregnancy in the Sumatran rhinoceros appeared similar to that in the domestic horse (Ginther, 1979, 1995) and white rhinoceros (Radcliffe et al., 1997). A 3–5 mm echogenic embryonic vesicle was detected using ultrasonography as early as day 14 after mating and was usually located in or near the uterine body (Fig. 4a). The embryonic vesicle was mobile within the uterine lumen and grew rapidly, expanding to 10 mm by day 16. The embryo developed from the ventral surface of the vesicle by day 21 (Fig. 4b). As the embryo grew, it rotated dorsally (Fig. 4c) before dropping down, suspended by the umbilicus, within the allantoic sac. A heartbeat was detected by day 26 and the fetal heart rate was 153 beats per min on day 52.
The first pregnancy occurred in September 1997 and was monitored closely until day 35 of gestation. The next evaluation (day 42) revealed embryo loss. The embryonic vesicle had collapsed and only remnants of fetal tissues were observed. The second pregnancy occurred in March 1998 and was examined using ultrasonography at 9–18 day intervals. By day 70, a completely formed fetus was visualized suspended from the umbilicus (Fig. 4d). On day 79, the fetus appeared normal showing limb mobility and a strong heartbeat. Examination on day 90 revealed no signs of a fetus or placental membranes. The only evidence that the female had been pregnant was a thickening of the uterine endometrium that slowly dissipated during the subsequent weeks. The third pregnancy (August 1998) was detected on day 16, and the female was not examined again until day 30 when the remains of a collapsed embryonic vesicle were observed in the caudal opening of the cervix.

Pregnancies were associated with an extended period of increased serum progesterone concentrations (pregnancies 1–3; Fig. 3b) and faecal progestins (pregnancy 1; Fig. 3a). The first two pregnancy losses were characterized by a decrease in progesterone concentrations accompanied by rapid follicular growth and re-mating within 2 weeks. Although the third pregnancy was lost by day 30, serum progesterone concentrations remained high for about 60 days. This pregnancy resulted from the only observed double ovulation. However, peak progesterone concentrations were not higher in the presence of two corpora lutea than with a single corpus luteum.

**Discussion**

A female Sumatran rhinoceros used in this study was found to be an induced ovulator. Although induced (or reflex) ovulation is not unusual it has not been documented in any other species of rhinoceros. Furthermore, this is the first report of induced ovulation within the Perissodactyla (Equidae, Tapiridae and Rhinocerotidae).

Similar to the domestic horse (Ginther, 1995), the Sumatran rhinoceroses developed large multiple antral follicles (> 50 mm in circumference and > 15 mm in diameter) on both ovaries; however, there were differences with respect to ovarian dynamics. For example, distinct follicular waves reported in horses (Ginther, 1993) were not apparent in the Sumatran rhinoceros during periods in which mating did not occur. Sumatran rhinoceros pre-ovulatory follicles (19–25 mm) were smaller than those reported in the horse (35–55 mm) (Ginther, 1995) and the African white rhinoceros (30 mm) (Radcliffe et al., 1997). Furthermore, follicles grew beyond preovulatory size when ovulation was not induced by mating. On the basis of these observations, it is apparent that attempts to breed the female when follicles reached 28–30 mm in diameter failed because the periovulatory phase and oestrus had passed. A somewhat similar observation has been reported in the dromedary camel. Preovulatory camel follicles are 9–19 mm in diameter, and larger follicles (> 30 mm in diameter) fail to ovulate after mating (Skidmore et al., 1996).

Luteinized follicles have been reported in horses, but are considered irregular and associated primarily with the transition from seasonal anoestrus to the spring breeding season (Ginther, 1990, 1995). In the present study, the female Sumatran rhinoceroses frequently developed luteinized follicles throughout the year. These follicles were determined to be anovulatory on the basis of (i) the ballottement test (Ginther, 1995); (ii) that collapse of the follicle was not observed; (iii) the follicle was ≥ 20 mm in diameter for at least 7 days; and (iv) tissue gradually displaced the fluid-filled centre over 11–23 days. These structures formed most frequently when animals were not mated and were usually preceded by the growth of a relatively large (≥ 80 mm circumference) follicle.

Although only one reproductively active animal was evaluated in this study, similar anovulatory haemorrhagic structures have been identified using ultrasonography in several Sumatran rhinoceroses in Indonesia and Malaysia, providing evidence that the formation of these structures is not an individual-specific characteristic (N. C. Schaffer, unpublished). However, the presence of anovulatory haemorrhagic structures may not be a normal occurrence but an artefact of captive management. Although luteinized follicle formation is not a common characteristic in most species, it has been reported in several induced ovulators (bulldog bats: Rasweiler, 1984; mink: Douglas et al., 1994; Zambian common mole-rats: Willingstorfer et al., 1998) and is thought to result from a lack of ovulatory stimulus. Wild female rhinoceroses are likely to mate and thus...
Fig. 3. Summary of the endocrine data from female Sumatran rhinoceros no. 29 during the 22 month study (from day 1 to day 647). (a) Faecal progestin metabolites measured from day 1 to day 375; (b) serum progesterone measured from day 151 to day 647 and (c) combined profiles where (a) and (b) overlap. Horizontal bars represent pregnancy intervals. Solid arrowheads indicate days of mating. Open arrows indicate the appearance of luteinized follicles.
ovulate when they come into oestrus. In contrast, captive animals are rarely in breeding situations and, therefore, ovulatory stimuli may be lacking. Mechanisms for inducing ovulation vary among reflex ovulators. Female rabbits will ovulate in response to another female mounting them (Goodman, 1998). In camels, seminal fluid induces ovulation (Chen et al., 1985). In cats, penile spines may contribute to the mechanical induction of ovulation (Aronson and Cooper, 1967), a theory supported by the fact that vaginal stimulation with a probe effectively induces ovulation in queens (Greulich, 1934). The stimulus for ovulation in the Sumatran rhinoceros appears to be associated with mating activity (mounting and attempted copulation), but does not require successful intromission as demonstrated on two occasions when the male failed to copulate fully, but the female ovulated. In both cases, although full penile penetration and ejaculation were not achieved, partial insertion of the penis into the vaginal opening and emission of fluid from the penis were observed. Therefore, both mechanical stimulation, caused by mounting and partial penile insertion, and seminal fluid exposure are possible mechanisms for inducing ovulation in this species of rhinoceros.

Blood was collected once or twice each day, so the exact timing of the LH surge relative to copulation was not characterized. However, it is clear that this species of rhinoceros differs from the horse, which shows an extended period of increased LH concentrations during oestrus and for several days after ovulation (Geschwind et al., 1975). Instead, the Sumatran rhinoceros appeared more like camels and cats, which experience a detectable increase in LH concentrations within 1 h after mating and an LH peak within 2–4 h (Concannon et al., 1980; Marie and Anouassi, 1986). LH concentrations were always baseline just before mating, increased up to 100-fold within hours after copulation and returned to baseline the next day. Therefore, in the Sumatran rhinoceros, a single mating appeared to elicit an LH surge that lasted < 22 h and induced ovulation.

Heistermann et al. (1998) reported a 25 day reproductive

Fig. 4. Ultrasound images of early pregnancy in Sumatran rhinoceros no. 29. (a) Day 14 after mating, arrow indicates 4 mm embryonic vesicle located within the caudal portion of the right uterine horn entering the uterine body; (b) day 21, the embryo (arrow) is visible developing from the ventral surface of the vesicle; (c) day 38, the embryo (arrow) is located dorsally within the vesicle; and (d) day 70, the fetus (arrow) is suspended vertically by the umbilicus.
cycle in a female Sumatran rhinoceros on the basis of faecal and urine endocrine monitoring over a 60 day period. The female rhinoceroses used in the present study mated with the male and ovulated at 21 day intervals, indicating a reproductive cycle similar to but slightly shorter than that reported by Heistermann et al. (1998). This small difference could be due to individual variation or the fact that the female in this study was mating. Regardless of this variation, it appears that the reproductive cycle of the Sumatran rhinoceros is more like that of the African black rhinoceros (25 days) (Schwarzenberger et al., 1993; Berkeley et al., 1997; Radcliffe et al., 1997) than the reproductive cycles of the African white (30–70 days) (Schwarzenberger et al., 1998; Patton et al., 1999) and Indian rhinoceroses (39–64 days), which are longer (Kasman and Lasley, 1981; Roth and Brown, 1999).

Both faecal progestin and serum progesterone profiles reflected physiological changes in the female reproductive status. Therefore, reproductive monitoring in this species could be conducted using faeces instead of serum. However, when the animals were not mating and luteinized follicles produced varied concentrations of progesterone ranging from 0.2 to > 1.0 ng ml⁻¹, the faecal progestin data were more difficult to interpret. If endocrine monitoring had been the only technique used in this study, the occurrence of induced ovulation may not have been detected. Fluctuations in progesterone concentrations, which are all characteristics of inactive ovaries, increased LH concentrations and baseline progesterone and successfully carried a term pregnancy (Berkeley et al., 1997). In addition, early embryo loss (by day 28) associated with endometritis has been documented using ultrasonography in a white rhinoceros (Radcliffe et al., 1997). However, the female Sumatran rhinoceros in this study was young, appeared healthy and did not show any uterine pathology. Furthermore, after the loss of the third pregnancy, the female experienced an extended luteal phase that lasted ≥ 30 days after embryo loss was detected. Therefore, at least in one case, premature luteolysis did not appear to be responsible for the failed pregnancy.

In pregnant mares, chorionic girdle cells invade the maternal endometrium 35–40 days after ovulation and form equine chorionic gonadotrophin (eCG)-secreting endometrial cups (Ginther, 1979). Serum concentrations of eCG reach peak concentrations about 60 days after ovulation. On the basis of DNA analyses, Sherman et al. (1997) suggested that pregnant African and Indian rhinoceroses do not produce a chorionic gonadotrophin. In support of this finding, data from the present study provide preliminary evidence that a chorionic gonadotrophin homologous to eCG is not produced by the Sumatran rhinoceros during early pregnancy. If the female Sumatran rhinoceros produced eCG, the LH assay, which uses an antibody to the LH/eCG β subunit, should have revealed an increase in eCG concentrations during the pregnancy that was sustained until day 79 of gestation.

The older female Sumatran rhinoceros used in the present study appeared infertile. The female had small inactive ovaries, increased LH concentrations and baseline progesterone concentrations, which are all characteristics of reproductive senescence (Vom Saal et al., 1994). In addition, the uterus contained a large mass. Unexplained uterine pathology has been detected in several Sumatran rhinoceroses in Malaysia (Schaffer et al., 1994; Kahn et al., 1999) and is becoming a serious concern.

Results from the present study provide an insight into several aspects of the reproductive physiology of the Sumatran rhinoceroses. Combined data from ultrasound and

![Fig. 5. Serum LH concentrations for female Sumatran rhinoceroses no. 29 (●) and no. 27 (□). Solid arrowheads indicate blood samples collected from no. 29 after mating (1–4 h after copulation). Open arrows indicate blood samples collected 1–3 h before mating.](image)
endocrine analyses provided evidence of patterns of follicular growth and induced ovulation and enabled documentation of early pregnancy for the first time in this species. Furthermore, the combined use of ultrasonography and progesterone monitoring proved a successful repeatable method for predicting oestrus and largely reduced the risk of aggressive interactions associated with introducing males to non-oestrous females.

The authors thank the Los Angeles and Bronx Zoos for loaning their female rhinos to the Cincinnati Zoo for breeding. The authors also extend their tremendous gratitude to the rhino keeper staff of the Cincinnati Zoo and Botanical Garden, especially P. Reinhardt, S. Yelverton and T. Tenhenfield for their dedication to the animals and for providing critical technical support throughout the project. The authors are also grateful to the Cincinnati Zoo Volunteer Observers for diligently recording animal behavioural data during introductions. Appreciation also goes to E. Maruska, Executive Director of the Cincinnati Zoo and Botanical Garden, and T. Foose, Asian Rhino Specialist Group Program Officer and International Rhino Foundation Program Director, for their helpful input on this project and long-term commitment to saving this species from extinction. This research was supported in part by the International Rhino Foundation and the Center for Research of Endangered Wildlife’s C. and E. Lindner Post-doctoral Fellowship.

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