Zainal Zahari ZAINUDDIN

Main Thesis

CHAPTER I

INTRODUCTION

The family *Rhinocerotidae* consists of five species of heavy land mammals found in the world today. They are characterized by a long nasal "horn" or "horns" which are not true horns but formed of a mass of compacted hairs. Rhinoceroses are bulky animals with thick, hairless skin, often falling in heavy loose folds. They live in transitional habitat between open grassland and high forest, grazing or browsing on the bushes or shrubs in Africa and Asia. All species are on the verge of extinction because of the conflict with land development.

The African species are the Black Rhinoceros (*Diceros bicornis*) and the White Rhinoceros (*Ceratotherium simum*) whereas the Asian species are the Indian or the Greater One-Horned rhinoceros (*Rhinoceros unicornis*), the Javan or the Lesser One-Horned rhinoceros (*Rhinoceros sondaicus*) and the Sumatran Rhino (*Dicerorhinus sumatrensis*). The Asian species are probably three of the rarest mammals in the world with the Javan and Sumatran being in imminent danger of extinction.

The rarity of the rhinoceros has been attributed to two factors. Firstly, the rhinoceros has been hunted for its horn, hide and blood for many centuries. Contrary to popular belief, rhino horn is not mainly used as an aphrodisiac but as cure to illnesses such as headaches, high fever and arthritis. Secondly, the extensive habitat destruction from

logging and forest clearance for agricultural development has isolated the small populations and reduced the amount of suitable habitat.

The world distribution of the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is presented in Plate 1. It is the species found in Peninsular Malaysia and is the smallest of all the rhino species, measuring a height of 1.2 m at the shoulder and covered with a fine coat of hair. Both female and male have two black anterior and posterior horns. In 1980, the population of Sumatran rhinoceros was estimated at 50 to 75 in Peninsular Malaysia.

Prospects for the long-term survival of the Sumatran rhinoceros in Malaysia are poor. The loss of adult animals to poaching and the almost complete lack of reproductive success are the immediate problems. The successful breeding of the Indian rhino in 1956 and 1958 at the Basel Zoo (Switzerland) has focused the role that zoos could play in preserving the rhino from extinction.

Realising that the Sumatran rhinoceros is on the verge of extinction, the Malaysian Department of Wildlife and National Parks initiated a long-term study in the 1980s for the conservation of the Sumatran rhinoceros. One of the strategies was to breed them in captivity.

Despite the dangers and difficulties of capturing the already threatened species, the Malaysian Department of Wildlife and National Parks acquired its first Sumatran rhinoceros in April, 1984. Since then, a total of 10 more animals have been recruited. Of the captive rhinoceroses in West Malaysia, one male and two females have died. But improvements in management, feeding and veterinary health care have helped to stabilize the captive population with no further mortalities.

Plate 1. Distribution of Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)
Map Credit: Francesco Nardelli

As of 1994, one male and six females form the captive breeding herd in West Malaysia. In May, 1987, a rhino gave birth at Zoo Melaka from a mating in the wild before its capture. Four unsuccessful matings of three female rhinos have been recorded in the captive breeding herd.

Although information on the reproductive biology is available for the Black, White and Indian rhinoceroses both in the wild and in captivity, similar information on the Javan and the Sumatran rhinoceros is lacking. This lack of information may be due to the difficult nature of their habitat, low population density and small number in captivity.

Successful breeding of the Sumatran rhinoceros in captivity has not been reported in any zoo in the world, highlighting the need for research on several aspects of the reproduction in both sexes. There are many gaps in our understanding of the reproductive biology of the Sumatran rhinoceros. In the long-term, the following questions need to be addressed if breeding in captivity is to be successful.

Is there a seasonality in reproduction?

- Can sexual receptivity be recognized?
- Is the male sexually aggressive towards the female?
- Can female reproduction be artificially controlled?
- How can pregnancy be diagnosed?
- Can semen be collected for long-term storage?

The ability to handle adult Sumatran rhinoceros in captivity in Malaysia has opened up a unique opportunity to apply hormonal assays, ultrasonography and semen analysis, routinely performed in domestic species. These diagnostic tests could provide valuable information to ascertain the success or failure of the Sumatran rhinoceros to breed in captivity. In the short-term, studies are needed on: the anatomy of the male and female reproductive organs; signs of oestrus (sexual receptivity) and monitoring the phases of the cycle; mating behaviour; and semen collection and evaluation

Objectives

A study was conducted in a captive group of Sumatran rhinoceroses with the following objectives :-

1. to describe the anatomical features of male and female genital organs;
2. to document male and female reproductive behaviour;
3. to monitor ovarian activity; and
4. to develop a technique of semen collection and evaluation.

CHAPTER III

MATERIALS AND METHODS

This study was conducted from 1990 to 1994 at Zoo Melaka, Melaka and the Sumatran rhinoceros Breeding Centre in Sungai Dusun, Selangor, Malaysia. Included in this study were seven wild-caught females, three wild-caught males and one captive born female Sumatran rhinoceros (*Dicerorhinus sumatrensis*). Their case histories are presented in Table 2.

General Management

Facilities

Zoo Melaka

The Sumatran rhinoceros enclosures at Zoo Melaka were circular and divided into 8 pie shaped units. Each unit consisted of a night stall (4 m x 4 m) and a connecting conical outdoor paddock.

Each paddock was 17.5 m long with the widest end measuring 23.8 m and the narrowest 4.6 m. The night stall was equipped with an overhead shower, light source, a water trough and a wooden feeding trough. The floor was of non-slipped tiles. Shade

trees (Angsana, *Pterocarpus indicus*) were planted within the paddock. The earth substrate in the outdoor compound was planted with carpet grass (*Axonopus compressus*) and Napier (*Pennisetum purpureum*).

Sungai Dusun

The enclosures at this facility consisted of a pie shaped enclosure with 8 night stalls in the centre, each connected to a paddock. One paddock was modified to contain a crush that measured 2.5 m long by 1.2 m wide by 1.5 m high. The crush was made up of vertical galvanised iron pipes, 4-inch diameter, on either side cemented into the floor. The front consisted of a double-leaf swing gate made up of 2-inch diameter metal pipes. The rear entrance consisted of three removable 4-inch diameter galvanised iron pipes that were slotted into the cemented flooring.

Feeding

All animals were fed on forages (30-40 kg daily), consisting of Kelompong Hijau (*Ficus variegata*) Tapak Gajah (*Macaranga gigantea*) Tapai (*M. triloba*) and Nangka (*Artocarpus rigidis*), fruits, vegetables, sweet potatoes and 3 kg concentrates (50 % Dairy Cattle Conditioner pellets and 50 % Pig Starter pellets) were also provided daily. The concentrates were mixed with 1-1.5 l of water and were hand-fed. Clean water with added vitamin and mineral supplements (Stresspak) was available *ad libitum*. Animals were let out into their respective paddocks, each of which had a mud wallow and a pool during the day and penned in individual stalls at night. Within the night stalls, animals were sprayed with water to induce defecation and to remove mud from the paddock.

Table 2

Case Histories of Sumatran Rhinoceroses

Given name	Breeding number	Sex	Capture date	Capture location	Comments
JERAM	ZM 1	F	30/04/94	Selangor	Adult (90 kg)
RIMA	ZM 4	F	15/12/85	Johor	Pregnant at capture
NAPANGGA	ZM 5	M	15/06/86	Sumatra	Adult
JULIA	ZM 6	F	06/07/86	Selangor	Adult
PANJANG	ZM 8	F	25/02/87	Selangor	Adult
MINAH	ZM 9	F	23/05/87	Melaka	Born in captivity; dam ZM4; sire wild
SRIDELIMA	ZM 10	F	01/07/87	Selangor	Adult
TANEGANG	SP 1	M	14/07/87	Sabah	Adult
MAS MERAH	ZM 11	F	26/08/87	Selangor	Adult
SHAH	ZM 12	M	01/03/87	Selangor	2.5 y* (446 kg)
SEPUTIH	ZM 13	F	11/07/86	Pahang	Adult (680 kg)

* estimated age

Female Reproduction

This study describes the gross anatomy of reproductive system, the ultrasound imaging of the uterus and ovaries and ovarian function.

Female Genitalia

Gross Anatomy

The anatomy of the reproductive system was based on 2 post mortem specimens of adult females (ZM 10 and ZM 6) which died in captivity. The reproductive tracts were examined grossly, measured and photographed. The description of the external genitalia was based on five live specimens (ZM 1, ZM 4, ZM 8, ZM 9 and ZM 11).

Ultrasonography

Ultrasonography was performed on six females (ZM 1, ZM 4, ZM 8, ZM 9, ZM 11 and ZM 13) and a male (ZM 12) using a real-time ultrasound machine (Aloka Echo Camera, Model SSD-210 DX II, Tokyo, Japan) with 5 MHz linear transrectal probe. All the animals were scanned in the restraining chute both at Zoo Melaka and Sungai Dusun. Prior to scanning, each animal was sprayed with water to initiate defecation and to remove mud or faecal materials from their bodies. The animal were then baited into the chute and secured with three 4 inch galvanized iron pipes inserted into the floor.

A well-lubricated gloved hand was inserted into the rectum to remove faeces, before the 5 MHz probe was slipped under the operator's hand into the rectum (Plate 2a). The ultrasound probe was then moved cranially to image the bladder, cervix, uterine

body, uterine horns, left ovary and right ovary. The structures were measured with the built-in callipers.

Ovarian Function

Ultrasonography

Using ultrasound imaging procedures as for the reproductive tract, the ovaries were scanned. Ovarian follicles and corpora lutea were measured (diameter) with the built-in callipers and photographed. Constant monitoring of the follicles was not possible due to the long distance to the facility and the availability of the echocamera. The difference in sizes of the follicles within an animal over a period of 1-30 days was recorded.

Progesterone Radioimmunoassay

Blood was collected by restraining the female in the chute. The ventral surface of the tail base was washed thoroughly and swabbed with 70% alcohol. The middle coccygeal vein was punctured with an 18 gauge needle which was attached to a winged infusion set (18G). As blood was visible in the tubing, the other the end of the infusion was inserted into a heparinized vacutainer tube (Plate 2b). Samples were collected at 3 to 4-day intervals for a two months from female ZM 8. Blood was also collected from female ZM 11following intramuscular administration of 3000 i.u of Pregnant Mare Serum Gonadotrophin (PMSG, Folligon, Intervet, Holland).

Blood was centrifuged, within 30 minutes of collection, for 15 minutes at 2500 RPM, plasma aspirated and stored in a labelled vial at -20° C until assayed.

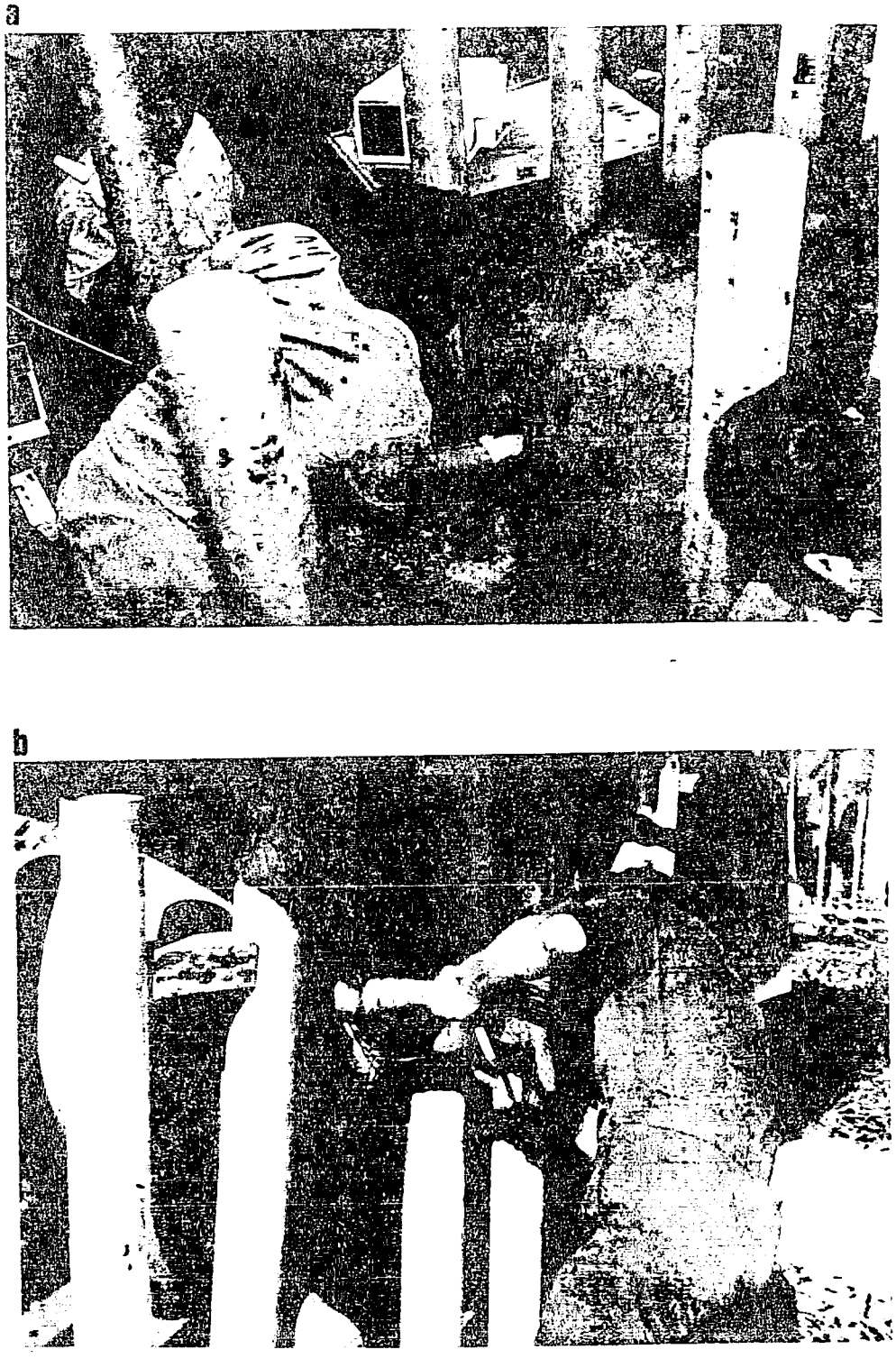

Plate 2. A Female Sumatran Rhinoceros being Subjected to (a) Ultrasonography and (b) Blood Collection from the Middle Coccygeal Vein.

Plasma progesterone concentration was quantified by a radioimmunoassay (RIA) using antibody (rabbit anti-progesterone serum against 11 alpha-succinyl bovine serum albumin conjugate) coated tubes and ^{125}I progesterone as the radioligand. The assay was performed as described in the IAEA manual (International Atomic Agency, 1984). The sensitivity of the assay was 0.1 ng/ml and the intra- and inter-assay coefficients of variations were 9.6% and 5.6% respectively. The progesterone radioimmunoassay (P4 RIA) was carried out at the Reproduction Laboratory, Department of Veterinary Clinical Studies Universiti Pertanian Malaysia, Malaysia.

Male Reproduction

Male Genitalia

Gross Anatomy

The anatomy of the external genitalia of the male Sumatran rhinoceros was studied in adults (ZM 5 and SP 1), estimated to be between 10 and 15 years old, and a subadult ZM 12) estimated age 2 1/2 years. The age of the subadult male, was on a comparison of the body weight of the captive-born animal (ZM 9) at Zoo Melaka. The examinations were done with the animal either standing in the chute or on lateral recumbency in the night stall. The penis was manipulated out of the prepuce by gently lubricating the lateral surfaces of the glans and body of the penis and slowly drawing it caudally. The scrotum was palpated for the presence of the testes. Measurements of the testes, penis, lateral projections, distances between anus-testes and testes-prepuce were taken using callipers (Mitutoyo, Japan) and a measuring tape (Stanley, USTM Reg.

1.217.360). The development of the penis and testes of the subadult male ZM 12 was monitored from recruitment at 2 1/2 years until it was 6 years old.

Ultrasonography

Ultrasonography was performed on one male (ZM 12) using the Aloka Echo Camera (Model SSD-210 DX II) with a 5 MHz linear probe. The scrotum was scanned with the animal standing in the chute. The testes was held in a dorsoventral position and the transducer moved along the dorsal surface of the scrotum to view the testis and epididymis. The prostate, seminal vesicles and the bulbo-urethral glands were imaged per rectum using the same transducer. The ultrasonic images were videotaped and printed using a thermal printer (Sony UP-860 CE).

Semen Collection and Evaluation

Semen collection and evaluation were conducted on ZM 12 (4 to 6 years of age) during the period 1991 to 1994, at the Sungai Dusun Sumatran Rhinoceros Breeding Facility. The semen collections were carried out in the chute. On several occasions rectal massage preceded penile massage. A well lubricated gloved hand introduced into the rectum was used to massage the accessory glands situated about 10 cm cranially from the anal sphincter. A rhythmic stroking was initiated by applying side to side pressure on the floor of the rectum in the region of the prostate and bulbo-urethral glands. The massage was applied for 5 minutes intervals followed by a rest of 1 to 2 minutes resting (Schaffer et al., 1990). The total time did not exceed 20 minutes. The penis was massaged with a well-lubricated gloved hand from the umbilicus to the prepuce. by an operator kneeling at the rear of the rhinoceros. Subsequently, the prepuce was to reveal the tip of the penis

and later the glans penis. With commencement of erection, the penis protruded with exposure of the two lateral projections. The penis was cleaned and held firmly with one hand immediately posterior to the lateral projection while the other hand firmly massaged the area anterior to the projections (Plate 3). The animal's response to the manipulation of the glans penis was indicated by a rhythmic lifting of the tail which was synchronous with the massaging.

The penis was continuously lubricated to reduce abrasions. The engorgement of the superficial vessels and stiffening of the penis with straightening of the flexure indicated imminent ejaculation. After 20 to 40 minutes of penile massage, semen either in drops or jets, was collected into a calibrated tube which is kept at 37 to 40°C. The evaluation of the semen includes volume, colour, pH, motility, live/dead ratio, abnormalities and concentration.

The artificial vagina (AV) developed for the Indian rhinoceros (Schaffer et al, 1990) was used. It consisted of a cylindrical double layered rubber sleeve with a conical end on one side (Figure 1). A calibrated tube is fitted at this end for the collection of semen. A screw capped opening was situated on the side for filling up with water. Penile massage was carried out prior to introducing erected penis into the AV which was filled with water at 38 to 40°C and lubricated with methyl cellulose. Stroking of the penis anterior to the lateral projections was continued until the animal responded by several forward thrusts which eventually resulted in ejaculation.

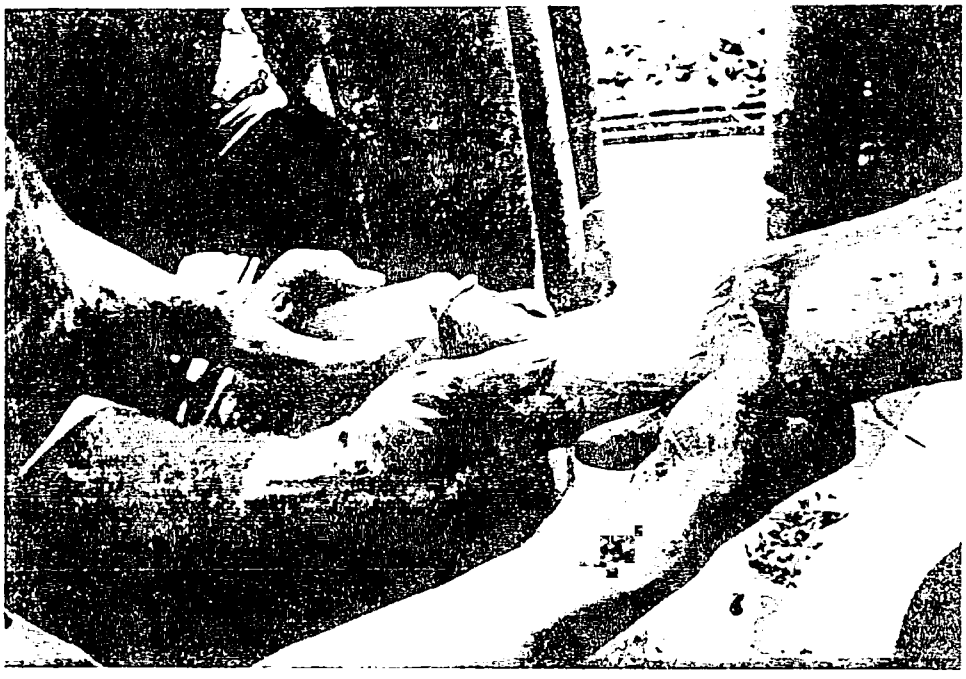

Plate 3. Semen Collection by Penile Massage in the Sumatran Rhinoceros

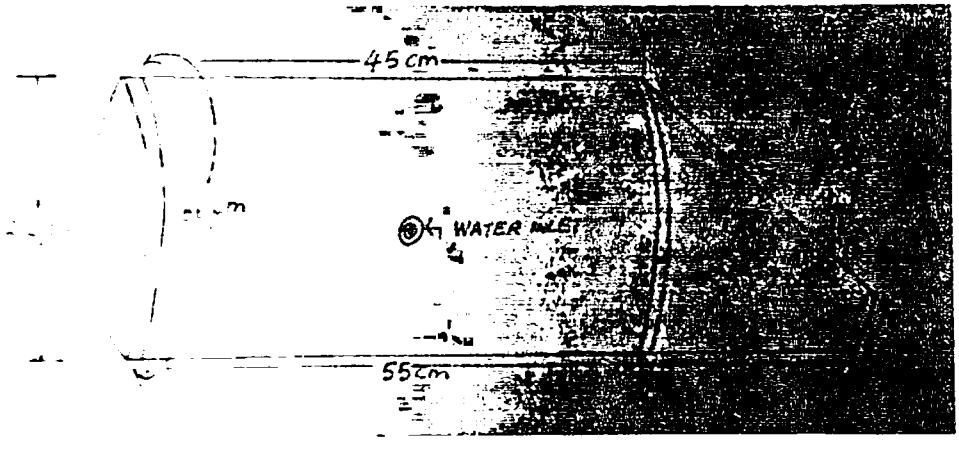

Figure 1. Diagram of an Artificial Vagina for Semen Collection in the Sumatran Rhinoceros

Reproductive Behaviour

The reproductive behaviour was studied by introducing the male to the female's outdoor paddock for a period of 1 to 2 hours every morning until behavioural oestrus (standing to be mounted) was noted. Once the day of oestrus was established, each female was tested for oestrus daily about three weeks from the previous oestrus. In severe cases of aggression, the animals were separated after one hour.

The behavioural parameters studied were: (a) precopulatory behaviour—vocalization, tail raising, urination and contact promoting behaviours with the head (snout) involving the flank, hind legs, neck, head, rump, perineum and external genitalia; (b) copulatory behaviour—penile exposure, erection, mounting and dismounting. The frequency, duration and the postures were recorded during mating.

The prepartum behaviour and parturient behaviour of ZM 4 was documented.

CHAPTER IV

RESULTS

Female Reproduction

Female Genitalia

Gross Anatomy

External genitalia In the live animals (ZM1, ZM4, ZM8 and ZM9), the labium consisted of an elongated vertical structure, with a deep convex groove on either side of the vulval opening. The average width and length in an adult were 8.1 cm and 6.9 cm, respectively. The vulval lips were thick, greyish to grey, wrinkled and densely covered with coarse hair. The dorsal commissure was rounded and the ventral commissure tapered to form a convex structure which slightly protruded caudally (Plate 4a).

The clitoral fossa was situated 2 to 3 cm cranially from the ventral commissure. A deep central depression courses cranially from the clitoral fossa. The clitoris was short, broad, 1.0 to 1.5 cm in diameter and was flattened dorsoventrally. The glans forms a pointed projection over the clitoral fossa.

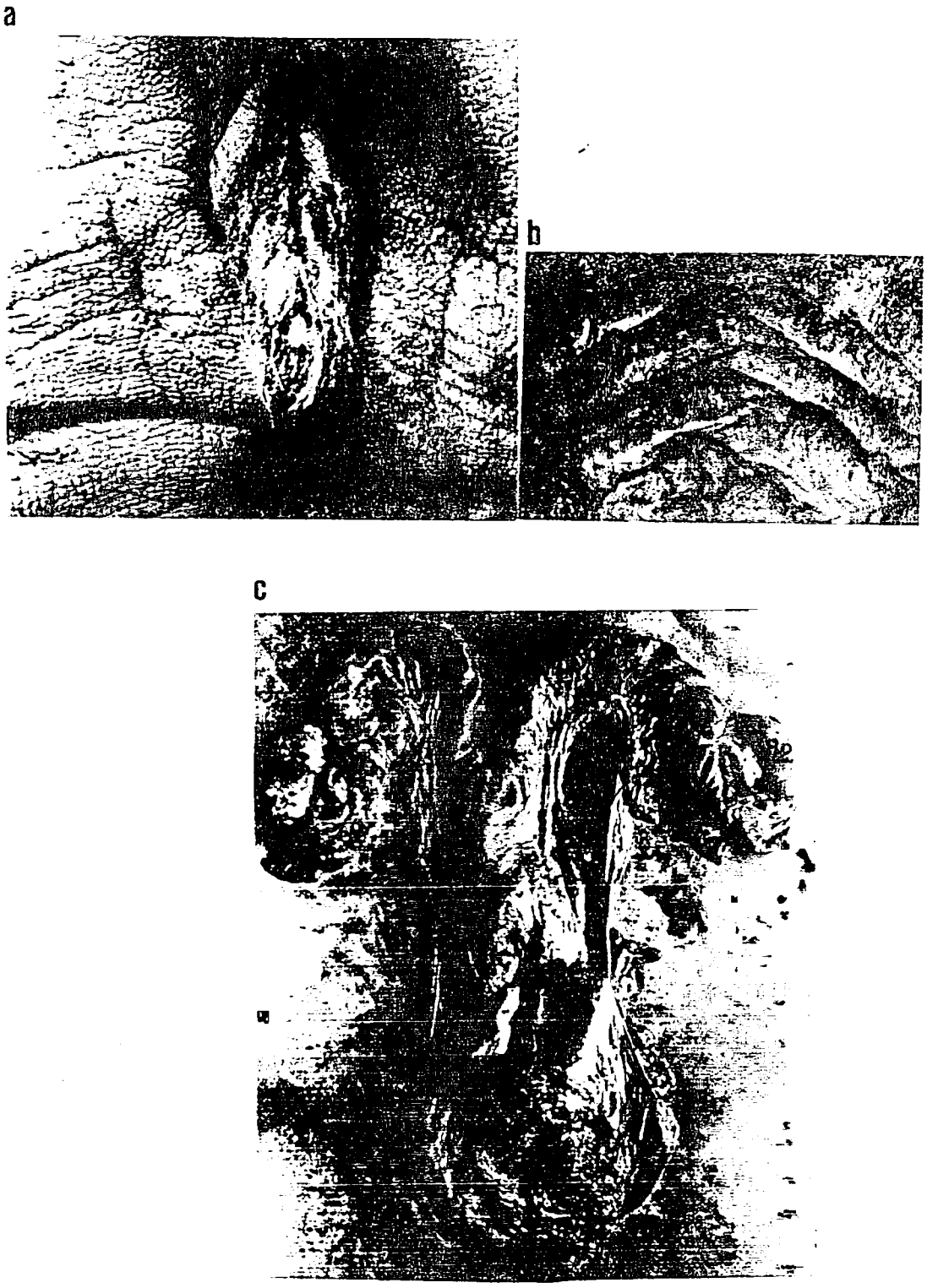

Plate 4. **Reproductive Tract of an Adult Female Rhinoceros.** (a) External Genitalia; (b) Transverse folds in the cervix; (c) Reproductive Tract, *V*, Vagina; *C*, Cervix; *U*, Uterine Body; *Uh*, Uterine body; *Uh*, Uterine Horn, *RO*, Right Ovary; *LO*, Left Ovary

Vagina and Cervix In the autopsied rhinoceros (ZM 6 and ZM 10), the vagina was 17.0 to 18.5 cm long and 5.3 to 7.6 cm in diameter, consisting of a thick muscular coat. The cervix was 5.6 to 7.0 cm long projecting into the vagina with 4 to 5 folds arranged transversely with increasing thickness cranially (Plate 4b).

Uterine Body The body of the uterus was relatively short measuring 3.5 to 4.2 cm in length and 2.3 to 3.1 cm in diameter. Grossly, the uterine horn was 30 to 34 cm long and blunt at the cranial end. Measurement of the reproductive tract of two female Sumatran rhinoceroses are presented in Table 3. The cervix, uterus, fallopian tubes and ovaries are illustrated in Plate 4c.

Uterine horn. Each horn traversed cranially and curved laterally before running caudally. Both uterine horns were suspended within the abdominal cavity by the broad ligament.

Fallopian Tubes. The broad ligament supporting the ovary and uterine horn formed a deep ovarian bursa in which the tortuous oviduct traversed through to end at the infundibulum. The fallopian tubes were long (> 20 cm when uncoiled), relatively narrow and flexuous. The fimbriated extremity covered the ovary (Plate 5a).

Ovaries. The ovaries were flattened and elongated. They were either oval, triangular or kidney shaped and were enclosed in the ovarian bursa (Plate 5b,c). In two females autopsied, they averaged 8.2 cm by 4.0 cm in length and width, respectively and weighed 70 to 100 gm. They were located 40 to 58 cm from the vulva. The ovaries were situated in the broad ligament and were attached to the sublumbar region. The tunica albuginea covering the ovary consisted of a thick and tough layer. The ovarian follicles and corpora lutea were seen to protrude from the surface of the ovary.

Table 3

Dimensions of the Female Reproductive Tract and Ovaries of Two Sumatran Rhinoceroses (Identification Numbers ZM 6 and ZM 10)

Structure (cm)	Animal Identity	
	ZM 6	ZM 10
Vagina		
length	18.5	17.0
width	7.6	5.3
Cervix		
length	7.0	5.6
Uterine horn length		
left	34.0	30.0
right	33.8	30.0
Uterine body		
length	4.2	3.5
width	3.1	2.3
Ovaries (length and width)		
left	8.4 x 3.8	8.0 x 3.0
right	8.2 x 4.6	8.0 x 4.5

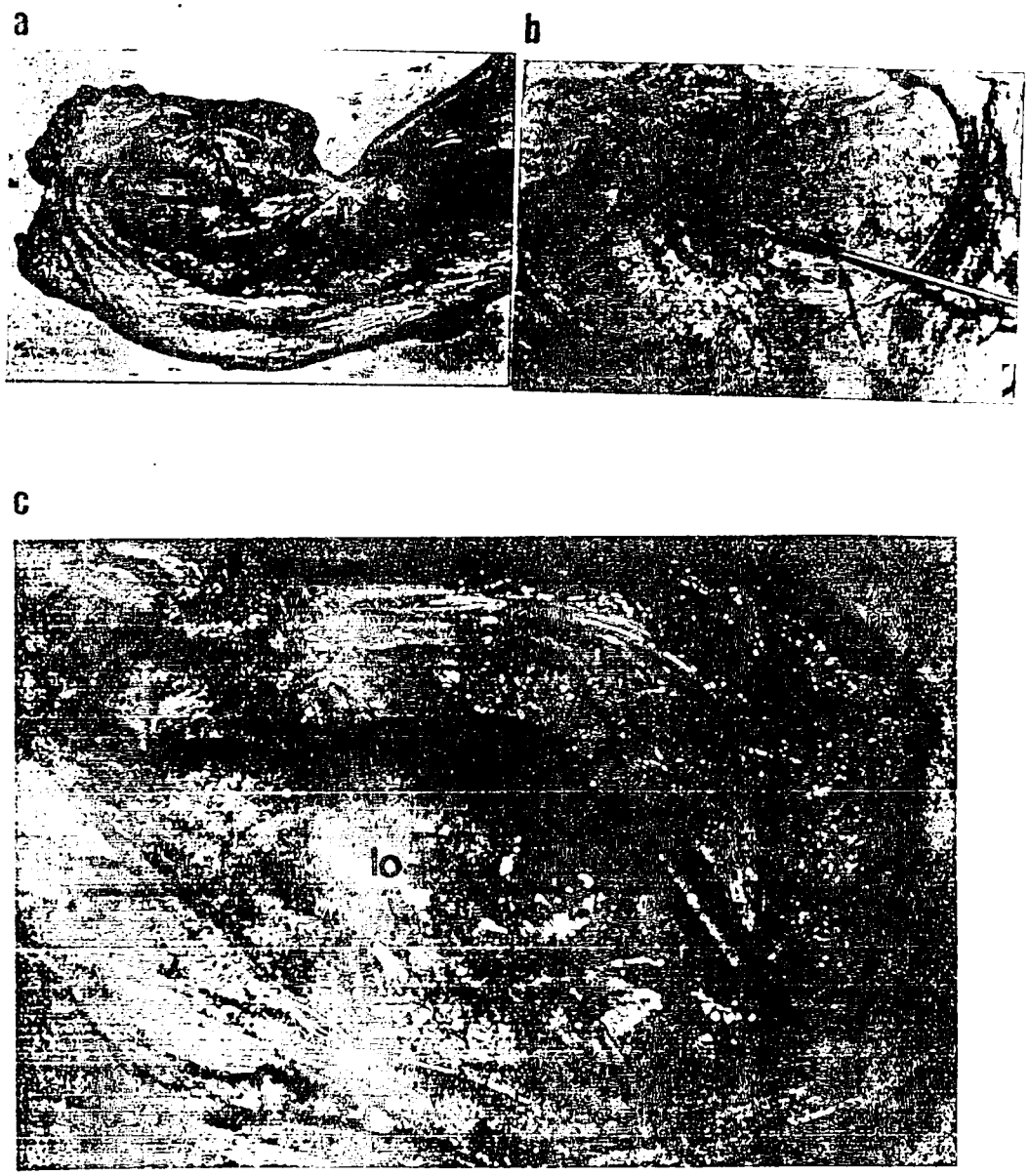

Plate 5. **The Relationship of the Fallopian Tube to the Ovary in the Sumatran Rhinoceros.** (a) The Fimbriae Covering the Ovary (→); (b) A Catheter Inserted into the Ostium of the Ovarian Bursa (→); (c) Left ovary (lo) exposed by deflecting the fimbria (f)

Ultrasonographic Images

The vagina is situated dorsal to the urinary bladder, primarily extending along the neck of the bladder. It was viewed as a mass of tissue, 2.5 to 3.0 cm in thickness caudally, decreasing to 1.7 cm immediately beyond the neck of the bladder.

The bladder was distinguished by its thin wall and hyperechogenic contents (Plate 6). The cervix was mostly located on the dorsal surface of the urinary bladder, often extending dorso-cranially, immediately over the pelvic brim. The cervix consists of a very dense series of alternating annular folds, reflected as hyperechogenic and hypoechogenic contours (Plate 6). The cross section of the cervix tapered from 5 cm, cranially to 1.5 cm, caudally. The annular folds started caudally as simple elongated interlocking projections which progressively became more inter-twined around the cervical canal, cranially. All the cervix examined were tightly closed.

Plate 6. **A Composite Ultrasonographic Image of the Cervix and Uterine Body of the Sumatran Rhinoceros.** *Ce*, Cervix; *Ut*, Uterus; *Ub*, Urinary Bladder; *Af*, Annular Folds

The uterus was located dorso-cranially to the urinary bladder, anterior to the cervix. Ultrasonic images of the uterus were seen as a rounded to oval thick walled structures with a transverse diameter from 1.6 to 2.5 cm, extending in length from 2.3 to 4.5 cm. A thick 0.5 cm layer of myometrium surrounded the uterus (Plate 7a). The endometrial layer of the uterus was seen as a dense structure with hyperechogenic specks ranging from 0.1 to 0.4 cm in diameter.

The uterine horn consists of a circular transverse section with a diameter of 1.5 cm. The layers of myometrium measured 0.1 to 0.4 cm in thickness. The inner segment of the uterine horn constituted a dense mass with hyperechogenic specks, reflecting the folds of the endometrial mucosa (Plate 7b). The perpendicular length of the uterine horn as measured between the uterine body and the ovary ranged from 15.5 cm in the smaller individuals (500 - 600 kg body weight) to more than 25 cm in larger animals (> 700 kg).

The ovaries were located 50 to 58 cm anterior to the vulva. The ultrasonographic image demonstrated an elongated oval to triangular outline . Ovaries measured 5.9 to 8.0 cm and 2.9 to 5.0 cm in length and width respectively (Table 4). The sizes of the ovaries were relatively larger during the later period of follicular development.

Ovarian Function

Ovarian follicles (Plate 7c), were distributed over the ovarian surface in all females examined by ultrasonography; they measured 0.5 to 3.2 cm in diameter and (Table 5). Corpora lutea were observed in some individuals, being ovoid and containing dense mass with hyperechogenic center (0.1 to 0.3 cm diameter)measured 2 to 3.5 cm by 1.3 to 1.9 cm in length and width respectively (Plate 7d).

Table 4

Size of Ovaries in Six Sumatran Rhinoceroses*

Animal Identity	Left ovary (cm) (l x b)	Right ovary (cm) (l x b)
ZM 1	7.5 x 4.3	7.2 x 3.2
ZM 4	7.0 x 4.5	6.3 x 3.4
ZM 8	7.5 x 3.8	8.0 x 4.0
ZM 9	5.9 x 2.9	6.6 x 3.8
ZM 11	7.8 x 3.8	8.0 x 5.0
ZM 13	7.3 x 4.5	7.5 x 3.9
Mean	7.2 (± 0.7)x 4.0 (±0.6)	7.3(±0.7) x 3.9 (±0.6)

*Determined by Ultrasonography

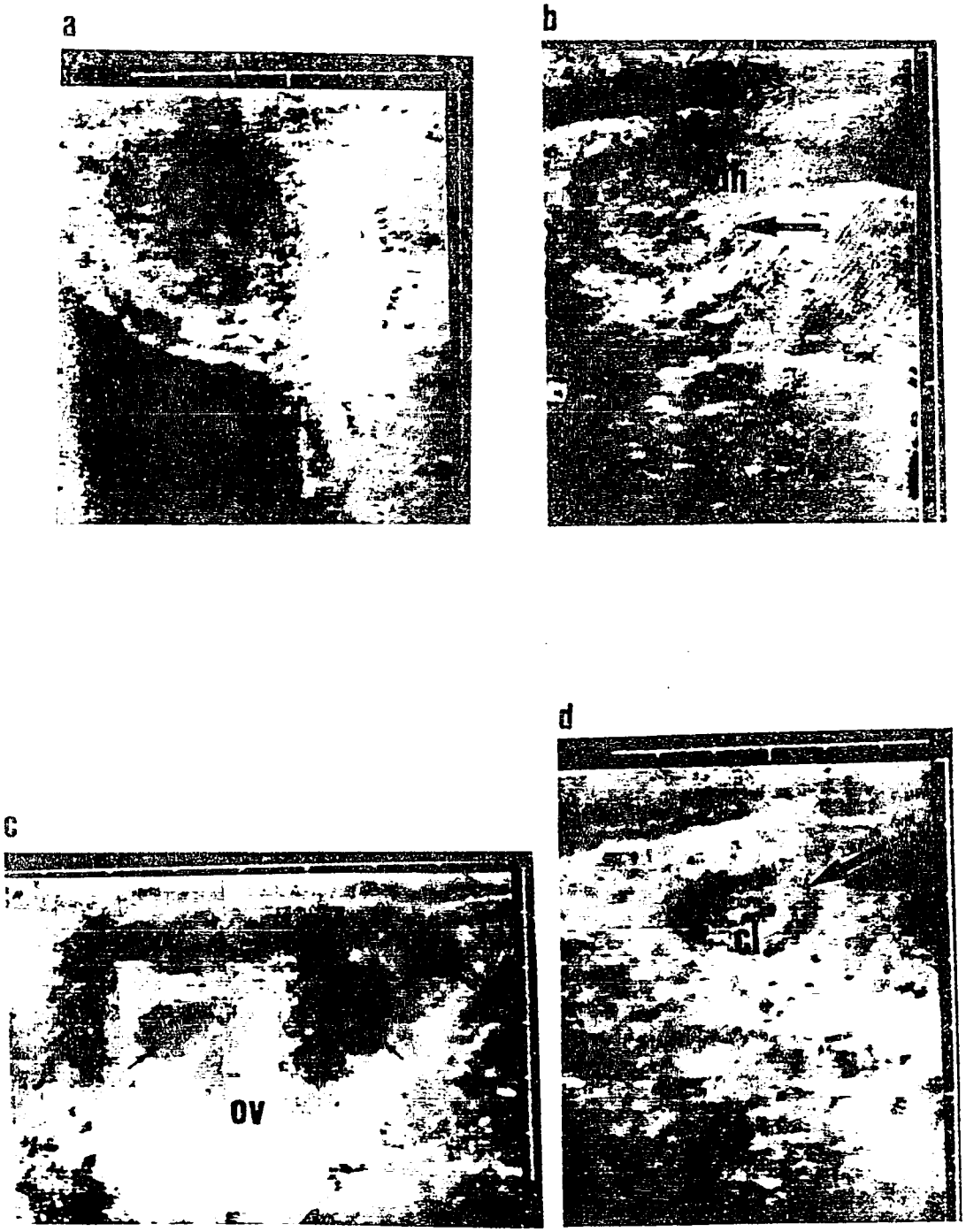

Plate 7. **Cross-sectional Ultrasonographic Images of Female Internal Genitalia of the Sumatran Rhinoceros.** (a) Uterine Body ↗, Urinary Bladder, *uh*; (b) Uterine Horn, *uh*; (c) Ovary, *ov*; Ovary Showing Follicies ↗; (d) Ovary Showing a Corpus luteum, *cl*

Table 5

Number and Sizes of Ovarian Follicles in Six Sumatran Rhinoceroses*

Animal Identity	No of Ovarian Follicles (diameter in mm)	
	Left ovary	Right ovary
ZM 1	3 (10-32)	1 (7)
ZM 4	3 (10-18)	1 (10)
ZM 8	2 (8-16)	4 (6-27)
ZM 9	3 (8-18)	NA
ZM 11	10 (5-21)	2 (8-13)
ZM 13	5 (8-19)	2 (5-12)

*Determined by Ultrasonography

NA: not available

The daily plasma progesterone concentrations representing two ovarian cycles are presented in Figure 2. The occurrence of luteal activity was indicated by progesterone levels exceeding 1.6 ng/ml in all cycles as in cattle. The cycle length based on progesterone levels was 21 days. The administration of FSH to an animal which showed no follicular activity based on ultrasonic images, resulted in a high progesterone profiles (2.0 ng/ml) that subsided to basal levels (0.5 ng/ml) after 49 days.(Figure 3).

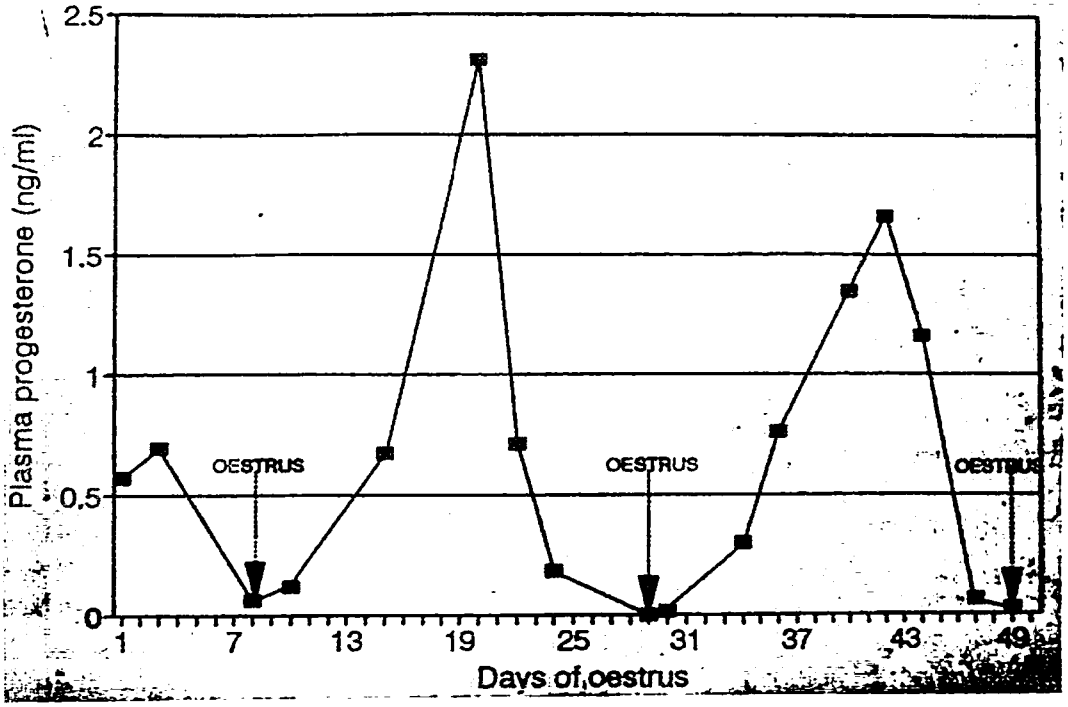

Figure 2. Plasma Progesterone Profile in a Cycling Sumatran Rhinoceros (ZM 8)

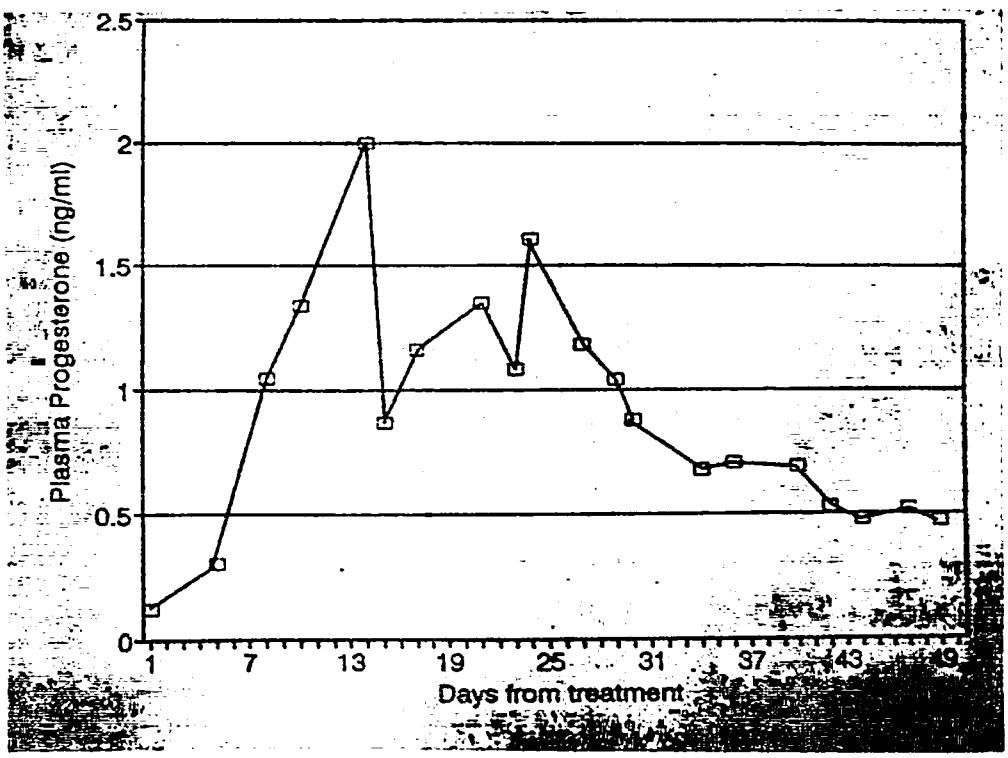

Figure 3. Plasma Progesterone Profile in a Gonadotrophin-treated Female Sumatran Rhinoceros (ZM 11)

Male Reproduction

Male Genitalia

Testes

In the adult, the testes are located extra-abdominally within a pendulous scrotum, 36 to 38 cm from the anus ventrally (Plate 8a). The right testis, excluding the tail of epididymis was 12.5 to 16.5 cm long, 5.5 to 6.3 cm wide. The left testis was smaller than the right, 11.5 to 15.4 cm long and 5.4 - 5.8 cm wide. The position of the epididymis of the right testis was horizontal but the left was inclined at an angle of 50° upwards. The width of the tail of epididymis was 2.8 to 3.2 cm. The dimensions of the reproductive organs in the adult are presented in Table 6.

In a subadult (ZM 12), the testes were not visible at 2 1/2 years but was palpable. The length and width of the right testis was 8.9 cm and 4.8 cm, respectively while the left measured 8.8 cm and 4.8 cm, respectively. At six years, the testis measured 5.5 cm and 12.5 cm in width and length, respectively. The left and right testes were positioned diagonally at 3 years old but at 6 years of age, the right testis shifted to a horizontal plane and the left testis was inclined at 50° dorsocranially (see Plate 8a inset).

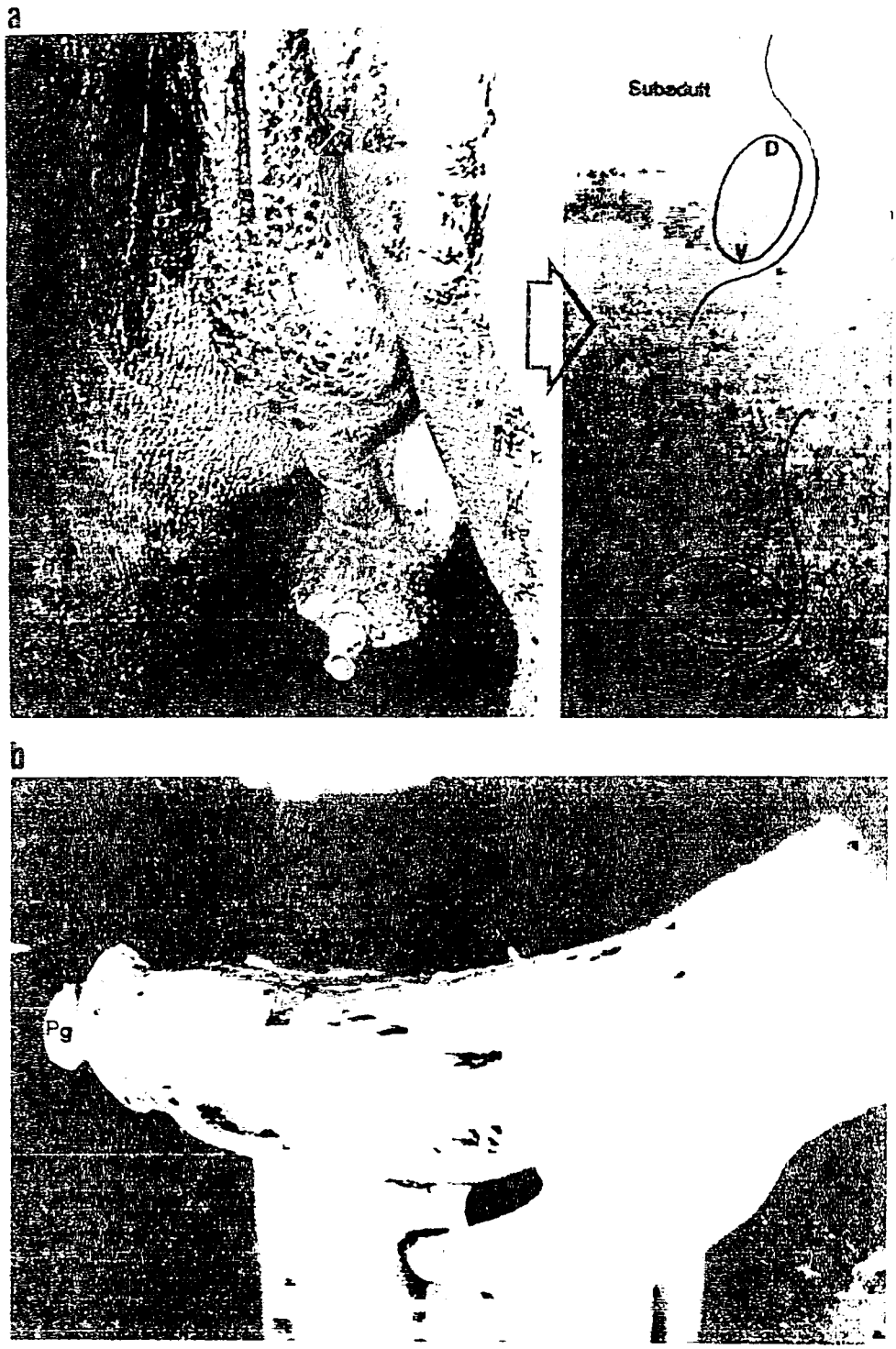

Plate 8. **Male External Genitalia of the Sumatran Rhinoceros.** (a) Testes located within a pendulous scrotum.*Inset*: Schematic representation of testicular descent. Note the Angle of Inclination of the Testes from a subadult to an adult. D, dorsal pole; V, ventral pole; (**b**) Penis with two lateral lobes attached at its base. *Pg, Process glandis*

Table 6

Dimensions of the External Genitalia of Three Male Sumatran Rhinoceroses

Structure (cm)	Animal Identity		
	ZM 5 >10yrs	SP 1 >10yrs	ZM 12 6yrs
Right testis			
length	16.5	15.0	12.5
width	6.3	6.0	5.5
Left testis			
length	15.4	14.4	11.5
width	5.8	5.7	5.4
Anus-testis length	38.0	38.0	36.0
Testes- prepuce length	NA	NA	16.5
Penis(relaxed) - length	30.6	28.7	26.5
Lateral projection			
length	7.3	6.8	6.0
width	4.3	4.3	4.0

NA: not available

Penis and Prepuce

The preputial orifice in the adult is situated 35 cm caudally from the inguinal region. Normally, the penis which was retained within the prepuce, was oval in cross-section and was curved caudally and directed ventrally. Two lateral projections were located on the greater curvature, 16.5 cm from the tip of the penis (Plate 8b). They were flat elastic structures measuring 6.0 cm long and 3.5 to 4.0 cm wide. In the 2 1/2 year old

animal, the lateral projection measured 4.8 cm (length) and 1.4 to 2.9 cm (width).

During penile stimulation and mating, the paired lateral projections expanded to twice their normal size. At the tip of the glans penis was the processus glandis which was a telescopic structure with an expanded thin, rounded border (Plate 8b). The tip was 1.38 cm in width and was oval in cross section. The glans penis enlarged, and was very firm during erection.

In the 2 1/2 year old subadult male (Plate 9), the penis was retained within the prepuce by 4 major attachments to the glans, 2 cm from the distal end. The attachments extended posteriorly to the lateral projections. The attachments involved four sections of the penis (1) the area 2 cm from the tip, (2) the area immediately anterior to the lateral projections, (3) the lateral projections to the body of the penis and (4) the fused left and right lateral projection.

The areas of attachments gradually started to separate from the penis and the first region was completely free at 2 years 10 months. The second region was free at 3 years after several attempts to get an erection. However, the third and fourth regions took an additional 8 months to separate itself. This was consistent with the animal attempting to get more erections in the night stall. The paired lateral projections were fused together throughout the entire length of the medial borders, and on to the proximal end of the glans.

Plate 9. **Penile Development in a Subadult Sumatran Rhinoceros. Note the Areas of Attachments of the Penis to the Prepuce →.**(a.) at 2.5 years old, note the attachments; (b) at 3 years, penis is protruding but prepuce is attached to the lateral projection; (c) at 3.5 years, the penis protruded completely from the prepuce but the paired lateral projections→ were still attached to each other

Ultrasonographic Image

The cross sectional view at the midline of the scrotum revealed that the testis was a circular structure, 5 cm in diameter. The left testis was 11 cm in length and 6 cm in width. The length and width of the right testes was 9.8 cm and 5.5 cm, respectively. The echotexture of the body was homogenous with hyperechogenic central densities (mediastinum testis). The epididymis was hypoechoic in comparison to the testis. Nonechoic dilations (0.5 - 0.8 cm) surrounded the head and body of the epididymis (Plate 10a). The head of the epididymis was 2.2 to 2.5 cm long and 1.5 to 2.5 cm wide. The tail of the epididymis was 5.5 to 5.6 cm long and 4.0 to 4.2 cm wide.

The accessory sex glands were easily observed by rectal exploration. The urinary bladder-urethral junction was located a few centimeters caudal to the pelvic brim, ventral to the sacral-coccygeal vertebra and was approximately 20 cm from the anal sphincter. The proximity of this junction represented the location of the seminal vesicles, prostate and bulbo-urethral glands.

The paired seminal vesicles were located lateral to the neck of the urinary bladder, cranial to the prostate gland (Plate 10b). They were elongated, approximately 2.4 cm in length and flattened structure extending cranio-dorsally along the bladder. The ultrasonographic image showed an irregular hypoechogenic lobules with hyperechogenic flecks. The walnut-shaped prostate gland was located slightly cranial to the bulbo-urethral gland and surrounded the pelvic urethra.

A diagram of the male reproductive sytem was constructed from dissected specimens, live animals and ultrasonographic images (Figure 4).

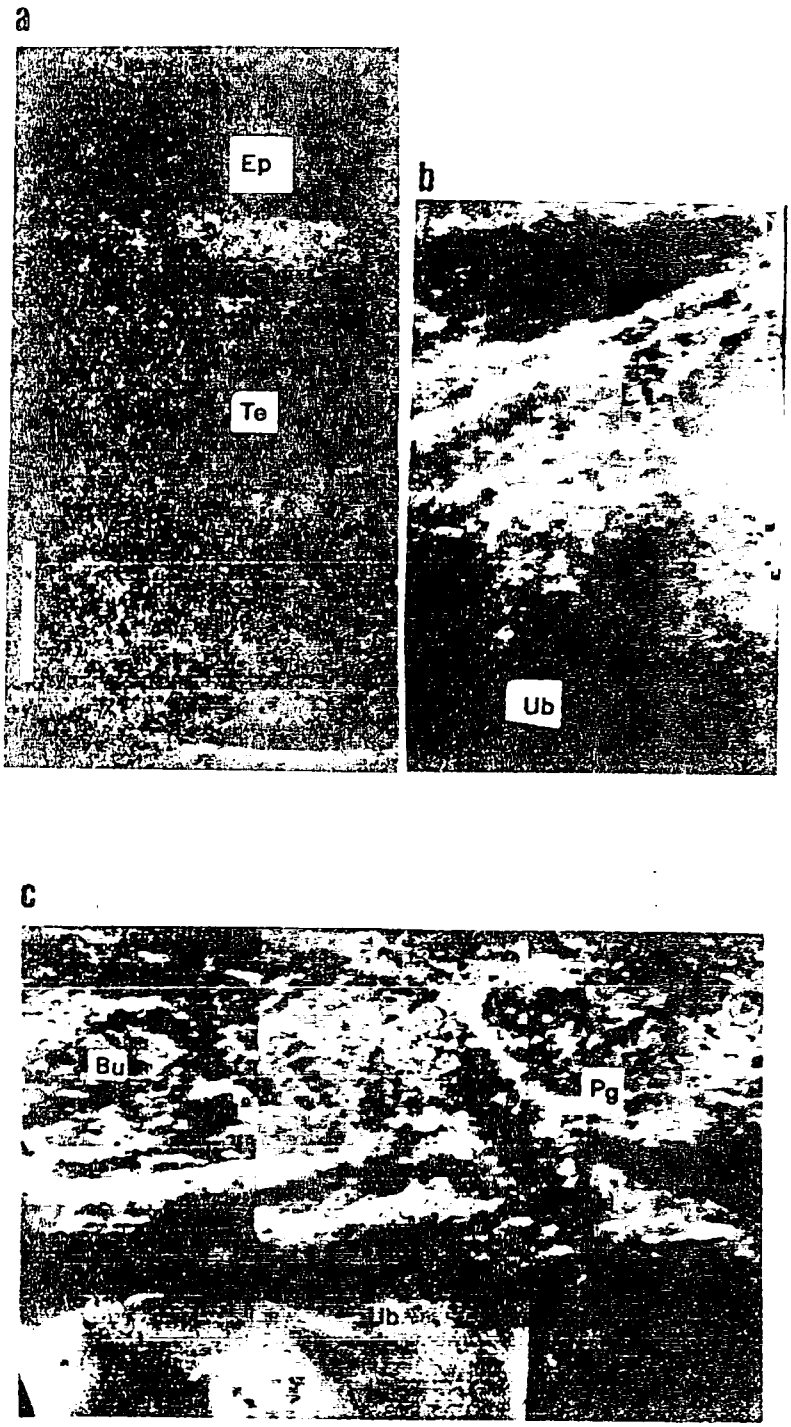

Plate 10. Ultrasonographic Images of Testis and Accessory Glands of the Sumatran Rhinoceros. (a) Testis; (b) Seminal Vesicles; (c) Accessory glands *Bu*, Bulbourethral Glands; *Ep*, Epididymis; *Pg*, Prostate Gland; *Te*, Testis, *Sv*, Seminal Vesicles; *Ub*, Urinary Bladder

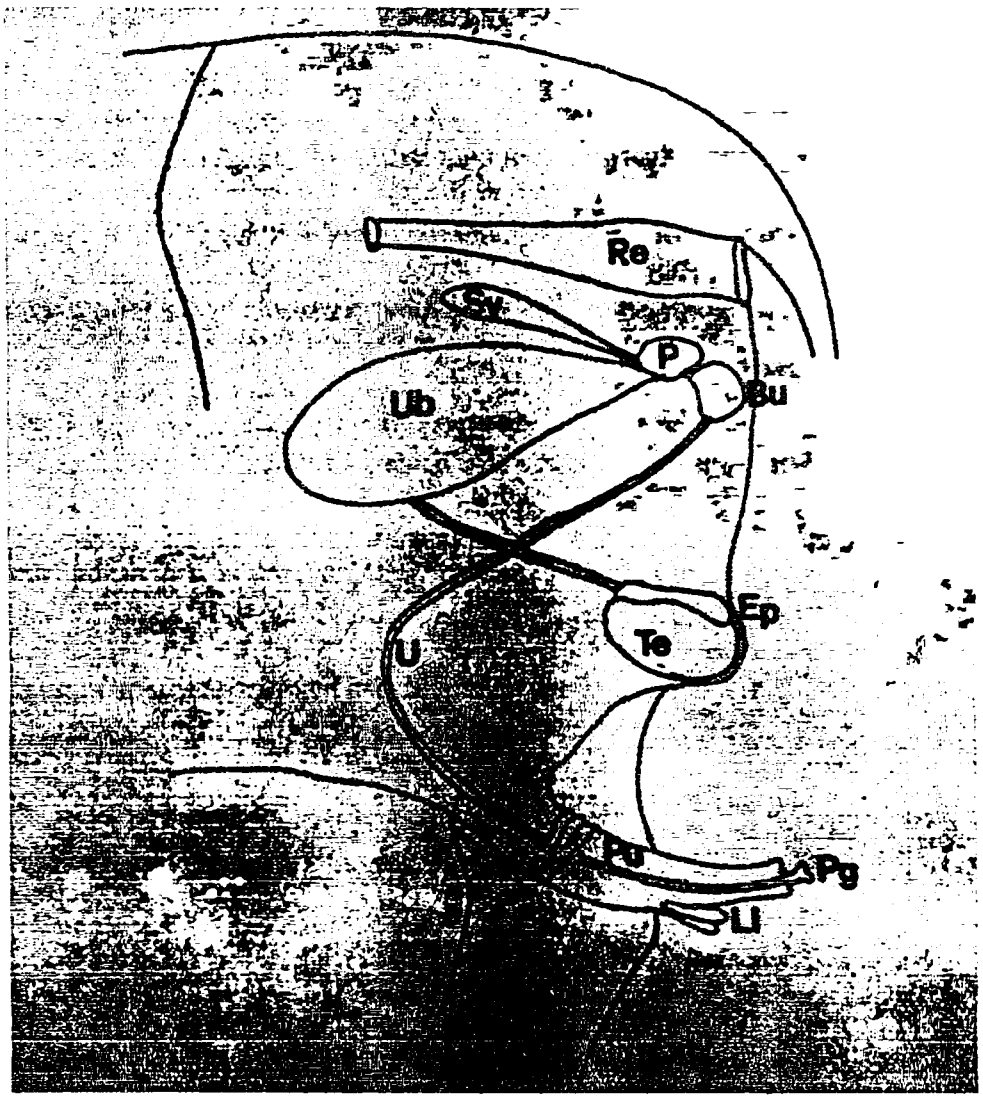

Figure 4. Diagram of a Saggital Section of the Male Reproductive Tract of the Sumatran Rhinoceros. *U*, urethra; *Ub*, urinary bladder; *Sv*, seminal vesicle; *Bu*, bulbourethral glands. The penile urethra (*Pu*) terminates at process glandis (*Pg*). The diagram also shows the lateral lobes (*Ll*) of the penis and the caudal flexure of the penis at rest and at urination. (Adapted from dissected and live specimens, and ultrasonographic images)

Semen Collection and Evaluation

Initially, rectal massage of the accessory sex glands followed by penile massage and "milking" of the penis resulted in 0.25 ml of semen. The semen was viscous, clear to brownish. An artificial vagina was used for subsequent collections following priming with rectal and penile massage. This combined method yielded higher volumes of semen ranging from 2.0 to 30.5 ml of semen. These samples were clear to brownish colouration, thick mucous to serous in consistency with a tendency to be stringy. The pH ranged from 7.5 to 8.0.

Samples from collection 1, 2, and 3 showed a sperm concentration of 0.8 to 1.9 x 10^6. The motility pattern ranged from zero to low. General and individual motilities were 38 to 68 % and 50 to 66 % respectively (Table 7). Abnormal tail constituted the highest form of sperm abnormality.

Table 7

Semen Parameters of Three Samples from a Sumatran Rhinoceros

Parameters	Collection 1	Collection 2	Collection 3
Volume (ml)	<1	20	25
Colour	yellow-cloudy	clear-brownish	clear-brownish
Wave pattern	nil	very low	low
General motility (%)	40	58	38
Individual motility (%)	50	66	63
pH	8	7.5	8
live/dead (%)	10	29	33
Detached heads (%)	4	2	3
Distal cytoplasmic droplets(%)	3	5	6
Proximal cytoplasmic droplets(%)	17	10	13
Abnormal tail (%)	47	34	38
Normal sperm (%)	29	49	40
Sperm concentration($\times 10^6$)	1.9	1.0	0.8

Reproductive Behaviour

Flehmen

The flehmen reaction was initiated by sniffing the female's urine and rump followed by lifting of the chin with exposure of the tip of the tongue. Exposure of the tongue tip and retraction of the tongue were repeated several times . The male will approach the female with its lower jaw raised to expose the lower canine. Squirt urination was observed on several occasions including normal daily urination, during excitement, being in new enclosure and in the presence of the female (Plate 11).

Masturbation

In the adult male, several incidence of masturbations (striking of the erected penis against the body wall) were observed in the morning prior to its morning feeding but without ejaculation. The male would demonstrate signs of flehmen, would urinate in small squirts, and as the penis erects, it protrudes from the prepuce. Quivering of the hind quarters was frequently observed.

Urine Spraying

During the study period, the male was observed to urine spray in 27.6 % of the days observed. Flow urination were observed on two occasions. Periodically, the female would approach the male's rear to sniff the urine. During the observation, the female only urinated in a flow.

Period of Sexual Inactivity

When the female was introduced into the male's paddock a series of responses was observed. The main areas of contacts were head, snout, neck, shoulder, forelimb, thorax, abdomen, flank, back, hindlimb and perineum (Plate 11).

In the male, during the first week of introduction, only 0.7 % of the contact promoting behviour involved the perineum and hindlimbs of the female. About 10% of the contact promoting behaviour involved the perineum of the male. The contacts involving the head and neck accounted for 70.4 % and 47.4 % in the female and male respectively (Table 8).

Vocalization was noted in the female throughout the study period. They ranged from a squeal to a blow and averaged 13.6 times per day.

In another pair, snout to snout contact was the first contact promoting behaviour observed. The female snorted and squealed as the male sniffed her rump. Whenever the male made contact with the perineum, the female would reverse. Rapid swinging of the tail was observed in the female. In the horn sparring behaviour, the female reversed and swung her head, initiating the male to charge. This behaviour was repeated several times.

Table 8

Forms of Contact Promoting Behaviour During Male-Female Encounters* in Captive Sumatran Rhinoceroses

Day	Sex	Contact Promoting Behaviour (%)						
		Head/snout	Neck	Flank	Back	Fore-leg	Hind-leg	Ano-genital
1	M	9 (45)	5 (25)	5 (25)	1 (5)	0 (0)	0 (0)	0 (0)
	F	10 (21)	10 (21)	6 (13)	16 (34)	2 (4)	2 (4)	1 (2)
2	M	11 (55)	7 (35)	2 (10)	0 (0)	0 (0)	0 (0)	0 (0)
	F	11(28)	7 (18)	2 (5)	11(28)	0 (0)	5 (13)	4 (10)
3	M	9 (36)	8 (32)	7 (28)	1 (4)	0 (0)	0 (0)	0 (0)
	F	9 (24)	9 (24)	6 (16)	10 (27)	0 (0)	1 (3)	2 (5)
4	M	13 (52)	4 (16)	7 (28)	1 (4)	0 (0)	0 (0)	0 (0)
	F	11(28)	6 (15)	7 (18)	10 (26)	1(3)	3 (8)	1 (3)
5	M	8 (31)	5 (19)	7 (27)	5 (19)	0 (0)	0 (0)	1 (4)
	F	9 (30)	6 (20)	3 (10)	8 (27)	1 (3)	3 (10)	0 (0)
6	M	11 (61)	4 (22)	0 (0)	3 (17)	0 (0)	0 (0)	0 (0)
	F	12 (60)	1 (5)	0 (0)	7 (35)	0 (0)	0 (0)	0 (0)

* During a period of 100 minutes

M : Male Snout on Female

F : Female Snout to Male

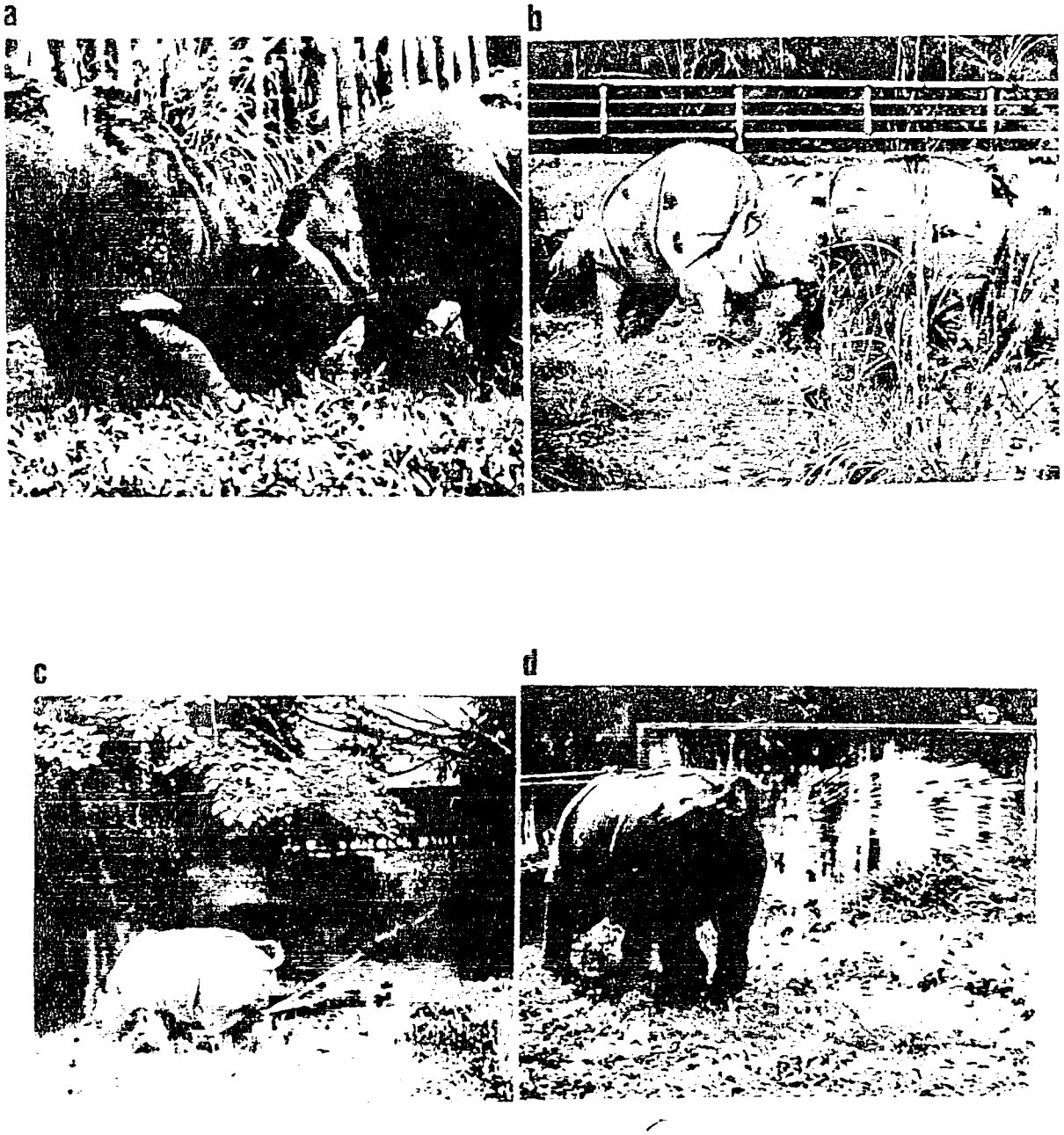

Plate 11. Contact Promoting Behaviour in Sumatran Rhinoceros . (a) Head to Head contact; (b) Head to Perineum; (c) Male Spraying Urine; and (d) Female Squirting Urine

Period of Sexual Activity

Proestrus

Figure 5 presents the contact promoting behaviour arranged between two periods of oestrus. The results show an increase in anogenital contacts as well as other male contacts with neck and shoulder of the female with approaching oestrus whereas there was no distinct pattern on female vocalization. Aggression involved head butting, biting, nose to nose nuzzling and horn clash. Severe lacerations were often inflicted on the female. The female displayed head bobbing when the male approached its perineum.

One day before onset of oestrus, both male and female displayed tail raising or tail swinging, lasting 5 to 10 minutes. Often, the female displayed spray-urination in the night stall. Feeding and defecation were not interrupted. In the paddock, chasing occurred over short distances. Both partners sniffed the ground frequently. Squealing was abrupt or continued for several seconds.

Oestrus

The day before oestrus, the female continued to vocalize (squeal and blow), followed by increased frequency of tail raising and swinging. The female reversed towards the male resulting in more male anogenital contacts. As the male placed its chin on the female's rump, she reacted by moving forward, initiating a driving reaction. Penile exposure was displayed on two occasions.

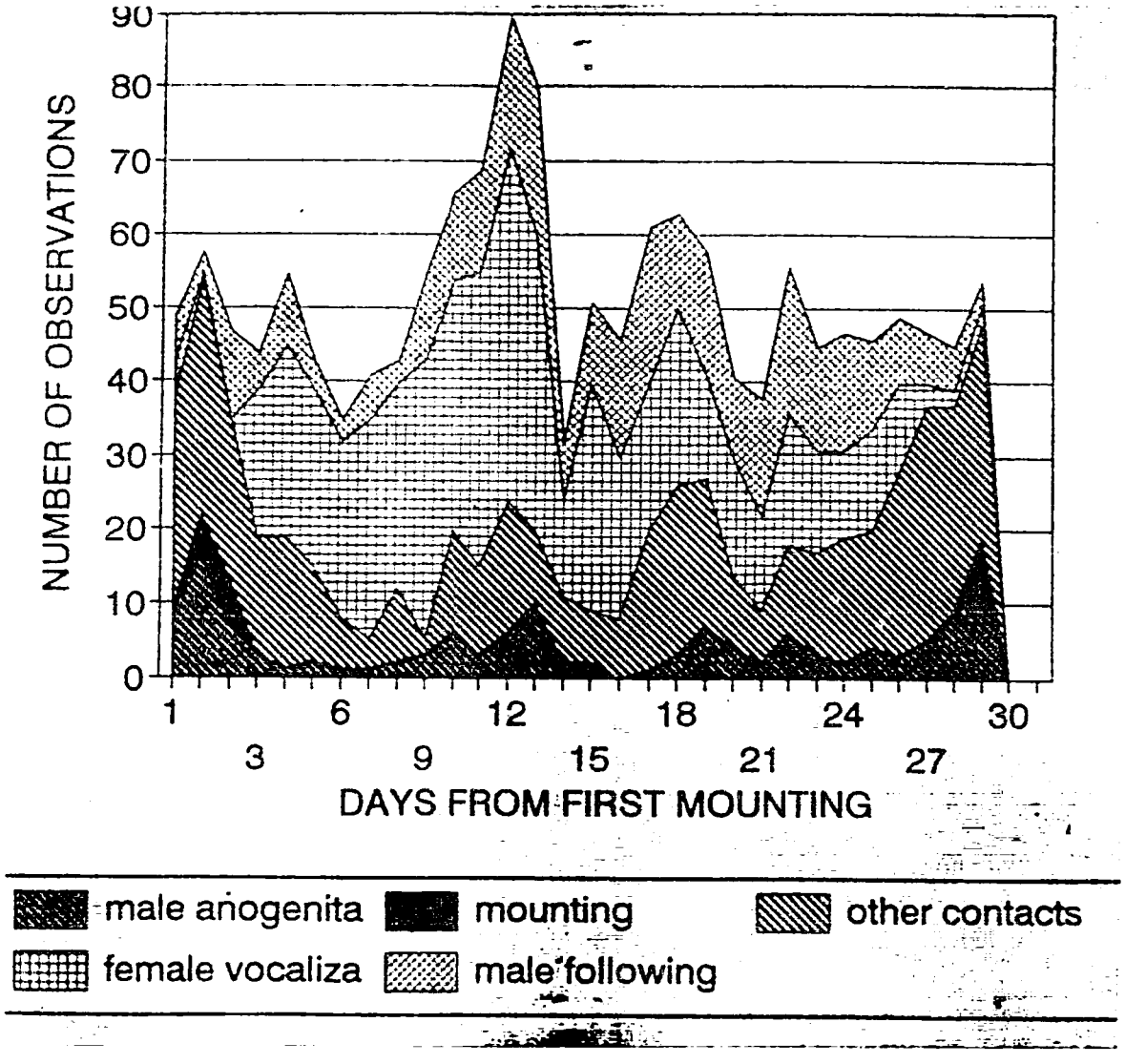

Figure 5. **Sexual and Related Behaviours in Sumatran Rhinoceros in Captivity.** The daily observations of 1 to 2 hours duration were made between 0800 and 1100 h. Note the increase in anogenital and other contacts with approaching oestrus

On day of standing oestrus, contact on the head and snout of the female was minimum whereas sniffing, licking and biting of the perineal region, on either side of the vulva were maximum. Rubbing of each others' flank and tail raising or swinging were observed with increasing frequency on the day of oestrus.

Copulation

Mounting was initiated when the female stood for the male. The male moved forward with the neck and shoulder touching the female's perineum and tail base. Subsequently, the male placed his lower jaw rest on the female's rump and slowly rotated it to the left and right using the chin as the pivot (Plate 12). The male then pushed forward and lifted its forelimbs onto the rump. The chin was placed firmly on the sacrum (chin resting behaviour). The male's tail was curled slightly and raised and the hindlegs were placed wide apart. Subsequently, the animal "rows forward" and positioned both its forelimbs on the lumbar region before finally securing at the shoulder fold (Plate 12). Mounting is usually preceded by erection which took about 5 to 100 seconds.

After full penile exposure, erection normally occurred within the next one minute followed by expansion of the paired lateral projections, one to two minutes later. Although the body of the penis was fully erected, the portion anterior to the lateral projections remained flexed posterio-ventrally. This flexed portion moved ventrally, then dorsally before the male started thrusting. A total of 36 thrusts was exhibited by the male during one mounting but without intromission.

Plate 12. Sequence of Events During Mating. (a) Male "Rows Forwards" to Position on Female Lumbar Region; (b) Partially Erected Penis Directed Caudally Showing Processes Glandis and Lateral Projections; and (c) Fully Erected Penis Seeking Vagina.

Pregnancy and Parturition

The animal keepers were not aware that ZM 4 was pregnant. They observed that it was very unapproachable, extremely aggressive and uncooperative. She could only be approached safely from the rear by securing a grip on her tail. One week before parturition, there was a swelling of the lips of the vulva. A day before parturition, the udder was moderately engorged and a thick serous straw colored fluid was expressed easily and in large quantity. There was no obvious enlargement of the abdominal region throughout the gestation.

Parturition occurred on the 23 May, 1987, at night between 1730 h and 0630 h, 430 days after capture. The female offspring weighed 24 kg. Its entire body was covered by short, curly black hair. The hooves were cartilagenous and very corrugated.

Plate 13. A Day-old Female Sumatran Rhinoceros Born at Zoo Melaka. (The dam had conceived before capture).

CHAPTER V

DISCUSSION

Study Limitations

The results of this study have demonstrated that the reproductive biology of the Sumatran rhinoceros can be studied in captivity by adapting reproductive technology used in domestic animals. However, there were major constraints imposed on this study The difficulties of recruitment from the wild population meant that the number of animals available for the study, particularly adult males, was limited.

Being an endangered species, the Sumatran rhinoceros had to be handled with care and patience to avoid stress that could have led to the animal's death. Since sedation was not permitted, animals had to be trained to enter the chute and this took several months.

Observations on the establishment of the oestrous cycle based on sexual behaviour was conducted with caution since it is well-known that the male rhinoceros could be aggressive and might inflict severe injuries to the female animals and animal handlers. As an alternative to using a male rhinoceros to determine sexual receptivity, this study was successful in establishing the oestrous cycle by measuring plasma progesterone levels in two females. It was also possible to monitor changes in the ovary such as development of follicles and luteal activity by transrectal ultrasonography.

Several months were spent in training the only male in captivity to collect semen by a combination of penile massage and artificial vagina. When used for natural mating this male could mount a female in oestrus but it failed at intromission and ejaculation. Despite all the limitations stated above, the study has provided many answers to our understanding of the reproductive biology in the Sumatran rhinoceros. They are discussed below

Female Genitalia

In the present study, a detail anatomical comparison with the other species of rhinoceros was not possible due to inadequate information on reproductive anatomy in the latter.

In general, the external and internal genitalia of the female Sumatran rhinoceros were similar to those of Black, White and Indian rhinoceros previously described (Schaffer et al., 1991). The thick muscular coat and longitudinal folds of the vagina were similar to that reported for the African rhinoceros species (Schaffer and Beehler, 1990). In addition, hymen or hymen remnants reported in the African and Indian rhinoceros species were not observed in the Sumatran rhinoceroses studied..

The cervix, cervical folds and canal were comparable to those of other rhinoceros species (Schaffer and Beehler, 1990). Similarly, no distinct demarcation of the internal and external os was observed due to the numerous thick cervical folds that encircled the lumen. The uterus was of the bicornuate type with a short body of the uterus and two cornua that course cranially as in other rhinocerses, horses and pigs. The ovaries were similar to the Black, White and Indian rhinoceroses but differ from those of the mare in the absence of an ovulation fossa (Frandson, 1976; Strauch et al., 1982; Schaffer et al., 1991).

The ultrasonographic images of the anatomy of female Sumatran rhinoceroses were comparable to the images of the other species of rhinoceroses (Schaffer and Beehler, 1990; Schaffer et al., 1991) and were markedly different from those of domestic animals. The cervix of the Sumatran rhinoceros was located just over the pelvic brim and was easily identified because of its close association with the urinary bladder. The cervix was convoluted with interlocking projections around the cervical canal confirming the postmortem findings in the present study. A convoluted cervix was also present in the Black, White and Indian rhinoceros (Schaffer et al., 1991).

The smaller body size of the Sumatran rhinoceros as compared with other species of rhinoceroses made it possible to image the ovaries with a hand-held rectal transducer. Ovaries were located at a distance of 50 to 58 cm cranially from the vulva. Ovarian activity was evident by the the large number of follicles on the ovaries of some females. Ovarian follicles appeared as black, circular to irregularly rounded structures. Irregularly -shaped follicles could be attributed to the compression by adjacent follicles, luteal structures or ovarian stroma as in the horse (Squires et al., 1988).

Female ZM 8, that displayed oestrus, had several ovarian follicles and luteal levels of progesterone typical of a female that was experiencing an oestrous cycle. In some instances, the follicles were greater than 20 mm in diameter. Whether they were follicles or ovarian cysts could not be differentiated. Further data are needed on size of normal follicles in the Sumatran rhinoceros as ovarian cysts could interfere with female fertility as in domestic animals.

Abnormalities of the uterus which were detected by ultrasonography in these females have been reported elsewhere (Schaffer et al., 1994). Several large cysts and tumours were diagnosed in the uteri of three females. Presently none of these females

have conceived and possibly uterine pathology could have impaired the attachment of the embryo to the endometrium.

Oestrous Cycle

Oestrus in captive Sumatran rhinoceros as determined by sexual receptivity to the male lasted about 24 hours and was similar to previous findings in other species of rhinoceroses (Tong, 1961; Baishya, 1978 - 1979; Goddard, 1970; Owen-Smith, 1972,1973; Laurie, 1978). However, a duration of 3 to 4 days and 1 to 6 days was reported in the Black rhinoceros (Dittrich, 1967; Goddard, 1970).

Signs of oestrus commonly observed in the Sumatran rhinoceros were increases in the frequency of urine spraying, tail raising or tail swinging, anogenital and other contacts involving the hind quarters and flank of the male. Aggression displayed by chasing and biting were observed a day before oestrus. Many observers have drawn a relationship between courtship behaviour and displays of dominance or aggression. However, the aggressive tendencies of the territorial White rhinoceros male seem strongly muted throughout the courtship period. Males quickly moved away from females when they were threatened (Owen-Smith, 1973).

Based on plasma progesterone levels, the oestrous cycle of one Sumatran rhinoceros in this study averaged 21 days. Although oestrus was not determined by sexual receptivity towards a male, the cyclic pattern of plasma progesterone which reached basal levels was highly suggestive of oestrus as in most domestic animals. In other species of rhinoceroses, cycle lengths based on changes in female behaviour were longer and showed wide variations: 38 to 58 days, 17 to 60 days, 28 to 35 days and 27

to 32 days (Baishya, 1978-1979; Jones. 1978; Yamamoto, 1967; Krishne Gowda, 1967, Goddard, 1970; Laurie, 1978).

In the Indian rhinoceros, the oestrous cycle length was determined by measuring urinary steroids—oestrone sulphate and pregnanediol 3-glucuronide (Kasman et al., 1986). The mean follicular phase was 14.8 day and luteal phase was 19 days (range 17 to 21), giving a cycle length of about 34 days. However, urinary oestrone conjugates and pregnanediol 3-glucorunide were not reliable in evaluating ovarian function in a nonpregnant Black rhinoceros (Ramsay, et al., 1987).

The administration of follicle stimulating hormone to an acyclic female resulted in luteal levels of plasma progesterone which persisted longer than in the cyclic female Although only one animal was treated, the results indicate that ovaries of the Sumatran rhinoceros could be stimulated with gonadotropins.

Male Genitalia

The penis of Sumatran rhinoceros as in the other species of rhinoceroses pointed caudally and urine was directed caudally. However, during mating or penile stimulation, the erected penis extended cranially along the ventral abdomen.

As in the other rhinoceroses (Cave, 1964), the Sumatran rhinoceros has a pair of lateral projections attached to the body of the penis, immediately posterior to the glans confirming a previous report by Forbes (1881). In the Sumatran rhinoceros, during penile stimulation leading to erection, the lateral projections expand more dorsolaterally as in the Indian rhinoceros whereas they expand more vertically in the Black and White rhinoceroses (Schaffer and Beehler, 1990). The significance of these lateral projections in copulation is not known. They probably serve as a locking mechanism during

intromission and ejaculation. It was unlikely that intromission could occur with previously expanded lateral projections on the penis.

The penis has a semitranslucent skin of prepuce dorsally as reported in the White rhinoceros (Groves and Kurt, 1972), but the scent glands reported in the prepuce and glans of the White rhinoceros (Cave and Aumonier, 1965; Cave, 1966) were not observed in the Sumatran rhinoceros.

The testes in the Sumatran rhinoceros were more prominent than in the other species of rhinoceros because the scrotum was more pendulous (Schaffer et al., 1991). The testes in the other species of rhinoceroses were usually hidden in the large penile sheath, particularly when they were drawn more cranially towards the inguinal canal. The long axis of the testis was parallel to the longitudinal axis of the animal as in other species of rhinoceroses, the stallion and the boar. Therefore, the probe had to be held vertically to image its maximum midsectional diameter.

The ultrasonographic images and dimensions of the testes and epididymides of the male in this study rhinoceros resembled normal images of bull testes. Whether the nonechogennic dilations surrounding the head and body of the epididymides were normal or pathologic need to established.

The accessory sex glands including the paired seminal vesicles and bulbo-urethral and the unpaired prostate were similar to those in other rhinoceros species (Schaffer et al., 1991).

The Sumatran rhinoceros ZM 12 exhibited flehmen at four years of age. Sexual maturity in the Indian rhinoceros commenced at 6 years and was completed at about 11 years. However, mounting could occur as early as 2 to 3 years (Baishya, 1978-1979). A 5-year- old male Indian rhinoceros displayed lipping the female's urine and performing

flehmen although it did not show interest in an oestrous female (Dixon and Macnamara, 1981). As the subadult male (Zm 12) approached sexual maturity and due to frequent erections, the penis detached itself from the prepuce in stages, starting from the glans towards the lateral projections. These semi-translucent attachments were separated with very little haemorrhages

Masturbation was observed in Sumatran rhinoceros as in the White rhinoceros (Owen-Smith, 1973).

Semen Collection and Evaluation

The method of semen collection in the Sumatran rhinoceros was similar to that used in the Black rhinoceros. In the Black rhinoceros, complete erection was obtained by applying pressure at the proximal part of the penis, with ejaculation observed 15 minutes after of continuous massage. On different occassions, 3 - 15 mls were obtained using this technique (Young, 1967). In the Sumatran rhinoceros, complete erection was obtained by applying rhythmic forward pressure of the penis from the distal end towards the lateral projections. This was followed by stroking the areas anterior to the lateral projections. Ejaculation was achieved by continuously stroking the glans in a forward series.

Training the rhinoceros resulted in an increase in sperm concentration. A significant improvement in sperm concentration was observed in the black rhinoceros after repeated application of the procedures (Schaffer and Beehler, 1988). Rectal massage was not sufficient to cause ejaculation but it was used as a priming technique for other collection methods (Carpenter et al., 1982). Similar findings were demonstrated in the Sumatran

rhinoceros. Rectal massage was often not tolerated by the rhinoceros. However, training animals to these procedures could improve semen quality.

In the Sumatran rhinoceros, the smaller volume and sperm concentration compared with other species of rhinoceroses was probably related to the collection technique.

The training of the animal to respond to manipulative procedures was important in obtaining semen of good volume and quality as noted in this study where improvements were seen with successive semen collection . An Indian rhinoceros at the Oklahoma City Zoo required a period of 10 months to train for semen collection (Schaffer and Beehler, 1988). The important factors to consider during semen collection from Sumatran rhinoceros are the animal, handler and the restraint facility.

The modification of the artificial vagina could improve the semen collection and would also provide more safety to the handlers. Modifications to the handle and orientation of the funnel would provide better expansion or erection of the penis. Two extra leather straps to the sides of the artificial vagina could be secured around the abdomen and chest of the animal. This would definitely stablize the artificial vagina and could ensure better contact and stimulation of the penis. The straps would also remove the weight of the artificial vagina on the handlers who could concentrate stimulating the glans penis and collecting the ejaculate in the callibrated tubes.

Mating Behaviour

The pattern of courtship of the Sumatran rhinoceros was comparable to the Black rhinoceros, except for the greater excitability in the latter. The horn jousting which reportedly takes place between the male and female Black rhinoceros, does not occur in the White rhinoceros. In the latter, attacks by the cow on bull were limited to the

ritualised clash of horns which serves as a distance increasing display (Goddard, 1966; Owen-Smith, 1973)

Copulation was prolonged in the other species of rhinoceros, ranging from 15 minutes to more than an hour (Laurie, 1978; Baishya, 1978 - 1979; Backhaus, 1964; Greed, 1967; Hallstrom, 1967; Owen-Smith, 1972, 1973; Jones, 1978). In the present study, intromission was not observed although mounting and erection in the male Sumatran rhinoceros were analogous to the Indian and White rhinoceroses (Laurie, 1978; Baishya, 1978 - 1979). During mating, intromission should occur before the lateral projections were fully expanded. In this study, the delay in intromission resulted in continued expansion of the lateral projections. As a result several attempts at intromission failed

The smaller size of the female Sumatran rhinoceros compared with that of the other species of rhinoceroses facilitated transrectal ultrasonography. The technique permited a rapid visual and non-invasive evaluation of the reproductive tract of the Sumatran rhinoceros and has the potential in monitoring follicular development and luteal activity. Follicles of 3 mm diameter or greater could be identified through the use of a 5 MHz transrectal transducer.

Future Research

The solitary behaviour of the male rhinoceros in the wild would necessitate that only females in oestrus be introduced into the males' enclosure in a captive breeding programme. Progesterone assays could provide an accurate means of determining the reproductive status and oestrus in the captive Sumatran rhinoceros. An alternate

approach is to regulate the oestrous cycle with hormones for planned dates of matings. Research on use of intavaginal devices available for cattle should be conducted.

Since the body and horns of the uterus of the Sumatran rhinoceros can be imaged, it is possible to use transrectal ultrasonography for pregnancy diagnosis as in cattle and horse. This technique would be particularly useful in a captive breeding programme to identify and rebreed those that failed to conceive.

Ultrasonography of the testes and the accessory glands could be used as a diagnostic tool for breeding soundness examination of males recruited from the wild and those already in captivity.

The recruitment of additional males for breeding is urgently needed. But poaching of male rhinoceroses has resulted in the limited recruitment of breeding males from the wild population.Therefore efforts should be made to maximise the use of breeding males in captivity. This could only be possible through artifical insemination. Studies should be undertaken for cryopreservation of semen of the Sumatran rhinoceros. It would then be possible to supply cryopreserved semen for artificial insemination of solitary females in zoos and in situations where natural mating is not possible or fails.

CHAPTER VI

SUMMARY

The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is on the verge of extinction in Malaysia. One of the strategies for its conservation was to breed them in captivity. Because there was a paucity of scientific information on the reproductive biology of the Sumatran rhinoceros, this study was conducted to obtain information on the anatomy of the male and female reproductive organs; signs of oestrus (sexual receptivity), mating behaviour and semen evaluation

Seven wild-caught females, three wild-caught males and one captive born female Sumatran rhinoceros (*Dicerorhinus sumatrensis*) were included in this study which was conducted from 1990 to 1994 at Zoo Melaka, Melaka and the Sumatran rhinoceros Breeding Centre in Sungai Dusun, Selangor, Malaysia.

The anatomy of the reproductive system was based on two post mortem specimens of adult females which died in captivity and two living adult male. Ultrasonography was performed on six females and an adult male using a real-time ultrasound machine. Blood samples were collected from the coccygeal vein and analysed for plasma progesterone by a radioimmunoassay .Observations were made on signs of oestrus in the female and mating behaviour. Although six ejaculates were collected from a male, 4 to 6 years of age by a combination of penile massage and an artificial vagina, only three were analysed for semen qualities.

Genitalia of the Sumatran rhinoceros were similar to those of the other species of rhinoceroses. The cervix consisted of several folds, the uterus was bicornuate with a short body and prominent horns and the ovaries were completely covered by the fimbriated end of the fallopian tube. The testes were located within a pendulous scrotum. Two lateral projections were located at the base of the penis. A well-defined processes glandis was present at the tip of the penis. The accessory glands and the testes could be imaged by ultrasonography. The volume of semen collected was about 25 ml and the concentration was about one million sperm/ml.

The average length of the oestrous cycle as measured by plasma progesterone levels was 21 days. Oestrus determined by receptivity towards the male was about 24 hours. Common signs of oestrus were: increase frequency of urine spraying, tail raising or tail swinging, anogenital and other contacts. Mounting was recorded but the inability of the male to achieve intromission was probably the reason for the failure of females to conceive.

It is concluded that the captive Sumatran rhinoceros can be restrained for ultrasonography, blood collection for progesterone level and collection of semen for evaluation

Future research should be directed towards detecting oestrus other than by testing with an adult male by monitoring ovarian activity with progesteone levels or ultrasosonography. Alternatively, the use of intravaginal progesterone devices to regulate the oestrous cycle could be considered for for planned dates of matings.

Studies are needed for cryopreservation of semen of the Sumatran rhinoceros. It

would then be possible to supply cryopreserved semen for artificial insemination of solitary females in zoos and in situations where natural mating is not possible or fails.