



UNIVERSITI PUTRA MALAYSIA

**REPRODUCTIVE BIOLOGY OF THE SUMATRAN RHINOCEROS
(*DICERORHINUS SUMATRENSIS*) IN CAPTIVITY**

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By

ZAINAL ZAHARI BIN ZAINUDDIN

**Thesis submitted in Fulfilment of the Requirements for
the Degree of Master of Science in the Faculty of
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Universiti Pertanian Malaysia**

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF PLATES	viii
ABSTRACT	xi
ABSTRAK	xi
CHAPTER	
I INTRODUCTION	1
Objectives	5
II LITERATURE REVIEW	6
Female Reproduction	6
Reproductive Organs	6
Age at Sexual Maturity	7
Oestrous Cycle	8
Ovarian Function	10
Gestation	11
Parturition	13
Birth in Captivity	13
Male Reproduction	14
Reproductive Organs	14
Age at Sexual Maturity	14
Semen Collection and Evaluation	15
Sexual Behaviour	17
Consort Period	17
Copulation	20
Post Copulatory Behaviour	21
Summary of the Reproductive Biology of the Rhinoceros	22



III	MATERIALS AND METHODS	24
	General Management	24
	Facilities	24
	Feeding	25
	Female Reproduction	27
	Female Genitalia	27
	Ovarian Function	28
	Male Reproduction	30
	Male Genitalia	30
	Semen Collection and Evaluation	31
	Reproductive Behaviour	34
IV	RESULTS	35
	Female Reproduction	35
	Female Genitalia	35
	Ultrasonic Images	40
	Ovarian Function	41
	Male Reproduction	46
	Male Genitalia	46
	Semen Collection and Evaluation	54
	Reproductive Behaviour	56
	Flehmen	56
	Masturbation	56
	Urine Spraying	56
	Period of Sexual Inactivity	57
	Period of Sexual Activity	60
	Pregnancy and Parturition	64
V	DISCUSSION	65
	Study Limitations	65
	Female Genitalia	66
	Oestrous Cycle	68
	Male Genitalia	69
	Semen Collection and Evaluation	71
	Mating Behaviour	72
	Future Research	73
VI	SUMMARY AND CONCLUSION	75
	REFERENCES	77
	BIOGRAPHICAL SKETCH	83

LIST OF TABLES

Table		
1	Gestation Length in the Family Rhinocerotidae	13
2	Case Histories of Sumatran Rhinoceroses	25
3	Dimensions of the Female Reproductive Tract and Ovaries of Two Sumatran Rhinoceroses (Identification Numbers ZM 6 and ZM 10)	38
4	Size of Ovaries in Six Sumatran Rhinoceroses	42
5	Number and Sizes of Ovarian Follicles in Six Sumatran Rhinoceroses	44
6	Dimensions of the External Genitalia of Three Male Sumatran Rhinoceroses	48
7	Semen Parameters of Three Samples from a Sumatran Rhinoceros	55
8	Forms of Contact Promoting Behaviour During Male-Female Encounters in Captive Sumatran Rhinoceroses	58



LIST OF FIGURES

Figure		
1	Diagram of an Artificial Vagina for Semen Collection in the Sumatran Rhinoceros	33
2	Plasma Progesterone Profile in a Cycling Sumatran Rhinoceros	45
3	Plasma Progesterone Profile in a Gonadotrophin-treated Female Sumatran Rhinoceros	45
4	Diagram of a Saggital Section of the Male Reproductive Tract of the Sumatran Rhinoceros	53
5	Sexual and Related Behaviours in Sumatran Rhinoceroses in Captivity	61

LIST OF PLATES

Plate		
1	Distribution of Sumatran Rhinoceros (<i>Dicerorhinus sumatrensis</i>)	3
2	A Female Sumatran Rhinoceros Being Subjected to (a) Ultrasonography, and (b) Blood Collection from the Middle Coccygeal Vein	29
3	Semen Collection by Penile Massage in the Sumatran Rhinoceros	33
4	Reproductive Tract of an Adult Female Rhinoceros	36
5	The Relationship of the Fallopian Tube to the Ovary in the Sumatran Rhinoceros	39
6	A Composite Ultrasonographic Image of the Cervix and Uterine Body of the Sumatran Rhinoceros	40
7	Cross-sectional Ultrasonographic Images of Female Internal Genitalia of the Sumatran Rhinoceros	43
8	Male External Genitalia of the Sumatran Rhinoceros	47
9	Penile Development in a Subadult Sumatran Rhinoceros	50
10	Ultrasonographic Images of Testis and Accessory Glands of the Sumatran Rhinoceros	52
11	Contact Promoting Behaviour in Sumatran Rhinoceros	59
12	Sequence of Events During Mating	63
13	A Day-old Female Sumatran Rhinoceros Born at Zoo Melaka	64

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science

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ZAINAL ZAHARI BIN ZAINUDDIN

Chairman: Professor M.R. Jainudeen (1990-1994)

Associate Professor Dr. M.K. Vidyadaran (1995)

Faculty: Veterinary Medicine and Animal Science

At Zoo Melaka, and the Sumatran Rhinoceros Breeding Centre in Sungai Dusun, seven wild-caught females, three wild-caught males and one captive born female Sumatran rhinoceros (*Dicerorhinus sumatrensis*) were studied from 1990 and 1994 to obtain information which could assist in the captive breeding of this endangered animal.

The anatomy of the reproductive system was based on two post mortem specimens and transrectal real-time ultrasonography in six adult females. 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 internal genitalia could be imaged by ultrasonography.

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 average length of the oestrous cycle as measured by plasma progesterone levels using the radioimmunoassay technique 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 failure of females to conceive.

Six ejaculates were collected from a male, 4 to 6 years of age, by a combination of penile massage and an artificial vagina. The volume of semen was about 25 ml and the concentration was about one million spermatozoa/ml.

It is concluded that ultrasonography, semen analysis and hormonal assays could provide valuable information for successful breeding of the Sumatran rhinoceros in captivity. Because of the aggressive behaviour of the male, testing females for sexual receptivity with a male should be replaced by ultrasonography and progesterone measurements for the prediction of oestrus. The recruitment of additional males for breeding is urgently needed. Improvements in semen collection and cryopreservation are needed for future use of artificial insemination if natural mating is not possible or fails.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Master Sains

**BIOLOGI PEMBIAKAN BADAK SUMATERA
(*DICERORHINUS SUMATRENSIS*) DI DALAM KURUNGAN**

Oleh

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Di Zoo Melaka dan di Pusat Pembiakan Badak Sumatera, Sungai Dusun, tujuh ekor betina dan tiga ekor jantan yang ditangkap dari hutan serta seekor Badak Sumatera (*Dicerorhinus sumatrensis*) betina yang lahir dalam kurungan dikaji dari tahun 1990 hingga 1994 bagi mendapat maklumat untuk membantu di dalam pembiakan spesis yang terancam ini di dalam kurungan.

Anatomi sistem pembiakan adalah berasaskan dua spesimen bedah siasat dan daripada kajian ultrasonografi dengan kaedah "transrectal" di dalam enam ekor Badak



Sumatera betina Serviks mengandungi beberapa lapisan, uterus adalah pendek, serta bercabang dua dengan tanduk uterin yang ketara. Kedua-dua ovari adalah tertutup terus dengan fimbria di penghujung tiub falopian. Kaedah ultrasonografi dapat menggambarkan organ-organ dalaman sistem pembiakan Badak Sumatera.

Testis terdapat di dalam skrotum yang "pendulous" Dua juluran sisi terdapat pada permukaan ventral penis Proessus glandis adalah terletak pada penghujung penis. Kelenjar-kelenjar aksesori dan testis dapat dilihat dengan menggunakan kaedah ultrasonografi

Purata panjang pusingan estrus yang diukur dengan paras plasma progesteron dengan kaedah radioimmunoassay adalah 21 hari Panjang estrus mengikut penerimaan seksual Badak Sumatera jantan adalah 24 jam Tanda-tanda yang lazim terdapat semasa estrus adalah: kekerapan menyembur air kecing, menaikkan pangkal ekor atau menghayun ekor, sentuhan-sentuhan anogenital. Proses mengawan adalah diperhati tetapi ketidakupayaan Badak Sumatera jantan untuk mengawan dengan sempurna adalah sebab utama badak betina tidak mengandung

Enam sampel ejakulasi telah diperolehi dari Badak Sumatera jantan, 4 hingga 6 tahun dengan kombinasi urutan penis dan penggunaan vagina tiruan. Sebanyak 25ml semen diperolehi dengan kepekatan lebih kurang satu juta sperma/ml. Adalah dirumuskan, kaedah ultrasonografi, penganalisan semen dan assei hormon boleh memberi maklumat yang penting untuk pembiakan Badak Sumatera di dalam kurungan. Disebabkan perangai yang agresif oleh badak jantan, pemeriksaan badak betina untuk penerimaan seksual

dengan badak jantan dari ultrasonografi dan penyukatan paras hormon bagi menentukan estrus adalah penting Badak-badak jantan untuk pembiakan perlu diberi keutamaan Peningkatan cara pungutan semen dan "cryopreservation" adalah perlu untuk kegunaan pernian beradas jika proses mengawan secara asli tidak berjaya

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.

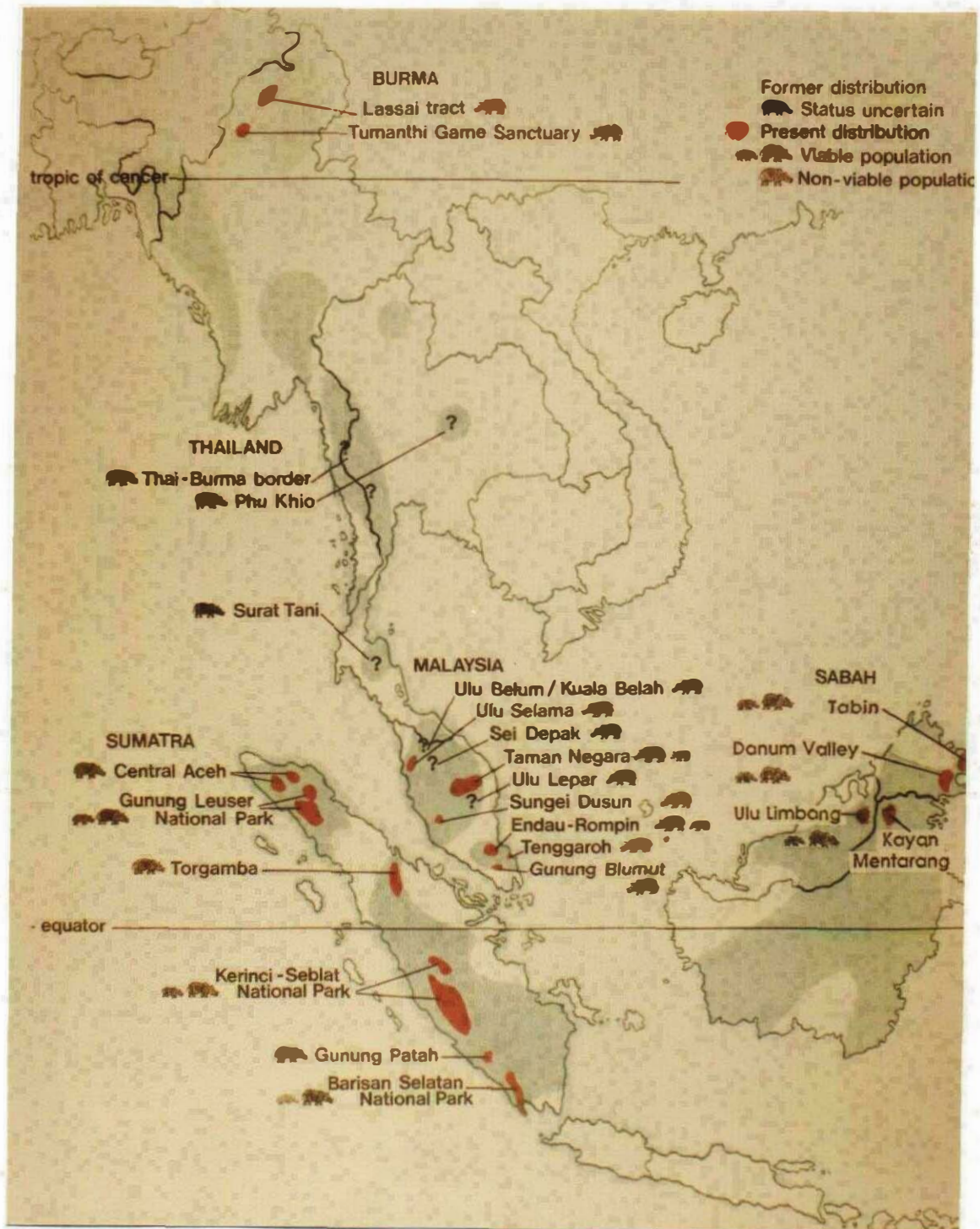


Plate 1. Distribution of Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)
 Map Credit: Francesco Nardelli

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.

CHAPTER II

LITERATURE REVIEW

This chapter reviews the reproductive biology of the extant species of rhinoceroses. It includes a description of the anatomy of the male and female reproductive organs, a detailed account of the oestrous cycle, current techniques of studying ovarian function and collection of semen, and finally a comprehensive account of sexual behaviour.

Female Reproduction

Reproductive Organs

The ovary of the Sumatran rhinoceros is kidney shaped, with an ovulation fossa which is oriented towards the infundibulum (Schaffer et al, 1991). Most of the ovary is covered by a thin tough layer of tunica albuginea limiting ovulation to a defined area. The ovary consists of an outer cortex and central medulla as in ruminants. It is oval and flat if quiescent or rounded during active folliculogenesis. The appearance of the corpus luteum on the surface of the ovaries suggests that ovulation occurs from the surface rather than into a fossa as in the mare. A broad infundibulum with extensive fimbria covering the entire ovary supports this assumption.

In recent years, ultrasonography has been used to study the reproductive organs of the live animal. Gross post-mortem descriptions and diagrams of female reproductive tracts in the Black, White and Indian rhinoceroses were compared to the in vivo

ultrasound images (Schaffer and Beehler, 1990) The distinguishing characteristics include a convoluted cervix with interdigitating folds that appear on ultrasound as dark and light swirls above the dark image of the bladder (Schaffer et al , 1991) The follicles are non-echogenic and appear as black, roughly circular ultrasonic images Compression by adjacent follicles, luteal structures or ovarian stroma can result in irregularly shaped images (Schaffer et al ,1991)

Age at Sexual Maturity

Generally, the female rhinoceros attains sexual maturity at 3.5 to 4 years of age, the first offspring being born at about 5 years of age (Baishya, 1978-1979) However, depending on the rate of growth and nutritional status, White and Indian rhinoceroses started cycling at about four years though they rarely conceived before the age of five (Jones, 1978)

Age at sexual maturity has been estimated as five years for the White rhinoceros, 3.8 to 5 years for the Black rhinoceros and 4.5 years for the Indian rhinoceros (Goddard, 1970, Owen-Smith, 1975, Tong, 1961) However, Heppes (1958) reported sexual maturity in a White rhinoceros at three years The Black rhinoceros only exhibited oestrus at six years of age in the Hanover Zoo (Dittrich, 1967) and the first mating occurred at seven years in the Kobe Oji Zoo, Japan, (Goddard, 1967). In contrast, at the Basel zoo, conception was reported in a captive born Black rhinoceros at 4.5 years (Lang, 1975) and another female showed oestrus at less than three years of age, mated at 3.25 years with parturition occurring at 4.5 years (Lang, 1961) In contrast, the Sumatran rhinoceros was reported to be sexually matured at the age of 20 years (Thom, 1943)

Oestrous Cycle

The rhinoceros is polyoestrous and the length of the normal oestrous cycle was highly variable ranging from 14 to 60 days (Jones, 1978; Goddard, 1970), but lengths of 28 to 35 days were more accurate (Yamamoto, 1967, Krishne Gowda, 1967; Goddard, 1970)

The oestrous cycle length of the Indian rhinoceros has been reported as 17 to 60 days at Whipsnade Park, England (Tong, 1961) and 27 to 32 days in the Indian rhinoceros at Chitwan valley in Southern Nepal (Laurie, 1979) A 17-day oestrous cycle was described for the Black rhinoceros (Greed, 1967) and 30 days for the White rhinoceros (Owen-Smith, 1973)

Seasonality of Oestrus

The rhinoceros has no restricted breeding season although most animals come into oestrus simultaneously in March to April In Assam, the peak mating season was February to June although mating occurs throughout the year (Baishya, 1978-1979).

In the White rhinoceros, oestrous cycles are continuous throughout the year but peak oestrous behaviour was observed from November through February (Greed, 1967; Tong, 1961), apparently stimulated by a flush of green grass A mating peak was observed in spring with subsequent calving period in autumn (Owen-Smith, 1973) In the Zululand and Uganda, mating peaks were observed from July to September and from February to May, respectively Similarly, in the southern subspecies of White rhinoceros, births were recorded in June and July (Smith, 1968, Bigalke et al , 1950)

In Sumatra, the rutting season in the Sumatran rhinoceros occurs in August (Brasser, 1926) A forester in Burma, reportedly saw a Sumatran rhinoceros mating in

July, 1933 (Ansell, 1947) Probably the mating of the Sumatran rhinoceros which resulted in the birth aboard the steamship "Orchis" in 1872 took place in May (Bartlett, 1873)

Duration of Oestrus

In the family, Rhinocerotidae, the duration of oestrus is approximately 24 hours (Baishya, 1978-1979) True oestrus lasted 24 hours in the White and Indian rhinoceros, (Tong, 1961, Goddard, 1970, Laurie, 1979, Owen-Smith, 1973) However, the duration of oestrus in the Black rhinoceros was longer and lasted from 1 to 6 days (Dittrich, 1967, Goddard, 1970)

Signs of Oestrus

Onset of oestrus in rhinoceros was characterised by gradual changes in behaviour patterns that include restlessness and frequent gargling sounds The vulva was swollen and becomes pink-red with an occasional mucous discharge from the vagina Sexual behaviour in the female rhinoceros was relatively inconspicuous and was less marked in the absence of the bull The signs of oestrus for each species of rhinoceros are described below

Indian rhinoceros In the wild state, external signs of oestrus were difficult to observe, however, the actively maintained association between adult males and females Indian rhinoceros (consort period) lasting more than 3 days ended in copulation (Laurie, 1979) An oestrous female released urine at short intervals and uttered a typical rutting call At Whipsnade Park, England a female continually emitted a shrill whistling sound and urinated at short and frequent intervals (Tong, 1958)

In captivity, signs of oestrus lasted 30 minutes to several hours (Dixon and MacNamara, 1981). A female became aggressive towards keepers, showed increased activity, constantly pacing and moving its head. A milky vaginal discharge, flashing of vulva, and spontaneous squirting of urine were also noted.

Urine squirting consisted of two rapid squirts in succession with the tail raised 20° to 45° and occasionally reaching 180°. Vulva flashing was accompanied by squirting of large quantities of urine, with the tail held horizontally. Flehmen was performed only after licking of her own urine or that of the male. During oestrus, both male and female undergo a period of sexual excitement (Gee, 1953). The sexual excitement of the male at Whipsnade Park lasted days after the end of oestrus (Tong, 1961). A high proportion of the observed oestrus periods did not result in conception (Laurie, 1979).

Black rhinoceros. The animal in oestrus repeatedly assumes a stance characteristic of urination; sprays urine in a ritualized manner while the vulva opens up and flashes. Simultaneously, the female utters a rhythmical whistling sound produced by forcing air in and out during breathing (Baishya, 1978-1979). In addition, the bull stood opposite the female, "sniffed" each other's mouths and made gargling sounds. The female then "attacked" the bull and butted hard into the flank. The attacks were tolerated by the male.

White rhinoceros. A male persistently accompanying a female over several days was a reliable indication of approaching oestrus which usually led to courtship and mating (Owen-Smith, 1973). Initially, the female warded off such attempts by the male but later tolerated him, leading to mounting and copulation. A male rarely associated with pregnant females or females with very young calves. The duration of the consort period was 4 to 20 days. Onset of oestrus occurred when the male made regular advances towards the female accompanied by "hiccing". This occurred 24 hours before copulation

The frequency increased with time until mating. The cow then repeatedly urinated in squirts. Vulval oedema was not a reliable indicator of oestrus under field conditions.

Ovarian Function

In domestic animals, endocrine activity of the ovaries can be monitored either by assay of hormones in blood plasma and urine by radioimmunoassay techniques or by monitoring follicular and luteal activity by rectal palpation and ultrasonography.

The evaluation of urinary steroids in the rhinoceros, provides information on the follicular growth, ovulation, formation of an active corpus luteum and the determination of pregnancy within 3 months post conception. In the Indian rhinoceros, the follicular phase was indicated by a gradual rise of urinary oestrone sulphate, peaking for several days, then sharply declining indicating the formation and regression of an ovarian follicle. The oestrous cycle ranged from 39 to 64 days with a mean follicular phase of 14.8 days (13 to 19 days). This was followed by a mean luteal phase of 19 days (range 17 to 21) (Kassam and Lasley, 1981). The combined oestrogen and progesterone metabolite profiles present a complete evaluation of ovarian steroid production in the mature female Indian rhinoceros (Kasman et al., 1986). The mean follicular phase based on the total urinary oestrogen excretion was 13 to 18 days (Kassam and Lasley, 1981). However, urinary oestrone conjugates and Pregnandiol 3-glucuronide (PdG) were not reliable in evaluating the ovarian activity in a non pregnant Black rhinoceros (Ramsay, et al., 1987). The excretion of urinary PdG increases in the latter pregnancy (Ramsay, et al., 1987).

Ultrasonography has revealed changes during the oestrous cycle and pregnancy, and has aided in confirming reproductive hormone levels during pregnancy in a black rhinoceros (Berkeley and Schaffer, 1992).

Ultrasonography has revealed changes during the oestrous cycle and pregnancy, and has aided in confirming reproductive hormone levels during pregnancy in a black rhinoceros (Berkeley and Schaffer, 1992)

Gestation and Birth Interval

Gestation periods for the family Rhinocerotidae are summarised in the Table 2. The period ranges from 450 to 530 days for the Indian rhinoceros, 419 to 480 days for the Black rhinoceros and White rhinoceros 476 to 550 days and 550 days for the Sumatran rhinoceros.

Intervals between births in the captive Black rhinoceros ranged from 24 to 36 months whereas in the wild population, it varied from 25 to 29 months (Goddard, 1967). The interval between parturitions was 30 - 39 months in the Indian rhinoceros. In the State Zoo, Assam, Gauhati, it averaged 3 years 8 months (Baishya, 1978-1979).

Parturition

Although several births have occurred in captivity, there is very little information published on the act of parturition in the rhinoceros (Owen-Smith, 1973). In a captive White rhinoceros, birth occurred in about 10 minutes. The dam licked the infant but ate only a small part of the afterbirth (Schuarte, 1969). The placenta of a Black rhinoceros was expelled four hours after parturition (Greed, 1967). In the Indian rhinoceros, parturition occurred in 40 minutes after onset of labour. The dam sniffed the neonate and exhibited flehmen, nuzzled and nibbled its hind feet. Nursing occurred after 3 hours 35 minutes with the female raising one of its hindlegs for easier nursing (Mackler et al.,