MUSTH AND REPRODUCTION IN THE AFRICAN ELEPHANT

Loxodonta africana

by

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Submitted in partial fulfilment of the
requirements for the degree of
M.Sc (Zoology)

in the
Faculty of Science
University of Pretoria
Pretoria

June 1988

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ABSTRACT

Endocrinological and histological data are integrated after investigations on the function of the temporal gland in the African elephant (Loxodonta africana) and the role of musth. Elephant bulls and cows from different reproductive conditions and herd structures were studied and their temporal gland activity described in relation to reproductive parameters.

Temporal gland activity occurs in elephants of both sexes from an early age with only mature bulls, over 24 years, experiencing musth periods. Increases in both plasma and temporal gland secretion testosterone concentrations are

correlated with an increase in temporal gland activity in bulls. Spermatogenesis in mature bulls was independent of season and no seasonal sexual cycle was evident. Individual differences existed between the chemical composition of temporal gland secretion of a full-musth bull and non-musth bulls.

Progesterone concentrations in elephant cows were highest during early pregnancy (5-8 months) and declined towards term. The mean plasma progesterone concentration in pregnant and non-pregnant elephant cows did not differ significantly from each other. No relationship between stage of pregnancy and temporal gland activity was apparent.

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ACKNOWLEDGEMENTS

I would like to thank Prof. J.D. Skinner for supervising this study and for his enthusiasm and guidance. During fieldwork in the first year of this study I was the recipient of a Foundation for Research Development postgraduate bursary.

The National Parks Board Of Trustees gave permission for research to be carried out in the Kruger National Park. I would especially like to thank Dr. A.J. Hall-Martin for his interest and organisation during my stay in the park. The advice and assistance of many colleagues and members of staff in Kruger National Park who contributed to this study are much appreciated.

Finally, I would like to express my gratitude to my parents who enabled me to go to university and their support throughout my studies.

CHAPTER 1

INTRODUCTION

General

In most published papers dealing with breeding habits of the African elephant (Loxodonta africana), the phenomenon of musth and temporal gland secretion are given considerable prominence. The functions of the temporal glands are not clearly understood and possible functions assigned to these glands are speculative.

Temporal glands are located on both sides of the head with their external orifices midway between the outer canthus of the eye and the external auditory meatus. The glands are embedded in subcutaneous fascia and situated in the temporal fossa. The name is derived from the glands' anatomical location. African elephants and Asian elephants (Elephas maximus) are the only known living mammals to possess temporal glands. Many other mammals, however, possess enlarged skin glands which release an exudate to the surface as does the temporal gland.

Musth in both elephant species is characterised by copious secretion from the temporal glands trickling down the cheeks. In Asian elephants, with occasional exceptions, only glands in bulls secrete during which time the bulls

exhibit musth (Jainudeen, McKay & Eisenberg 1972b). Temporal glands are functional in all age groups and both sexes in the African elephant, except in very young calves (Perry 1953 , Laws 1970).

Secretory activity of temporal glands is accompanied by increased plasma testosterone concentrations (Jainudeen, Katongole & Short 1972a , Hall-Martin & Van der Walt 1984 , Rasmussen, Buss, Hess & Schmidt 1984) ; urinary testosterone increase (Poole, Kasman, Ramsay & Lasley 1984) ; and visible physical behavioural changes, including increased aggression and dominance displays (Schmidt 1978 , Poole & Moss 1981 , Poole 1987).

In Asian elephant bulls, musth has been compared to rutting behaviour in ungulates and during musth free-living elephant bulls search out and associate with cows (Eisenberg, McKay & Jainudeen 1971). This musth period is popularly believed to be a cyclic peak of sexual stimulation and activity in bulls. Musth in Asian elephant bulls is cyclical, usually annually or biennially, during approximately the same month each year but varying both in duration and month of occurrence per individual (Jainudeen et al. 1972 b).

Poole (1987) gave the first detailed description of the characteristics and behavioural changes associated with musth in the African elephant. Temporal glands of cows and

immature elephants have a watery secretion at times, but they do not show musth. The physical and behavioural characteristics of musth in African and Asian elephants were found to be remarkably similar (Poole 1987). Seasonality, duration and recurrence of musth periods in the two species also resembled each other. The only difference was that Asian elephants experience musth at an earlier age (Eisenberg et al. 1971 , Jainudeen et al. 1972a) than do African elephants (Poole & Moss 1981 , Poole 1987). Poole (1987), furthermore concluded that musth in African elephant bulls can also be considered a rutting period.

Hall-Martin (1987) gave an explanation for musth as an alternative mating strategy and an adaptation to maximize reproductive success in some African elephant bulls. Little research has, however, been done on morphology and activity of temporal glands apart from Estes & Buss (1976) who studied development of the glands and gave a microanatomical description.

Reproductive physiology of the African elephant has been studied in some detail. A large number of corpora lutea associated with low concentrations of progesterone is one of the most intriguing aspects of elephant reproduction which was investigated in the present study. Possible relationships between female reproductive parameters and temporal gland activity were investigated. Histological

features of the testes were furthermore studied in relation to seasonality in breeding and occurrence of musth.

In Africa when elephant populations greatly exceed the carrying capacity of an area, a cycle of environmental degradation can be set in motion (Croze 1974 , Caughley 1976 , Laws 1969 & 1970 b & Barnes 1983). Part of the solution to this management problem usually involves culling of elephants and the utilization of products in a commercial manner.

Deductions were mostly made from work on such culled specimens during the present study, each of which represents a point in the life cycle. Their interpretation depended on linking up these isolated points to reconstruct the sequence of events that makes up the reproductive cycle as a whole.

The broad aim of the present study was to obtain information on aspects of reproduction with emphasis on reproductive endocrinology and histologically determined temporal gland activity in elephants of both sexes. Detailed objectives are delineated in each chapter.

Study area

Description. The Kruger National Park lies almost entirely within the Eastern Transvaal Lowveld. The area is bordered in the north by the Limpopo river and in the south by the Crocodile river and encompasses almost 20 000 km^2. The eastern boundary is formed to a large extent by the Lebombo mountain range, while the western boundary is enclosed by a fence.

Rainfall is highly erratic and usually confined to the summer months (September to April)(Gertenbach 1980). Annual rainfall varies from approximately 430 mm (Pafuri) to 740 mm (Pretoriuskop), compared to the long-term annual average of around 500 mm in the park. Between July 1984 and June 1985, 755 & 867 mm were recorded in areas I and III respectively and from July 1985 to June 1986 rainfall averaged 496 and 512 mm in the two areas. These average rainfall figures were recorded at six stations in area I and 10 in area III. The stations are distributed over each area and average rainfall may subsequently be regarded as being representative for each.

Topographically the K.N.P. is undulating with scattered rocky outcrops of granite or basalt. The highest points above sea level (847 m) are in the south-west. There is a gentle slope towards the Lebombo flats in the east which are between 180 - 240 m above sea level (Gertenbach 1983).

Drainage by six main rivers, the Limpopo, Levubu, Letaba, Olifants, Sabie and Crocodile rivers, and numerous semiperennial streams, is generally west to east. A number of dams and permanent water holes are also present throughout the park.

The most recent and up to date vegetation maps of the study area are those of Van Wyk (1972 & 1984) and Gertenbach (1983).

Elephant influence. Prevailing dry conditions between 1981 and 1984 in the Kruger National Park have made a considerable impact on the vegetation. Many tree species were affected and although particular species and the way in which they were affected differed in various areas, the following species suffered most : Common star-chestnut (Sterculia rogersii), White seringa (Kirkia acuminata), Marula (Sclerocarya caffra), Knob thorn (Acacia nigrescens), Umbrella thorn (A. tortilis), Silver cluster-leaf (Terminalia sericea) (K.N.P. Annual report 58, 1983/84).

Elephants, apparently as a result of the drought and grass shortage, have done extensive damage to a wide range of shrubs and trees in the park. Such damage constitutes an important management problem, a partial solution for which is to reduce elephant numbers by culling.

Animals. In the K.N.P., an annual aerial census of animals is conducted where total numbers of elephants are counted (Table I). Management objectives are to maintain a population of around 7 500 individuals, the estimated carrying capacity of the park in a dry year (Smuts 1975).

The present study is based on a sample of 171 elephants collected during culling operations between August 1984 and October 1985. Animals were culled from both breeding herds and bachelor groups, their herd structures are presented in Table II. Locations of the herds at the time of sampling is shown in Figure 1. All data on breeding herds were collected in area I and those on bachelor groups in areas I and III of the park (Fig. 1). Whole family groups were taken where possible and between two and five individuals were taken from bachelor groups per culling operation.

Table I. Elephant population numbers in the Kruger National Park from 1975 -1985.

Year	Total
1975	7 408
1976	7 275
1977	7 715
1978	7 478
1979	*
1980	7 454
1981	7 343
1982	8 051
1983	8 678
1984	8 273
1985	6 887

* no census.

Data from K.N.P. Annual report 58, 1983/84 & 60, 1985/86.

Herd structures (Table II) were given for elephants in different reproductive condition, determined after examining reproductive tracts. Age was not used because of wide age fluctuations at which animals attained puberty. Young elephant cows reached puberty between 7 - 10 years ($\bar{x}$ = 8,1 ± 1,0 years ; N = 10) and bulls from 10 - 14 years ($\bar{x}$ = 12,6 ± 1,4 years ; N = 8). The youngest pregnant cow was estimated to be 7 years with a 6,6 month old fetus.

Table II. Herd structures for elephants collected from breeding herds and bull associations during culling operations.

Herd no.	Date	Animal no.	Section	Herd structures Imm. ♀	Imm. ♂	Pub. ♀	Pub. ♂	Mature ♀	Mature ♂	Tot.
A	17.7.84	6,7,8,10	Skukuza						4(2)	4
B	18.7.84	13,29,31	Croc.bridge						3(1)	3
C	19.7.84	9,11,14-16	"						5(2)	5
D	20.7.84	17,18*,19	Skukuza						3(1)	3
E	24.7.84	25,26,28,30	Pafuri						4(4)	4
F	25.7.84	32,33,34,35	"						4(3)	4
G	26.7.84	36*,37,39,40	"						4(4)	4
H	27.7.84	41,42,43,44	Punda Maria						4(2)	4
5	26.3.85	D45 - D59	Woodlands	2	3	1	3	3(1)	3(1)	15
6	27.3.85	D61 - D69	Shangoni	2	1	1	1	4(1)	-	9
7	28.3.85	D70 - D83	Shingwedzi	5	6	1	-	6(4)	-	18
8	29.3.85	D87 - D95	"	-	3	2	-	4(2)	-	9
9	1.4.85	D96 - E07	Shangoni	7	4	-	-	7(3)	-	18
10	2.4.85	E14 - E26	"	4	7	-	-	7(3)	-	18
11	8.5.85	G23 - G38	Shingwedzi	5	3	-	-	6(6)	4(-)	18
12	9.5.85	G41 - G49	"	3	2	-	1	5(3)	-	11
13	10.5.85	G53 - G65	"	5	5	-	1	7(4)	-	18
14	14.5.85	G71 - G74	Punda Maria						4(2)	4
15	15.5.85	G75 - G83	"	2	1	-	1	5(3)	-	9
16	20.5.85	G84 - G92	"	3	1	-	-	5(3)	1(-)	10
17	21.5.85	G100- H06	"	2	4	-	-	3(1)	-	9
18	22.5.85	H10 - H18	"	5	1	-	-	5(3)	-	11
19	23.5.85	H19 - H26	"	3	2	-	-	4(3)	-	9
20	24.5.85	H27 - H36	"	2	3	-	-	5(3)	1(1)	11
21	27.5.85	H38 - H57	"	2	3	3	1	7(5)	-	16
22	28.5.85	H54 - H71	"	5	4	2	-	8(7)	-	19
23	1.10.85	O1 + O2	Croc.bridge						2(1)	2
24	2.10.85	AE1 + 2	"						2(1)	2
25	3.10.85	AE3 + 4	Skukuza						2(1)	2
26	11.10.85	AE5 + 6	"						2(-)	2
27	14.10.85	AE7 + 8	Kingfisher/s						2(1)	2
28	15.10.85	AE9 + 10	"						2(-)	2
29	16.10.85	AE11 + 12	"						2(-)	2
30	17.10.85	AE13 + 14	"						2(-)	2
31	21.10.85	AE15 + 16	Satara						2(2)	2
32	22.10.85	AE17 + 18	"						2(1)	2
33	23.10.85	AE19*+ 20*	Nwanedzi						2(2)	2
34	24.10.85	AE21*+ 22*	Satara						2(1)	2
35	25.10.85	AE23*+ 24*	"						2(2)	2

No. of pregnant ♀♀ and social sexually mature ♂♂ (>24 years) in parenthesis.
* single living bulls

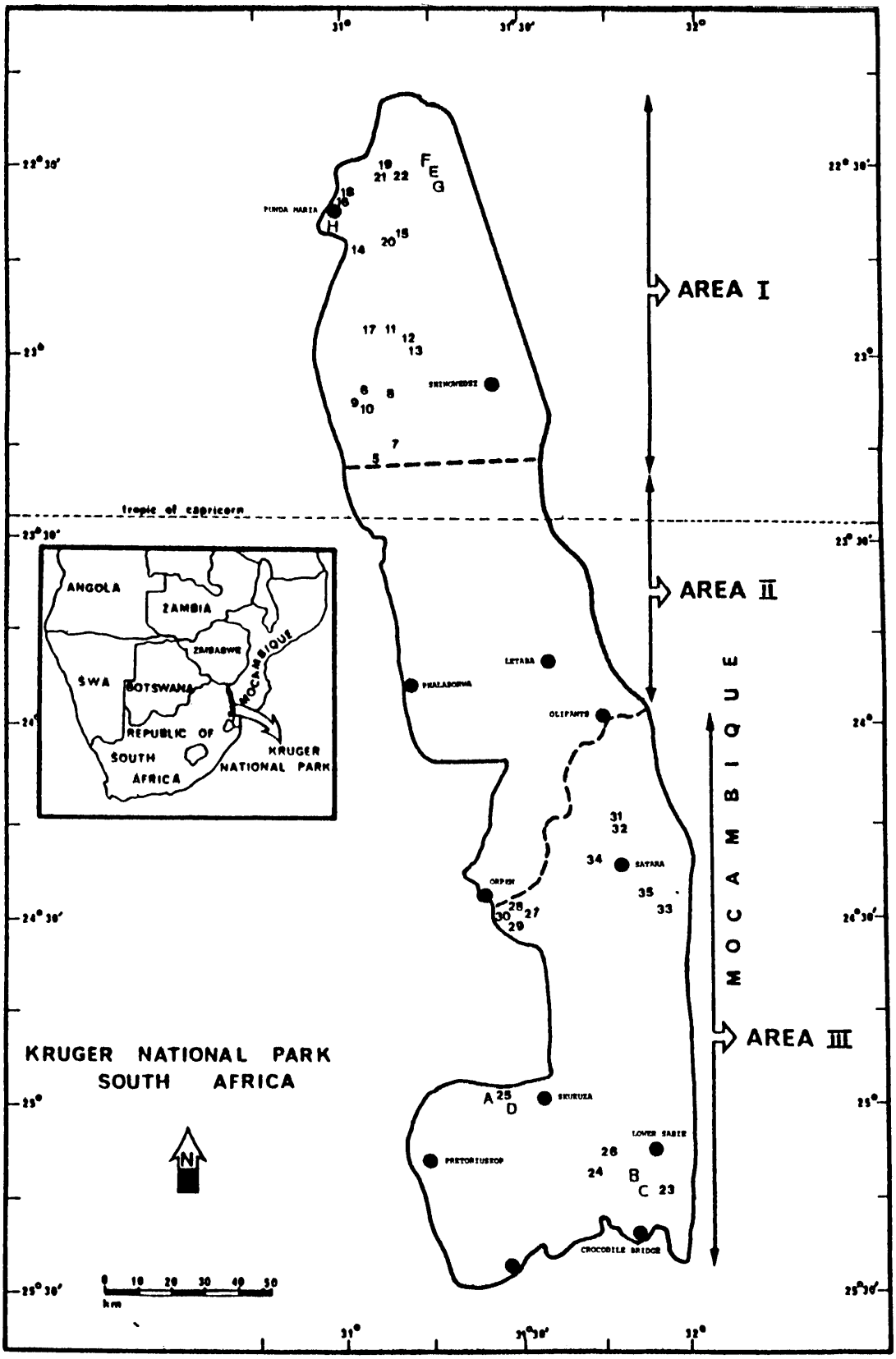

Figure 1. Map of the Kruger National Park showing (i) Its separation into three districts (North, Central and South) (ii) Herd numbers of localities where elephants have been culled during the present study.

CHAPTER 2

TEMPORAL GLAND HISTOLOGY

Introduction

Both the African elephant (Loxodonta africana) and the Asian elephant (Elephas maximus) possess a pair of large temporal glands on each side of the head which open to the surface at the temporal pore. When the glands are actively secreting, the discharge trickles down the sides of the head in copious amounts. Only bulls experience musth periods which have recently been shown to occur in African elephant bulls by Poole & Moss (1981). Although the temporal glands of cows and immature elephants have a watery secretion, they do not show musth.

Although the existence of temporal glands has been known for a long time, there is considerable disagreement as to its function. Evidence suggests that the glands become active when an individual is stressed or aggressive (Buss, Rasmussen & Smuts 1976 ; Hall-Martin & Van der Walt 1984 ; Rasmussen et al. 1984). Possible functions ascribed to the glands include chemical communication (Buss et al. 1976 ; Wheeler, Rasmussen, Ayorinde, Buss & Smuts 1982), maintenance of the hierarchical status in bull associations (Hall-Martin & Van der Walt 1984) and group member recognition (Short, Mann & Hay 1976).

Most reports on the temporal gland of the African elephant have been descriptive, based mainly on morphological examination of glands from bulls. Estes & Buss (1976) were the first to examine the histological structure of the glands in the African elephant, and found it to resemble that of Asian elephants (Fernando, Jayasinghe & Panabokke 1963). The glands are apocrine, enclosed in a fibrous capsule and consist of compound tubular alveoli grouped together to form lobules which are separated by septa of dense connective tissue in both Asian and African elephants (Fernando et al. 1963 ; Estes & Buss 1976). The presence of myoepithelial cells was suggested but not confirmed in the African elephant (Estes & Buss 1976). Myoepithelial cells are present in many apocrine glands and in mammary tissue they are associated with the neurohormonal mechanism of milk release (Linzell 1955).

Although constituents of temporal gland secretion have been examined (Adams, Garcia & Foote 1978 ; Wheeler et al. 1982), secretions and activity of the glands have never been studied in any detail. The purpose of the present study was to examine the histology and secretory activity of temporal glands in African elephants in relation to functional activity, activity in the field, reproductive condition, herd structure and age with emphasis on differences between the sexes and musth in bulls.

Materials and methods

Animals Temporal glands (N=141) were collected from elephants of both sexes and different ages which were culled in the Kruger National Park, South Africa, as part of a management programme. Samples were collected at different times of the year between August 1984 and October 1985.

Glandular activity was noted in the field prior to and immediately after immobilization with a neuromuscular relaxant (Scoline or Suxamethonium Chloride, Holpro. Chemical Co., Johannesburg, South Africa). The criterion for determining whether glands were active in the field was presence or absence of secretion trickling down the cheeks. When secretion in bulls commenced due to stress caused by the chase and darting, they were considered not to be in musth. Secretion from temporal glands is the prime indicator of musth accompanied by dripping of urine from the penis, behavioural changes including increased aggression and dominance displays (Poole & Moss 1981, Poole 1987).

Samples of temporal glands were collected from sexually mature, pubertal and immature elephants associated with family units and mature bulls from bull associations. The cows were considered as lactating when milk could be expressed from the mammary glands.

Age determination to the nearest year was based on the replacement of molar teeth in the lower jaw (Laws 1966).

Histology Temporal glands were removed between 1 - 6 h after death and weights recorded. Tissue samples were excised from the mid-portion of the glands, fixed in Bouins fluid, transferred to 70% ethanol after 48 h and, following routine paraffin embedding, sectioned at 3-5 µm. Sections were stained with Erlich's haematoxylin and counterstained with Rabie's eosin following standard procedures.

An index of glandular activity was obtained by measuring alveolar lumena size and glandular tissue volumes, using a light microscope with a side tube . Thirty to 40 randomly chosen alveoli per individual, transversely sectioned, were traced onto a sheet of paper at 600X magnification. The areas of each alveolar lumen and the glandular tissue were determined on a Li-cor area meter (Lincoln, Nebraska, U.S.A.). Average lumen area was expressed as a percentage of average total alveolar area for each gland and subtracted from 100 to give an indication of the change in glandular tissue volume and glandular activity. Activity was classified as : (i) inactive (40 - 59,9 %), (ii) moderately active (60 - 74,9 %), (iii) active (75 - 84,9 %) and (iv) fully active (>85 % glandular tissue)(Fig. 2).

The Students t-test was used in tests of significance at a 1 % level of confidence.

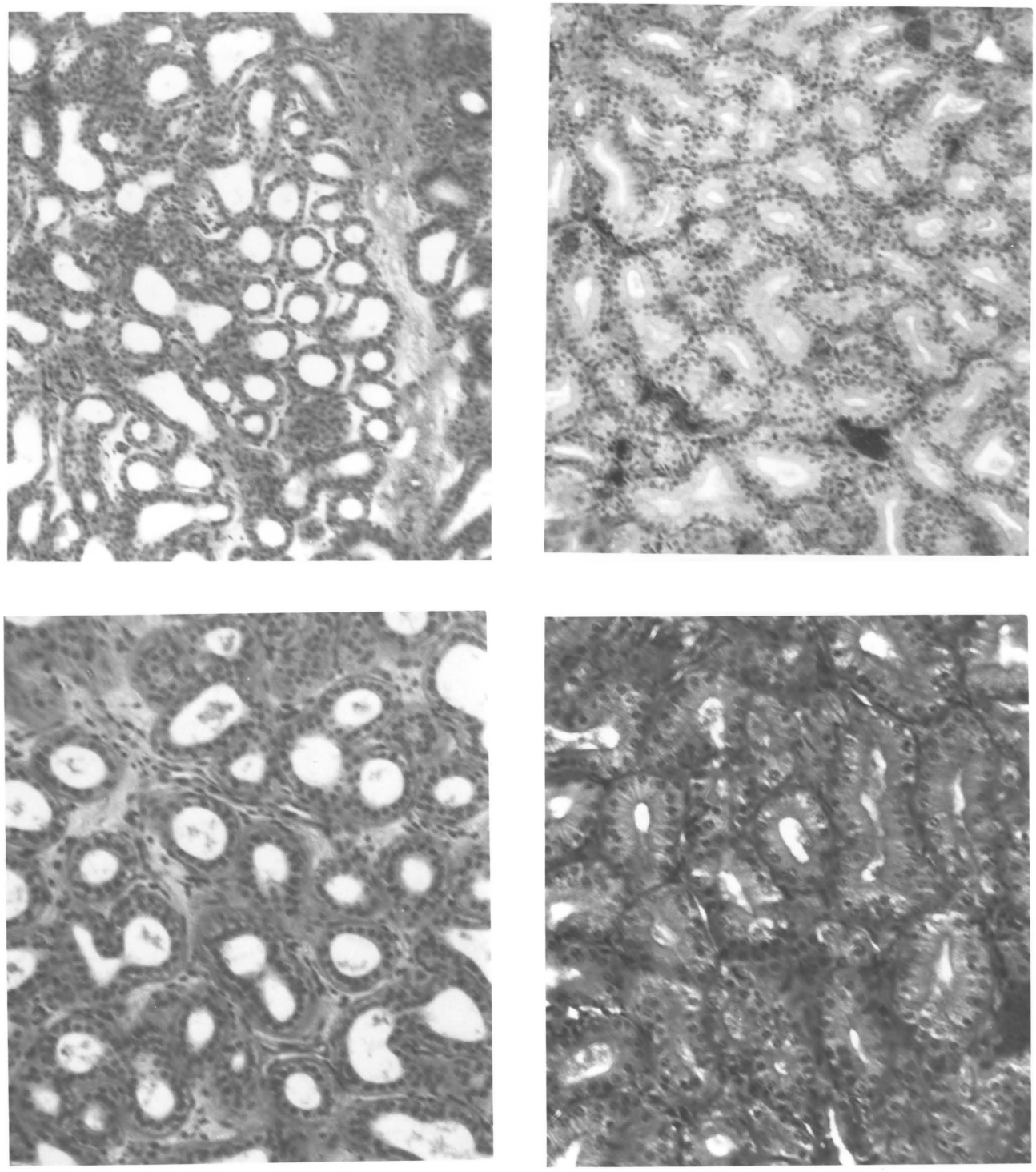

Figure 2. Histological section through the lobules of (a) inactive, (b) moderately active, (c) active and (d) fully active temporal glands, showing epithelial cells of the alveoli (150X magnification).

Results

An increase in glandular activity was accompanied by an increase in the amount of glandular tissue due to hypertrophy of cells and a decrease in alveolar lumen size. Cell height generally characterizes gland activity (Estes & Buss 1976), and in moderately active glands (60 - 74,9 % activity) both active and inactive lobules frequently occurred adjacently in the same gland. A generally higher variance of cell height could be found in these glands than in those at either extreme. In fully active glands (>85 % activity) all lobules were active and secretion was present in the collecting ducts.

The percentage of glandular tissue, lumena size and secretory activity of the temporal glands of immature, pubertal and mature elephants of both sexes was of the same order (Table III). No significant differences were found between glandular activity, as determined histologically, between immature bulls and cows ($t = 1,13$; d.f. = 23), pubertal bulls and cows ($t = 0,35$; d.f. = 10) or mature bulls in bachelor associations and mature cows ($t = 2,13$; d.f. = 90). In two bulls, considered to be in full-musth, glandular tissue predominated over lumena and there was a marked increase in the activity of the glands ($\bar{x} = 97,7 \pm 1,8$ %) which differed significantly ($t=14,1$; $p>0,01$) from that of other mature bulls in bull associations ($\bar{x} = 69,2 \pm 11,8$ % ; N=55)(Table III).

Table III. Temporal gland (T.G.) activity in elephants from different reproductive conditions.

Reproductive condition	Age interval (years)	Herd status	T.G. activity (% ± s.d.)
♂ immature	2,5 - 6	Breeding	63,5 ± 13,7 (11)
♀ immature	2,5 - 6	"	69,5 ± 13,1 (14)
♂ pubertal	7 - 13	"	66,0 ± 12,8 (4)
♀ pubertal	7 - 8	"	68,6 ± 10,8 (8)
♂ mature	>14	Bull ass.	69,2 ± 11,8 (55)
♂ mature	> 9	Breeding	63,8 ± 12,0 (37)
♂ musth	30	Lone	96,4 (1)
♂ musth	41	Lone	98,9 (1)

No. of animals in parenthesis.

No significant difference (t = 1,56) was found between glandular activity of bulls younger than 30 years ($\bar{x}$ = 68,5 ± 11,5 ; N = 40) and bulls over 30 years ($\bar{x}$ = 74,0 ± 12,1 ; N = 12). Temporal gland activity in post-pubertal bulls (14 - 24 years)($\bar{x}$ = 69,1 ± 11,5 % ; N=21) did not differ significantly (t = 0,48) from that of physiologically mature bulls (>24 years)($\bar{x}$ = 70,7 ± 12,2 % ; N=30) in bull associations. There was, however, in 95% of the bull associations (which ranged from 2 - 5 individuals), one bull with temporal glands which were significantly (t = 7,01) more active ($\bar{x}$ = 80,5 ± 8,7 % ; N=17) than those of other bulls in the same association ($\bar{x}$ = 62,3 ± 8,7 % ; N=32). Bulls which were solitary and not in musth had relatively active temporal glands ($\bar{x}$ = 77,2 ± 6,4 % ; N=4), which were significantly (t = 4,2) more active than those of bulls in bull associations ($\bar{x}$ = 62,3 ± 8,7 % ; N=32).

Within breeding herds, female and immature elephants in the whole spectrum of reproductive condition had some degree of glandular activity (Fig. 3). Mature bulls associated with breeding herds at the time of sampling did not have active temporal glands (mean activity = 52,7 ± 2,7 % ; N=3) and none of these bulls were considered to be in musth. Temporal gland activity commenced in immature bulls and cows whereafter there was a wide fluctuation with increasing age (Fig. 4 a & b). Activity did not differ significantly (t = 0,32 ; d.f.= 13) between immature and pubertal bulls.

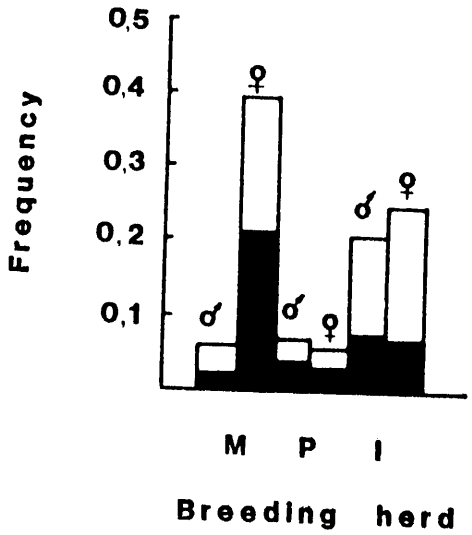

Figure. 3. Frequency distribution of mature (M), pubertal (P) and immature (I) elephants and their temporal gland activity, field and histologically determined (shaded area), in 17 breeding herds.

Temporal gland activity did not differ significantly ($t = 0,19$; $d.f. = 20$) between immature and pubertal or mature non-pregnant ($\bar{x} = 62,0 \pm 10,3$ % ; N=13) and pregnant elephant cows ($\bar{x} = 66,0 \pm 9,9$ % ; N=24)($t = 1,15$). No significant difference ($t = 0,13$) in glandular activity between the first ($\bar{x} = 66,0 \pm 11,7$ % ; N=9) and second half of gestation ($\bar{x} = 65,5 \pm 9,0$ % ; N=15) was evident and no significant difference was found between pregnant, non-lactating ($\bar{x} = 63,3 \pm 10,1$ % , N=12) and pregnant, lactating cows ($\bar{x} = 68,6 \pm 10,6$ % ; N=15)($t = 1,07$).

The temporal glands from elephant cows of all ages were secreting in the field. In 19,7 % of the elephants in breeding herds, excluding mature males, secretion could be seen trickling down the face. Mean temporal gland activity for cows with moderately active (> 60%) glands was 70,2 ± 10,1 % , five of which had active glands (>75%). Five mature cows (>20 years) with the most active glands ($\bar{x}$ = 80,6 ± 3,8 %) all showed visible secretion of temporin. One of them was close to term (~1 month) and lactating, two were pregnant and non-lactating and the other two non-pregnant and non-lactating. Immature elephants from both sexes showed temporin secretion in the field. Field activity of temporal glands was high in pubertal bulls, 80 % of them with secretion trickling down the cheeks. This was confirmed by determination of glandular activity from histological sections.

Weight of the temporal glands increased exponentially with age in bulls, but not in cows (Fig. 5 a & b). No growth spurt was observed during puberty in any of the sexes. Average temporal gland weight in pubertal bulls ($\bar{x}$ = 277,1 ± 77,0 g ; N=7) was significantly higher (t = 3,74 ; p > 0,01) than that of pubertal cows ($\bar{x}$ = 158,7 ± 30,1 g ; N=6). The highest mass recorded for a single gland was 4700 g in a 41 year old bull which was in full musth at the time of sampling.

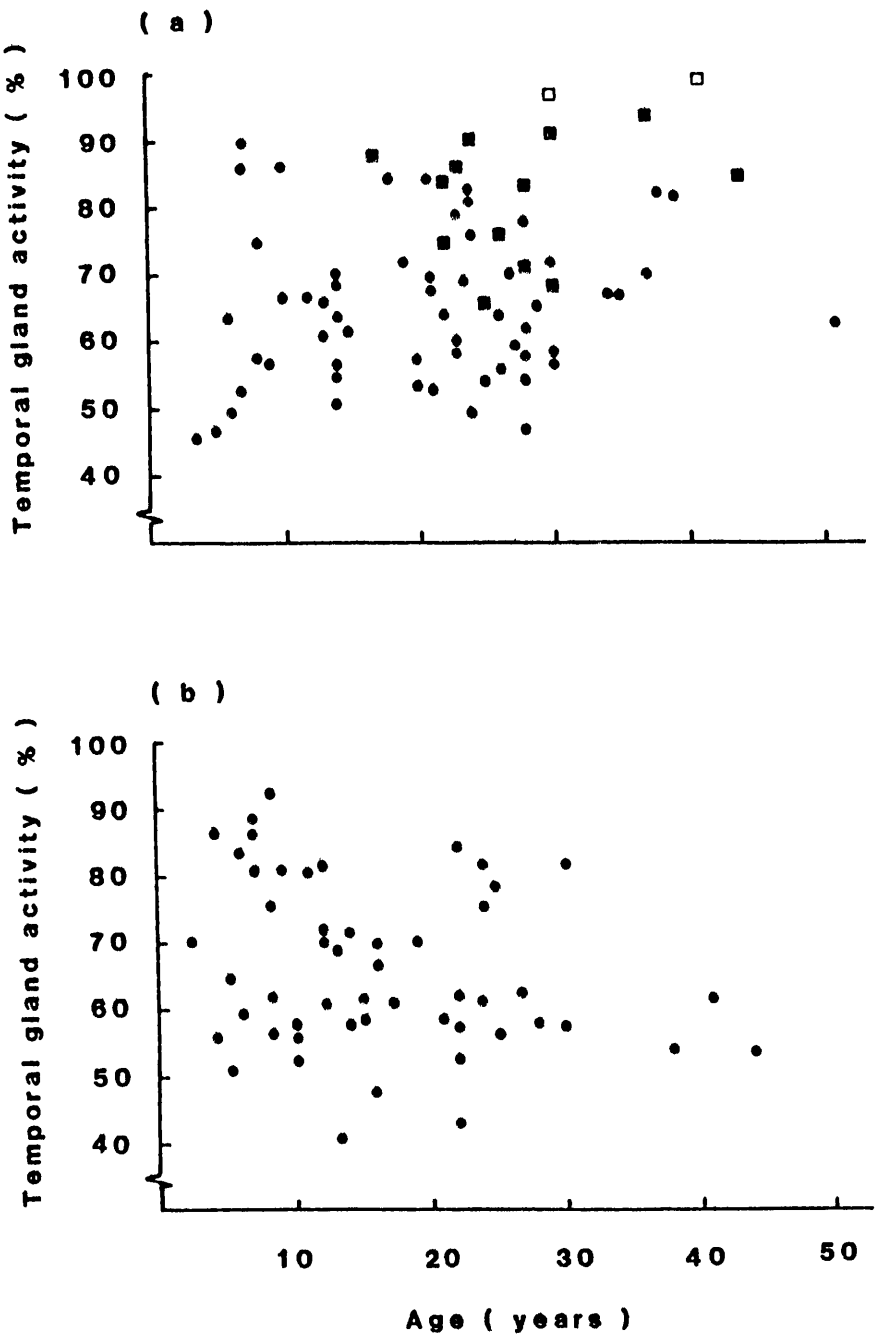

Figure 4. Changes in temporal gland activity with age in elephant bulls (a) and cows (b). Full-musth bulls (□) and those with more active glands in bull associations (■) than others are indicated.

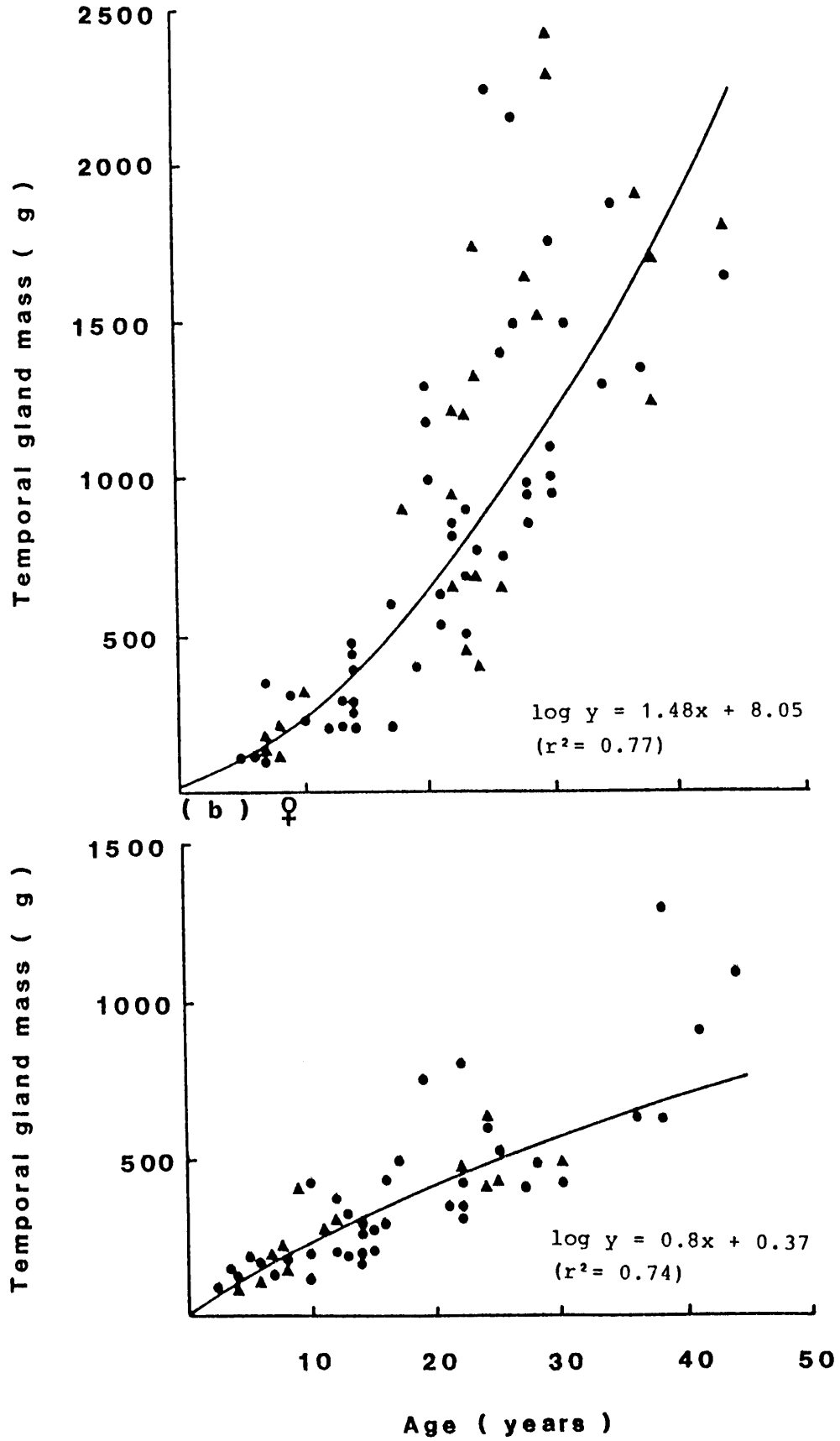

Figure 5. The relationship between active (▲) and inactive (•) temporal gland mass and age in elephant bulls (a) and cows (b).

Discussion

The present study did not reveal any difference between non-musth bulls and cows in any of the reproductive groups with respect to temporal gland activity which commenced at an early age. Musth bulls showed a marked increase in glandular activity accompanied by a conspicuous secretion trickling down the cheeks.

One or more musth periods per year is exhibited by 62 - 75 % of Asian bulls, starting at 21 years of age and increasing to 90 % by 30 years (Jainudeen et al. 1972 a). In contrast, Rasmussen et al. (1984) found that only 4,5% of adult African bulls, all over 30 years old, apparently had musth periods associated with temporal gland secretion. The present study indicates that temporal gland activity did not differ significantly between bulls younger than 30 years and those over 30 years or between post-pubertal (14 - 24 years) and physiologically mature bulls (>24 years) in bull associations. Immature and pubertal bulls also showed some glandular activity although none of the younger bulls were considered to be in musth. The two bulls which were in full-musth were 30 and 41 years of age respectively.

In most (95%) of the bull associations one of the bulls had temporal glands more active than the other bulls. Plasma testosterone concentrations were found to be higher

in these bulls than in the others in the same association (Chapter 3). The increase in temporal gland activity with increase in testosterone concentrations has been described for both African elephants (Hall-Martin & Van der Walt 1984 ; Rasmussen et al. 1984 ; Chapter 3) and Asian elephants (Jainudeen et al. 1972 a). Hall-Martin & Van der Walt (1984) found high plasma testosterone levels and active secretory temporal glands in the highest and second highest ranking bulls in an elephant population with individuals of known hierarchical status, suggesting that a possible role of temporal glands is to signal social status within a bull hierarchical structure. Data from the present study supports this hypothesis and shows that the temporal gland might serve as an aid to signal social status or dominance amongst musth bulls, solitary free-ranging bulls and bulls within non-permanent bull associations.

Copious secretion from temporal glands is frequent in African elephant bulls as well as cows (Buss et al. 1976) and care should be taken in the classification of musth in African bulls since the secretion alone is not indicative of musth. Two bulls in the present study were in full musth and ascribed to the behavioural and physical conditions for musth as described by Poole (1987). The effects of succinyldicholine on temporal gland secretion is not known. Cortisol concentrations increase after anaesthetization of elephants with succinyldicholine and then decrease again, suggesting that animals experienced physiological stress

(Hattingh, Wright, De Vos, Levine, Ganhao, McNairn, Russel, Knox, Cornelius & Bar-Noy 1984).

There is no relationship between temporal gland activity and age after the start of activity in immature elephants of both sexes (see Fig. 3). The wide fluctuation is most likely due to individual cyclicity in temporal gland activity in both sexes with no true increase or decrease in activity with age. Puberty occurred much later and over a longer timespan in bulls (10-14 years) than in cows (7-10 years) in the present study. Poole & Moss (1981) and Poole (1987) also indicated individual variation in duration and timing of musth in African elephant bulls.

Laws (1970 a) stated that the glands grew at a similar rate up to the age of 20 years, whereafter growth rate accelerated in both sexes. Short et al. (1976) detected no sex difference in weight or histological appearance of the temporal glands. In contrast, the present study indicates that the exponential growth rate of the temporal gland in bulls is high and accelerated after puberty. Growth rate in cows was much slower with no increase after puberty. Individual variation is shown once maturity is reached in both sexes. Active glands were found not always to increase in weight, however, active glands in musth bulls did show a marked increase in weight.

Activity of the temporal glands appears to be seasonal in bulls (Estes & Buss 1976 , Poole & Moss 1981) peaking during the wet season (Poole 1987). Histologically determined temporal gland activity and the presence of secretion in the field was found to occur in both sexes throughout the sampling period in the present study. The full-musth bulls (N=2) were, however, both collected during August 1984, which was a dry month in the areas of collection with an average monthly rainfall of 2,6 and 2,7 mm respectively.

Lactation did not seem to have any effect on temporal gland activity. Secretion from temporal glands in elephant cows occurs during excitement, stress or social interactions with higher ranking animals and in competitive situations (Hall-Martin & Van der Walt 1984). Buss *et al.* (1976) hypothesized that the exudate from the glands of elephants from all age groups in breeding herds might function in olfactory communication, possibly by keeping family groups together. In mature cows the temporal gland secretion might also serve to signal their social status in the matriarchal hierarchy. Due to the nature of collection of material in the present study, matriarchal cows could not be positively identified to determine whether their temporal glands were active.

Wheeler *et al.* (1982) found no correlation between either sex or age and composition of the secretion from temporal

glands. The present study illustrates that there is no difference in glandular activity between the sexes and that only mature bulls experience musth. A detailed behavioural study with special consideration of the temporal gland activity and secretion will be necessary to clarify the function of the glands.

CHAPTER 3

TESTOSTERONE AND TEMPORAL GLAND ACTIVITY

Introduction

Unlike musth in Asian elephant bulls (Elephas maximus), the function of the temporal gland of the African elephant (Loxodonta africana) is not clearly understood. Physical and behavioural characteristics of musth in African elephants have recently been described (Hall-Martin 1987 , Poole 1987). The occurrence of temporal gland secretion and musth periods in the African elephant is complicated since the copious secretion is not always indicative of musth (Buss et al. 1976). Only male African elephants experience musth periods. Although the temporal glands of cows and immature elephants have a watery secretion, they do not show musth.

Jainudeen et al. (1972 b) showed that with the onset and development of musth, serum testosterone concentrations increased in the Asian elephant. This was confirmed by Rasmussen et al. (1984) who indicated that a similar pattern was evident in the African elephant. Hall-Martin & Van der Walt (1984) reported on the plasma testosterone levels in three African elephant bulls of known hierarchical status, one of which was a dominant bull which experienced regular musth periods. They suggested that the

plasma testosterone levels may be correlated with, or are responsible for, the male hierarchical status as exhibited by these three bulls but did not provide data to validate their findings. On the other hand, published data indicate that the level of testosterone in the blood is a consequence of low or high social status and not a causal factor (Rose, Holaday & Bernstein 1971 , Sapolsky 1982).

Estes & Buss (1976) studied the microanatomy and development of the African elephant's temporal gland whereas the present study is the first to relate temporal gland histology to its activity. Morever, in the present study testosterone concentrations were measured in elephant bulls from different age classes, bulls associated with breeding herds, those in bull associations and lone bulls. The differences in degree of temporal gland activity were measured histologically to evaluate the correlation between testosterone concentrations and temporal gland secretions, testosterone in the different age classes and herd associations.

Materials and Methods

Animals. Seventy six plasma and temporal gland samples (N = 73) were collected from elephant bulls culled in the Kruger National Park, South Africa, as part of a management programme. All samples were collected between 14h00 and 16h00 from August 1984 to October 1985.

Temporal gland secretions (N = 6) were collected as soon as the animals were immobilized with a neuromuscular relaxant (Scoline or Suxamethonium Chloride, Holpro Chemical Co., Johannesburg, South Africa) and before they were dispatched. A needle (19G) connected to a 20ml syringe was inserted into the duct of the gland to collect the secretion. Samples were sealed in plastic vials , kept cool and within two hours of collection stored at -20 °C. Secretion ceased after death when no more fluid could be collected. Heparinized blood samples, taken after the elephants were killed, were centrifuged within two hours after collection, the plasma removed and then stored at -20 °C until assayed.

Temporal gland activity was noted in the field prior to and immediately after immobilization. The most obvious criterion for determining glandular activity in the field was to look for the secretion trickling down the face. Bulls which started secreting from the temporal glands due to stress caused by chasing were considered not to be in

musth. Plasma and temporal gland tissue samples were collected from immature, pubertal and sexually mature bulls associated with family units and post-pubertal and physiologically sexually mature bulls from 19 bull associations. The age classes for the reproductive conditions are presented in Table IV. Age determination to the nearest year was based on the replacement of molar teeth in the lower jaw (Laws 1966) or shoulder height (Laws 1966 , Hanks 1972 a , Sherry 1978) in 12 cases where lower jaws were not available.

Testosterone assay. Testosterone concentrations of both plasma and temporal gland secretions were estimated by radioimmunoassay as described by Van Aarde & Skinner (1986). Duplicate plasma aliquots (0,05 or 0,10 ml) were extracted with 4 ml diethylether (Merck, Darmstadt, F.R.G.). Extracts dried under a stream of nitrogen were dissolved in 0,1ml phosphate buffer (pH 7,0) containing 1% methanol. Standards ranging from 3,9 - 1000 pg testosterone (Δ^4 -androsten-17β-ol-3-one ; Sigma Chemical Co., Dorset, U.K.) per 0,1ml phosphate buffer, and buffer blanks, were prepared in duplicate and included in each assay. Antiserum in phosphate buffer (0,1ml) at a dilution of 1:8000 was added to standards, reagent blanks and plasma extracts. This was followed by the addition of [1,2,6,7, - ^{3}H] testosterone (sp.act .349 mCi/mg ; Radiochemical Centre, Amersham, Bucks, U.K.) in 0,1ml assay buffer (~10 000 c.p.m.). The contents of each tube were mixed thoroughly

and incubated for 60 min at 37 °C and then at 4 °C for 30 min. Separation of antibody-bound and free testosterone was carried out at 4 °C by adding 0,5 ml dextran-coated charcoal consisting of a suspension of charcoal (Activole ; Merck, Darmstadt, F.R.G) in assay buffer (0,25g/100ml) containing 0,025 g Dextran T-40 (Pharmacia, Uppsala, Sweden) to the contents of each tube. Extraction efficiency and the original volume of plasma extracted were taken into account when calculating the concentrations of testosterone in plasma samples.

Validation. The antiserum was raised in rabbits against testosterone-3-carboxymethyl-oxime conjugated to bovine serum albumin as described by Millar & Kewley (1976). Cross-reaction with the major naturally occurring steroids was <0,1% , except for dihydrotestosterone for which it was 5,1%. Sensitivity of the assays, defined as twice the standard deviation of the blank values ranged from 65 to 106 pg/ml (mean 78,1 ± 21,4 pg/ml ; n=5). Buffer blanks included in the assays contained less than 3,9 pg testosterone per 0,1 ml. Recovery of ^{3}H-testosterone from plasma volumes (0,05 or 0,1 ml) was always >87 % (mean 97,7 ± 6,7 %) and for temporal gland secretion (TGS) 90,6 ± 2,4 % (n=3). Intra-assay coefficients of variation were 5,4 and 9,4 % for plasma and TGS samples respectively. Interassay coefficient of variation was 7,3 %.

Histology. Temporal glands (N = 73) were removed from bulls as soon as possible after death. Tissue samples for histological preparation were excised from the mid-portion of the glands, fixed in Bouins fluid, transferred to 70% ethanol after 48 h and following routine paraffin embedding, sectioned at 3-5 µm . Sections were stained with Erlich's haematoxylin and counterstained with Rabie's eosin following standard procedures.

Alveolar lumena size and glandular tissue volume were measured to obtain an index of glandular activity as follows : slides were viewed under a light microscope with a side tube for projecting the field of view onto a drawing surface. Thirty to 40 randomly chosen alveoli per individual were traced onto a sheet of paper from the image projected onto the drawing surface at 600X magnification. The areas of each alveolar lumen and the glandular tissue were determined on an area meter (Li-cor., Lincoln, Nebraska, U.S.A.). Average lumen area was then expressed as the percentage of average alveolar area and subtracted from 100 for each temporal gland to give an indication of the percentage of glandular tissue and activity (Fig. 6). The classification for the four categories of temporal gland activity is presented in Table VI.

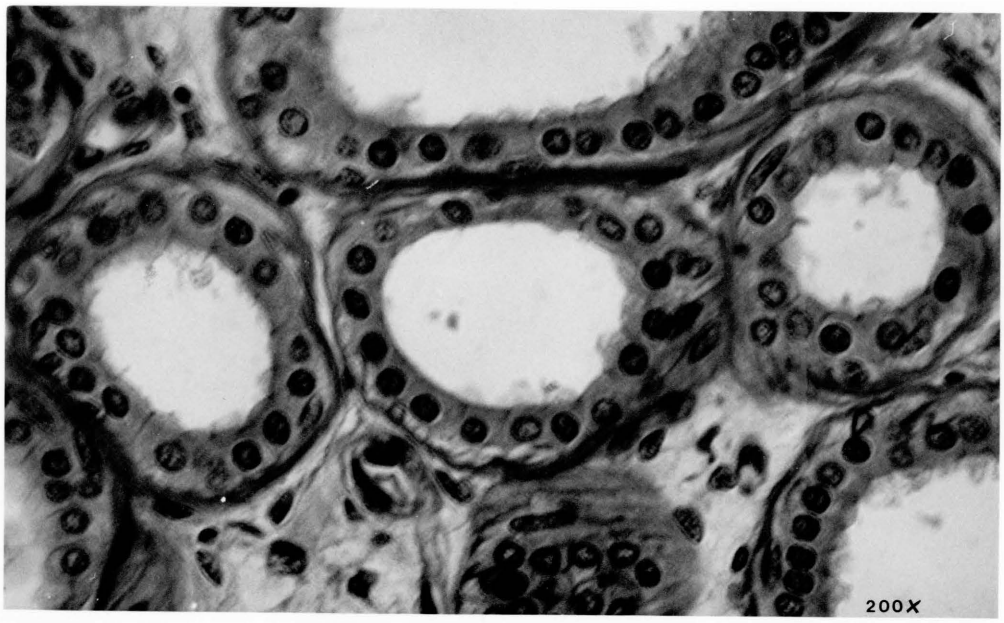

Figure 6. Histological section through the lobules of an inactive temporal gland.

Results

Plasma testosterone

In bulls 14-50 years of age (N = 65), plasma testosterone concentration averaged 1,51 ± 2,46 ng/ml (range 0,07 - 13,7 ng/ml)(Fig. 7). Testosterone concentrations for bulls in the different reproductive conditions are presented in Table IV. Bulls which started secreting temporin due to stress and disturbances caused by chasing , did not have high plasma testosterone concentrations ($\bar{x}$ = 1,28 ± 1,63 ng/ml ; N = 14).

Within most bull associations (N = 17) there was usually only one bull with a higher testosterone concentration than the others in the same group (Fig. 7). The mean value for bulls with high testosterone concentrations was 3,93 ± 3,54 ng/ml (N = 16) and for the others in the group 0,56 ± 0,56 ng/ml (N = 33).

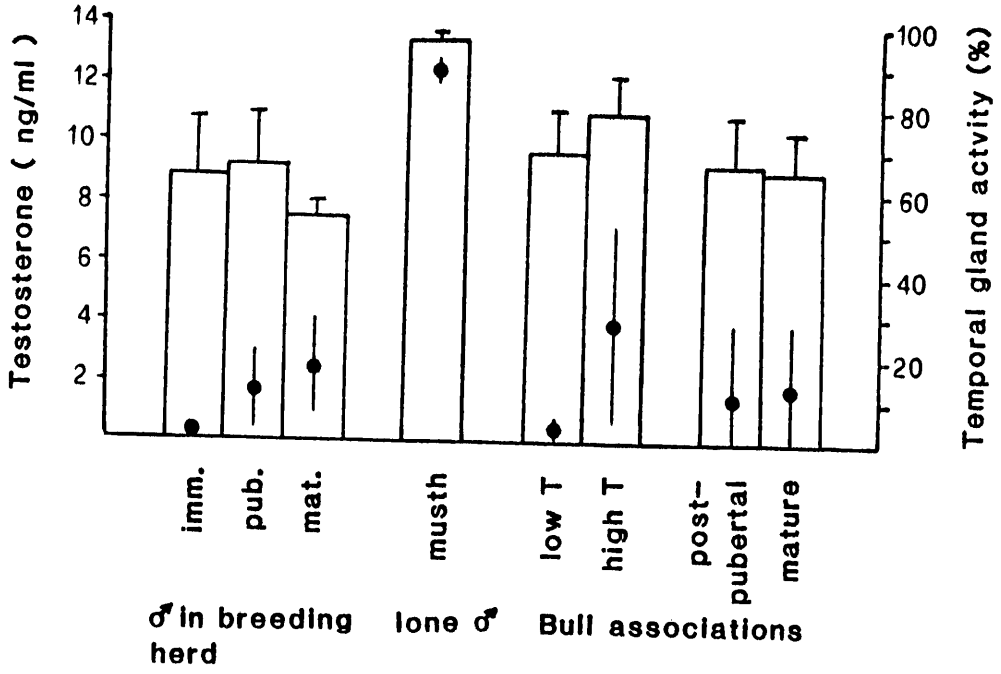

Figure 7. The relationship between testosterone concentration (●) and temporal gland activity in African elephant bulls in different herd groupings.

Table IV. Plasma testosterone (T) concentration and temporal gland (TG) activity in elephant bulls from different reproductive conditions.

Reprod. condition	Age (years)	Herd association	T. conc. (ng/ml)	T. range (ng/ml)	TG act. (%)
Immature	2,5-6	Breeding	0,16±0,14	0,07-0,46	63,5±13,7
Pubertal	7-13	Breeding	1,54±1,56	0,21-4,10	66,0±12,8
Post-pub.	14-24	Bull ass.	0,97±2,08	0,06-9,25	69,1±11,5
Mature	>24	Bull/lone	1,72±2,88	0,07-13,7	70,7±12,2
Mature	>24	Lone	0,28±0,36	0,07-0,70	77,2± 6,4
Full-musth	30	Lone	12,77	-	96,4
Full-musth	41	Lone	12,00	-	98,9

Testosterone in temporal gland secretion (TGS-T)

TGS-T ranged from 0,38 to 18,1 ng/ml in the six samples collected (Table V). Bulls in musth had the highest plasma testosterone (12,77 and 12,00 ng/ml respectively) and TGS-T levels for these bulls (18,07 and 11,72 ng/ml respectively) were markedly higher than values for elephants not in musth ($\bar{x}$ = 0,78 ± 0,47 ; N = 4). TGS-T and plasma testosterone concentrations were positively and significantly correlated (r = 0,79 ; p < 0.01).

Table V. Testosterone concentrations (T) in plasma and temporal gland secretion (TGS).

Elephant herd association	Age (years)	State of musth	Plasma-T (ng/ml)	TGS-T (ng/ml)
Herd bull	17	Non-musth	9,25	0,38
Herd bull	22	Non-musth	0,06	0,58
Herd bull	27	Non-musth*	0,15	0,68
Herd bull	44	Non-musth*	0,09	1,46
Lone bull	30	Full musth	12,77	18,07
Lone bull	41	Full musth	12,00	11,72

* In these animals secretion started due to stress from chasing

Temporal gland histology

The amount of glandular tissue, lumenal size and secretory activity of the temporal gland of immature, pubertal and mature bulls of different ages were very similar (Fig. 8). Mature bulls associated with breeding herds did not have particularly active temporal glands ; none of these bulls were considered to be in musth. Bulls that were in musth (N=2) had a high percentage of glandular tissue and temporal glands which were more active. Temporal gland activity did not differ significantly ($t = 0,49$) between the post pubertal bulls (14-24 years) and physiologically mature bulls(>24 years) in bachelor associations (Table VI).

Table VI. Temporal gland activity in relation to testosterone concentration in elephant bulls from bachelor groups and solitary living individuals.

TG activity	Glandular tissue (%)	Testosterone (ng/ml ± s.e.m.)	TG activity ($\bar{x}$ % ± s.d.)
Inactive	40 - 59,9	0,54 ± 0,21 (13)	55,6 ± 3,3 (13)
Some activity	60 - 74,9	0,92 ± 0,34 (19)	67,1 ± 3,2 (19)
Active	75 - 84,9	0,84 ± 0,39 (14)	80,6 ± 3,4 (14)
Fully active	> 85,0	7,50 ± 2,17 (7)	91,7 ± 4,8 (7)

No. of animals in parenthesis

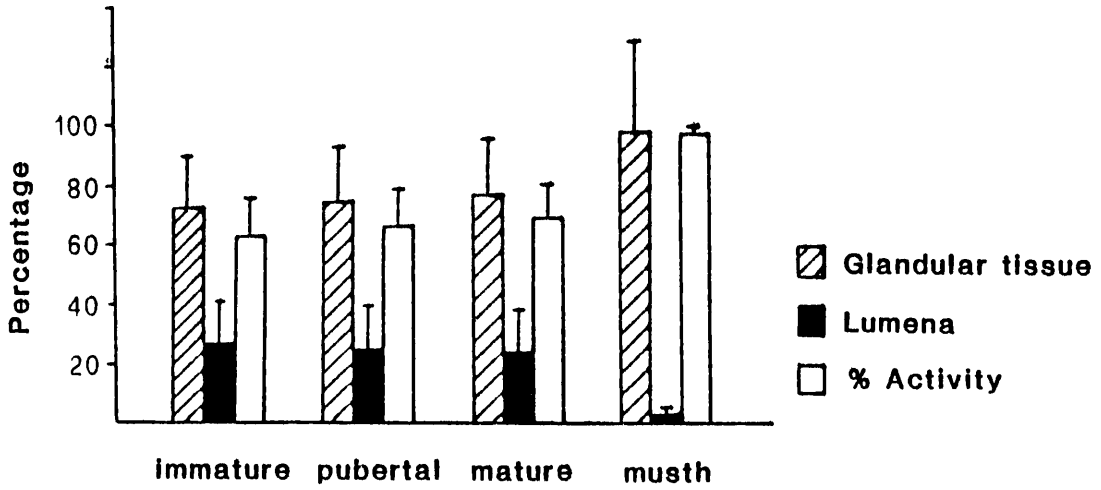

Figure 8. The relationships between the amount of glandular tissue, lumena size of the alveoli and secretory activity of the temporal gland in the African elephant.

Within each bull association there was usually one bull with temporal glands which were more active than those of other bulls in the same association. Combined temporal gland activity of these bulls ($\bar{x}$ = 80,5 ± 8,7 % ; N = 17) differed significantly (t = 7,01 ; p > 0,01) from that of all bulls with less active glands ($\bar{x}$ = 62,3 ± 8,7 % ; N = 32). In 94 % of the bull groups, the bull with the active temporal glands had the highest plasma testosterone concentration (Fig. 7). The two lone musth bulls had high testosterone concentrations in the plasma and TGS and very active temporal glands (% activity = 96,4 and 98,9 % respectively). Bulls which were not in musth had much lower testosterone concentrations and less active temporal glands (mean % activity = 69,2 ± 11,8 %). Testosterone concentrations for the different temporal gland activity classes are presented in Table VI. A significant difference (t = 3,08 ; d.f. = 21) was evident between active and fully active glands.

Discussion

The present study indicates that plasma testosterone concentrations in African elephant bulls varied greatly which explains why there is no correlation between plasma testosterone levels and temporal gland activity for all bulls (r = 0,33). However, the positive relationship between temporal gland activity and testosterone levels is clearly illustrated in musth bulls and bulls with fully active temporal glands (see Table VI). This agrees with the results of Rasmussen et al. (1984), who furthermore state that the temporal glands are sensitive to low concentrations of circulating testosterone . Even in bulls not in musth, the TGS showed high testosterone levels (Table V), supporting the latter hypothesis. The highest testosterone levels occurred in lone bulls which were in musth, had highly active temporal glands and other musth characteristics as described by Poole (1982).

The histological examination of the temporal glands revealed that activity commenced in immature elephants. Glandular activity in mature males aged 14 - 24 years did not differ significantly from that of mature males aged >24 years. Poole et al. (1984) found that only 4,5% of adult African bulls, all older than 30 years, had apparently had a musth period associated with temporal gland secretion. The present study shows that younger bulls do have temporal

gland activity, although the two bulls which were in musth were 30 years or older.

Mature bulls associated with breeding herds did not have very active temporal glands and none of them were considered to be in musth. Testosterone concentrations were much lower than those of bulls in musth. Bulls in musth would have enhanced mating opportunities due to their aggressiveness and possibly their high rank when encountering breeding herds where oestrous cows are present. Musth males have been observed to be more successful at guarding and mating oestrous cows than are non-musth bulls, while oestrous cows attempt to stay close to a bull in musth but not to non-musth bulls (Poole 1982).

Aspects of African elephant social organisation have been described by Douglas-Hamilton (1972) and Martin (1978). The effects of interbull relationships, how bull association groups function and the relation between increased aggression and bull dominance-submission structuring have not been described in detail for the African elephant. The present study indicated that in 94% of bull herd associations, where the number of animals ranged from 2-5, there was usually one bull with an active temporal gland and significantly higher testosterone concentrations than the remaining bulls. The bulls with active temporal glands did not always secrete from the glands at the time of sampling but glandular activity was confirmed

histologically. African bulls with high plasma testosterone concentrations and active temporal glands, compared to the other individuals in the same bull association and non-musth Asian bulls (Rasmussen et al. 1984 , Jainudeen et al. 1972 b) support the concept of bull ranking and suggest that high testosterone levels are positively correlated with increased aggression and temporal gland activity for the maintenance of dominance-submission structuring in bull groups. Poole (1982) suggested that bulls with active temporal glands and high testosterone concentrations were able to inhibit musth in lower ranking individuals ; data from the present study support this hypothesis. Concerning the changes of leadership among bulls, fighting is the most important determinant, but in many cases rank is undoubtedly determined without a fight, merely through withdrawal or assumption of a submissive posture by the weaker, less aggressive or smaller individual. Unlike Asian bulls where aggressive behaviour has been described by Jainudeen et al. (1972 b), this has not been adequately described in African bulls although interactions do occur.

Buss et al. (1976) concluded that there was individual recognition by members of bull herds after field observations where subordinates frequently and repeatedly challenged dominants, probably attempting to gain a higher position in dominance, and that the temporal gland secretion serves as an aid for such individual recognition. There is however no evidence to support this hypothesis

since it has not been demonstrated that individual odours are involved or that individual discrimination of group members is possible (Halpin 1986).

The present results are consistent with those of Poole et al. (1984) who measured high urine testosterone concentrations in musth, pre-musth and sexually active bulls. Sexually inactive, not in musth bulls had low testosterone concentrations in the urine (Poole et al. 1984). Their "pre-musth" bulls (group D) would correspond to the bulls with high plasma testosterone concentrations and active temporal glands in the present study. A solitary bull identified as dominant by field observations (Rasmussen et al. 1984) had high serum testosterone, as well as moderately high temporal gland testosterone concentrations. Detailed observations demonstrated an increased incentive towards a rise in dominance rank during musth (Poole et al. 1984 , Rasmussen et al. 1984 , Poole 1987).

The present study shows that there is a correlation between plasma testosterone and the activity of the temporal gland in bulls with fully active glands. All bulls did not show this relationship. Glandular activity occurs from an early age and it is proposed that a role of the temporal glands is to signal social status within the bull hierarchical structure to facilitate the formation of dominance hierarchies.

CHAPTER 4

HISTOLOGICAL FEATURES OF THE TESTES

Introduction

Detailed descriptions on the histological features and development of the testes of the African elephant (Loxodonta africana) were given by Johnson & Buss (1967 a & b). There is an indication of a seasonal cycle in testicular activity and individual reproductive cyclicity for elephant bulls was suggested by Johnson & Buss (1967 b). Field observations by Buss & Smith (1966) supported this hypothesis where mature bulls displayed varying degrees of sexual interest toward oestrous cows.

Buss & Johnson (1967) furthermore suggested that members of bull associations which showed non-aggressive behaviour, and the high proportion of such individuals with low testicular content, could be in a depressed phase of sexual activity whereas others were undergoing pubertal development. Johnson & Buss (1967 b) concluded that there was no cessation of spermatogenesis after puberty, however, they claimed that there was cyclical variation in Leydig cell function.

The phenomenon of musth which refers to a set of visible physical and behavioural changes displayed periodically by mature elephant bulls occurs in the African elephant (Poole & Moss 1981). Peaks in activity of temporal glands in Asian elephants have been likened to peaks in rutting behaviour in ungulates (Eisenberg, McKay & Jainudeen 1971) and it is popularly believed to be a cyclic peak of sexual stimulation and activity in bulls. Elephant bulls will, however, still mate when not in musth (McGaughney 1963, Jainudeen *et al.* 1971, Poole 1987). Eisenberg (1980) suggested that a high rate of spermatogenesis may accompany the musth period when plasma testosterone concentrations are high.

Objectives of the present study were to investigate whether there is any evidence for the occurrence of cyclical reproductive activity in African elephant bulls with respect to spermatogenic and androgenic activity. Leydig cell abundance and size with age were investigated in relation to circulating plasma testosterone concentration. The present study was furthermore aimed at investigating relationships between spermatogenic production, testicular exocrine function and temporal gland activity.

Materials and methods

Animals. Samples were collected from elephant bulls culled in the Kruger National Park, South Africa, as part of a management programme between 14h00 and 16h00 from August 1984 to October 1985.

Material was collected from elephants (N = 67) in bull associations, breeding herds and from solitary living individuals. Animals were immobilized with a neuromuscular relaxant (Scoline or Suxamethonium Chloride, Holpro. Chemical Co., Johannesburg, South Africa).

The testes were removed as soon as possible after death and weighed separately on a triple beam balance scale after removing the ductuli efferentes and wolffian duct. Sperm smears from the terminal section of the epididymis (Jones & Brosnan 1981) were examined in the field for assessment of reproductive status (Laws & Parker 1968). Elephants were then classified as immature (no sperm), pubertal (very few sperm) and sexually mature (many viable sperm).

Age determination to the nearest year was based on replacement of molar teeth in the lower jaw (Laws 1966). Shoulder height was used when lower jaws were unavailable (Laws 1966 , Hanks 1972 a , Sherry 1978).

Histology. Tissue samples for histological examination were excised from the mid-portion of testis and fixed in Bouin's fluid, transferred to 70 % ethanol after 48 h and following routine paraffin embedding, sectioned at 5-8 µm. Sections were stained with Erlich's haematoxylin and counterstained with Rabie's eosin following standard procedures.

Mean diameter of seminiferous tubules was determined by means of an eyepiece micrometer after calibration using a micrometer slide. A minimum of 30 readings was used to obtain a mean value.

Phase analysis procedure. A series of 9 phases of the seminiferous epithelium as described by Johnson & Buss (1967 a) were used in the analysis. Each phase represents a certain cellular association in the seminiferous epithelium and in aggregate represents one spermatogenic cycle (LeBlond & Clermont 1952). The criteria to delimit each phase is presented in Table VII. Testicular sections were scanned under low power magnification (400 X) and each transversely sectioned tubule was assigned a phase value. Between 160 - 200 tubules per individual were sampled for an evaluation of the relative frequency of each phase.

Table VII. Criteria to delimit each spermatogenic phase (after Johnson & Buss 1967 a).

Phase no.	Description
Phase 1	Immature tubule, sparse germ cell population and with no lumen.
Phase 2	Immature tubule, first indications of spermatocyte formation.Sertoli cells generally abundant.
Phase 3	Primary spermatocytes present , secondary spermatocytes may be infrequently present in very low numbers, and very low numbers of spermatids.
Phase 4	As for 3 , except spermatids are sparsely present in the tubule.
Phase 5	No spermatozoa are present, abundant spermatids which are either undergoing elongation in the central area of the tubule or becoming associated with Sertoli cells.
Phase 6	Spermatid bundles well organized, maturation divisions occurring among primary and secondary spermatocytes.
Phase 7	Spermatozoa bundles are moving centrally,toward lumen of tubule.
Phase 8	Spermatozoa have completed their central movement and are now located in the lumen.
Phase 9	Spermatozoa absent from tubule, abundant spermatids which are not yet elongating.

Leydig cell analysis. Estimates of Leydig cell abundance and descriptions of the cytoplasm were obtained from slide preparations. Cells were counted (Buss & Johnson 1967) in a series of 10 sample areas of interstitial tissue under 400 X magnification. An ocular grid representing 1050 um was used to determine the boundaries of each sample field. Cell diameters were measured with an ocular micrometer.

Testosterone assay. Circulating plasma testosterone concentrations were determined by means of radioimmunoassay as described by Van Aarde & Skinner (1986)(See Chapter 3).

Temporal gland histology. Activity of temporal glands in the present study was determined from histological slides as described in Chapter 2.

Results

Testicular mass increased linearly with age. Mean mass of paired testes for pubertal bulls was 1300 ± 440 g (N = 7) and for mature bulls 3890 ± 930 g (N = 50). High plasma testosterone concentrations (>0,70 ng/ml) are accompanied by an exponential increase in testicular mass (Fig. 9). This mass increase was not followed by a higher rate of spermatogenesis as judged from the phase analysis technique.

Diameter of seminiferous tubules increased with an increase in testes mass and age (Figs. 10 & 11). No seasonal variation in tubule diameters were evident for mature bulls although there was considerable individual variation. Tubule diameter for immature bulls aged 4 - 9 years was 85,0 ± 17,7 µm (N = 9); pubertal bulls 155,0 ± 15,5 µm (N = 6) and for mature bulls 193,0 ± 13,1 µm (N = 50).

The mean percentage of each spermatogenic phase for the different reproductive groups is represented in Fig. 12. Phases 1 & 2 were prominent in immature elephants, phase 3 in pubertal bulls and the whole spectrum was present for mature bulls, including a quantity of immature tubules (Fig. 12). There was no apparent relationship between seminiferous tubule maturity as determined from tubule phase values (Johnson & Buss 1967 a) and interstitial cell cytoplasm.

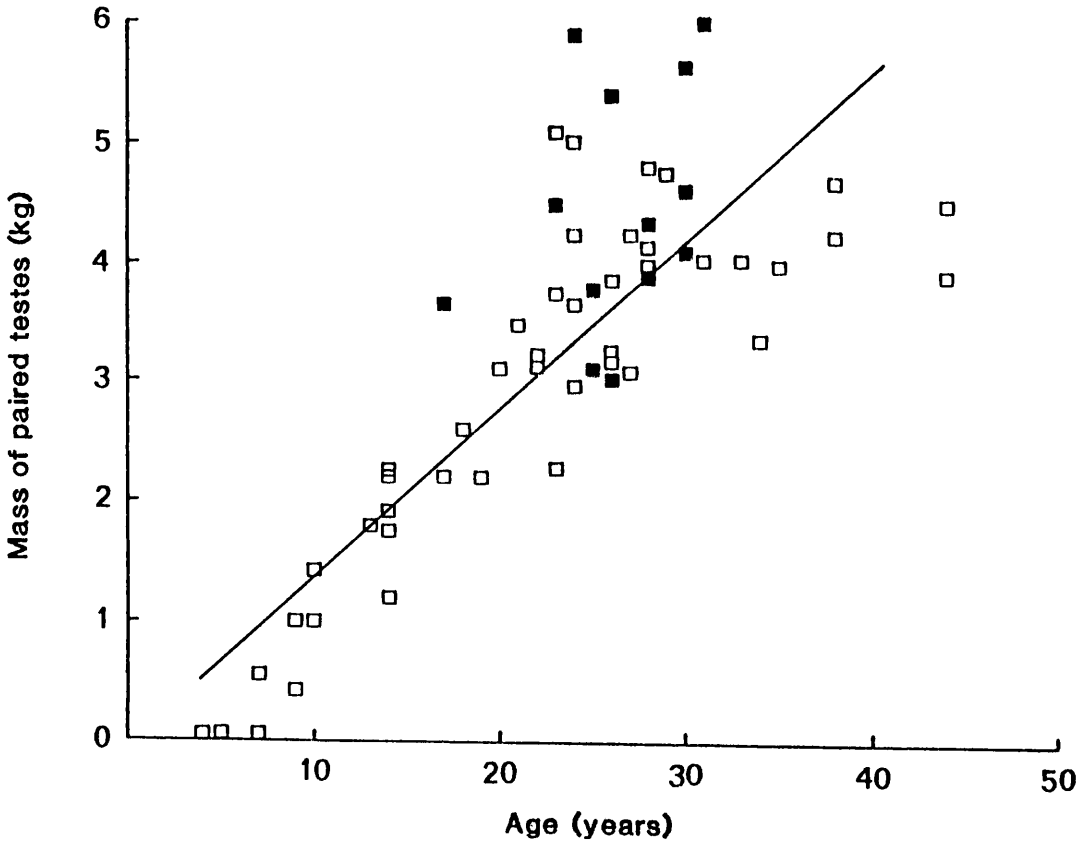

Figure 9. The relationship between mass of paired testes and age (equation y = 143,4x + 19,9 ; r^2 = 0,71). Testes mass where high plasma testosterone concentrations (> 0,70 ng/ml)(■) were measured, is indicated.

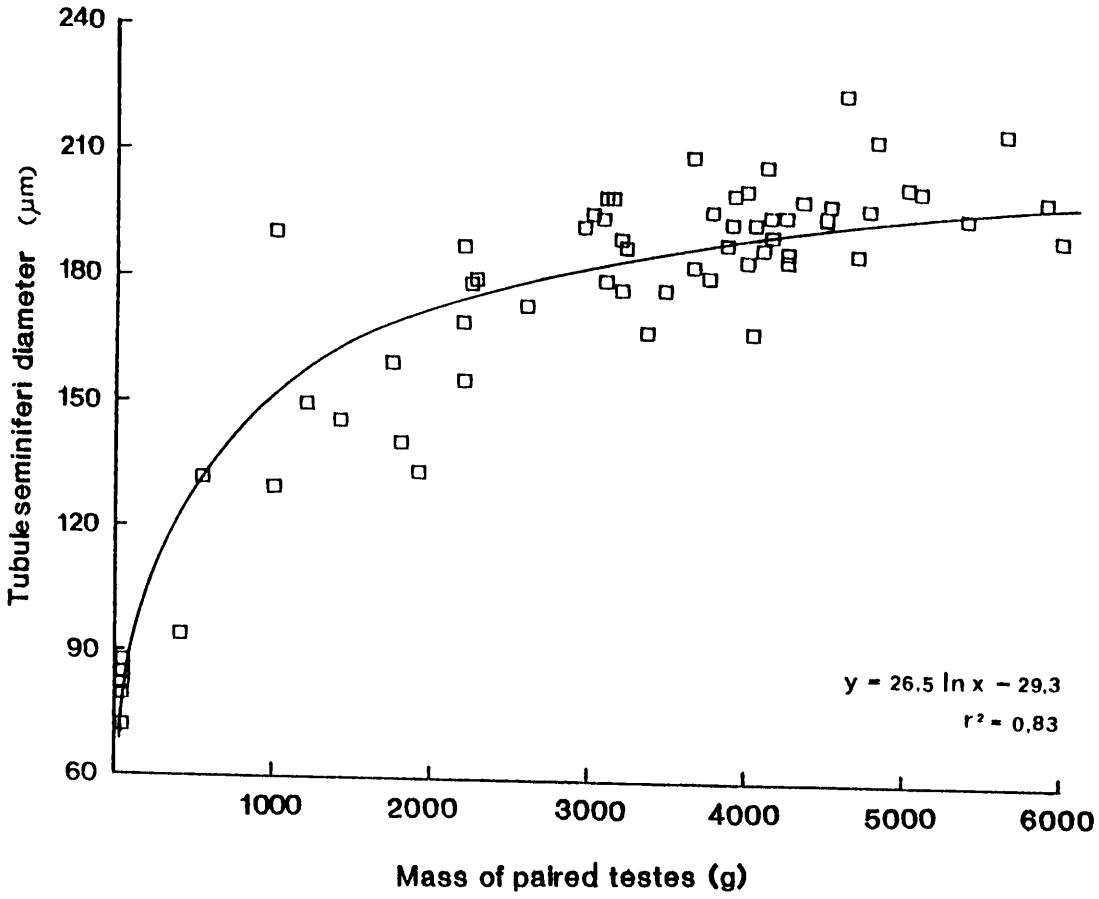

Figure 10. The relationship between seminiferous tubule diameter and combined testicular mass.

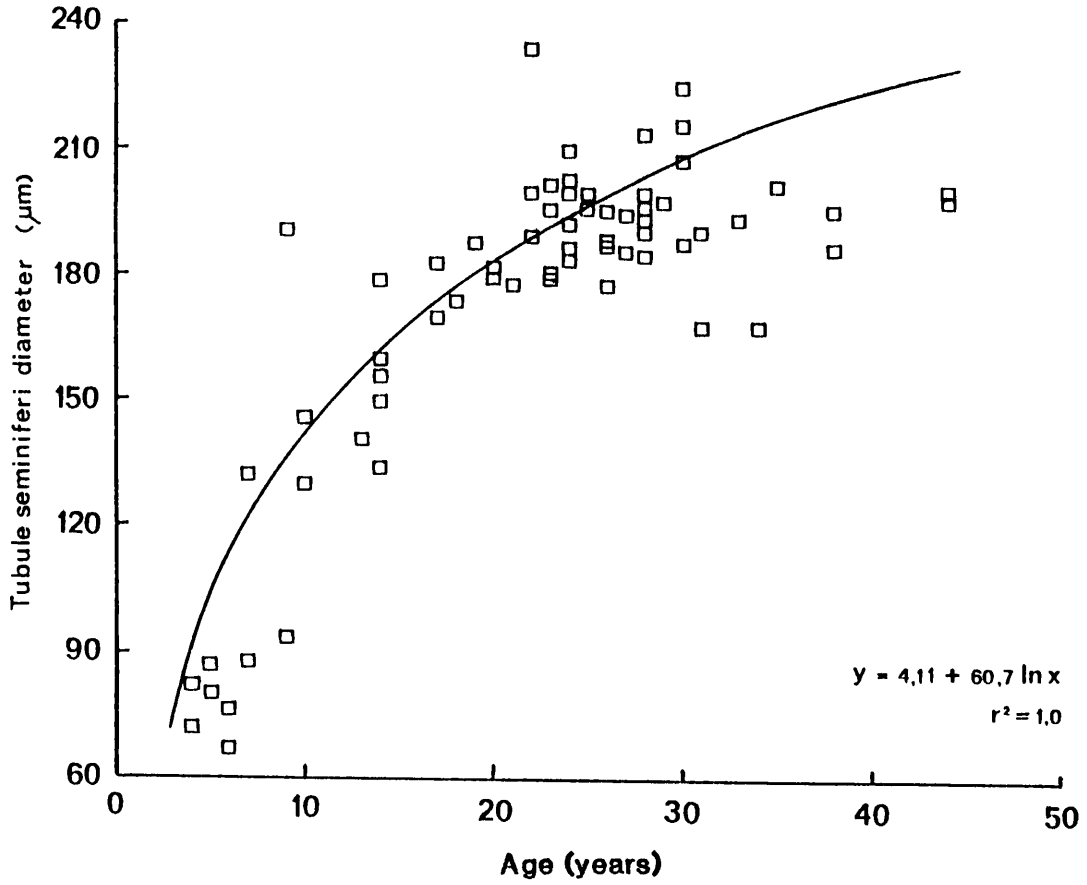

Figure 11. Seminiferous tubule diameter in relation to age in elephant bulls.

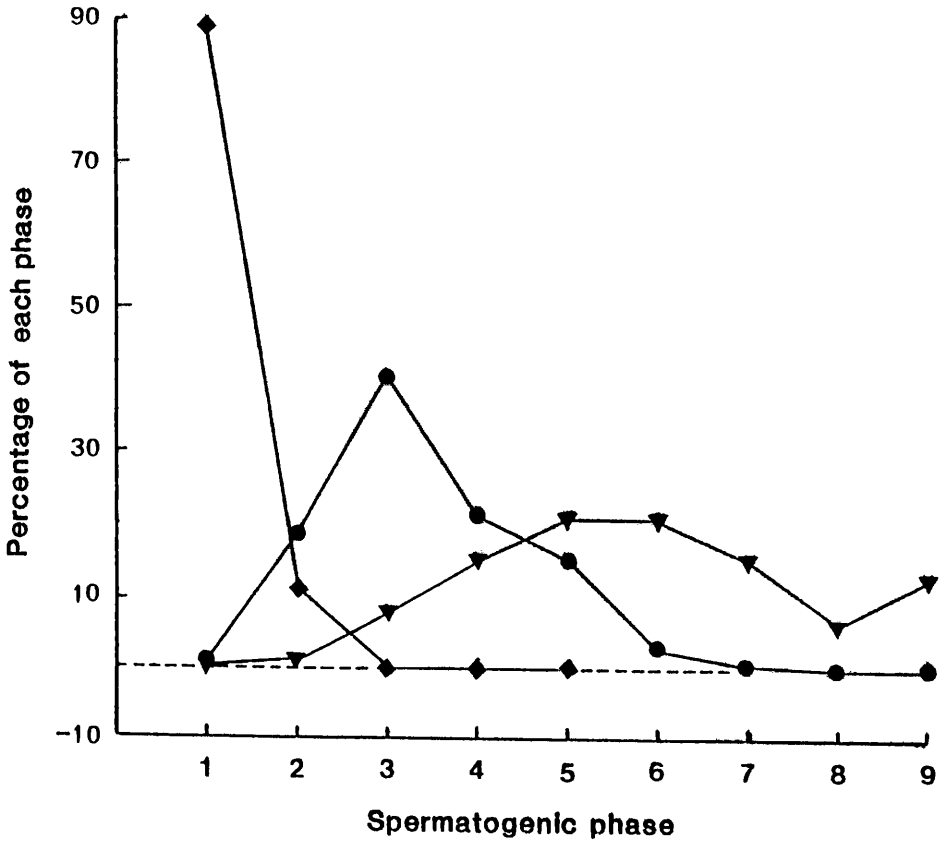

Figure 12. Mean percentage of each spermatogenic phase for immature (♦) , pubertal (●) and mature (▼) elephant bulls.

The student's t-test was used in comparing the average percentage of phases below phase five (resting phases) for each period of data collection in mature elephant bulls. There were no significant differences between the months March - May and October ($t = 0,08$; d.f. = 24) ; March - May and August ($t = 0,65$; d.f. = 30) ; August and October ($t = 0,83$; d.f. = 42).

When number of elephants with no spermatozoa and those with spermatozoa forming in the tubules (phase 8) were compared for the different months , evidence showed that production of sperm in mature bulls is independent of the season (Chi square = 0,48 ; $p > 0,01$).

Only two bulls with just mature spermatogenic phases (5-8) in their testes were present. Neither of these bulls had high Leydig cell size (11,9 & 11,7 μm) or numbers (8,2 & 10,0 per 1000 μm^2 of interstitium), high paired testes mass (3 900 & 3 200 g) or large tubule seminiferi diameters (194 $\pm$ 4 & 190 $\pm$ 18 μm respectively).

Leydig cells are abundant and prominent in the interstitium, number of cells per 1 000 μm^2 of interstitium was 9,8 $\pm$ 1,6 (range = 7,7 - 12,9 ; N = 67) for all bulls combined. Histological sampling techniques indicated no change in Leydig cell numbers other than individual variation. Both vacuolated and non-vacuolated Leydig cells were found, none of the 67 bulls studied had only non-vacuolated cells.

Vacuolated cells were found in immature, pubertal and mature bulls in breeding herds and bull associations.

Leydig cell diameter increased with age (Fig. 13), no relationship with the other variables were evident. Diameter of the cells averaged 11,0 ± 0,9 µm (range 8,2 - 14,0 µm ; N = 67). Abundance and size of Leydig cells showed no consistent relationship with the diameter of seminiferous tubules, season or circulating plasma testosterone concentrations. High Leydig cell numbers, large diameters and both vacuolated and non-vacuolated cells were frequently associated with low plasma testosterone concentrations and vice versa. Circulating testosterone as low as 0,07 ng/ml was measured in bulls with active spermatogenesis.

Seminiferous tubule diameters differed significantly when bulls with active and inactive temporal glands were compared, none of the other histological features examined differed significantly (Table VIII). The largest mean tubule seminiferous diameter ($\bar{x}$ = 225 ± 14 µm) was measured in a bull in full musth.

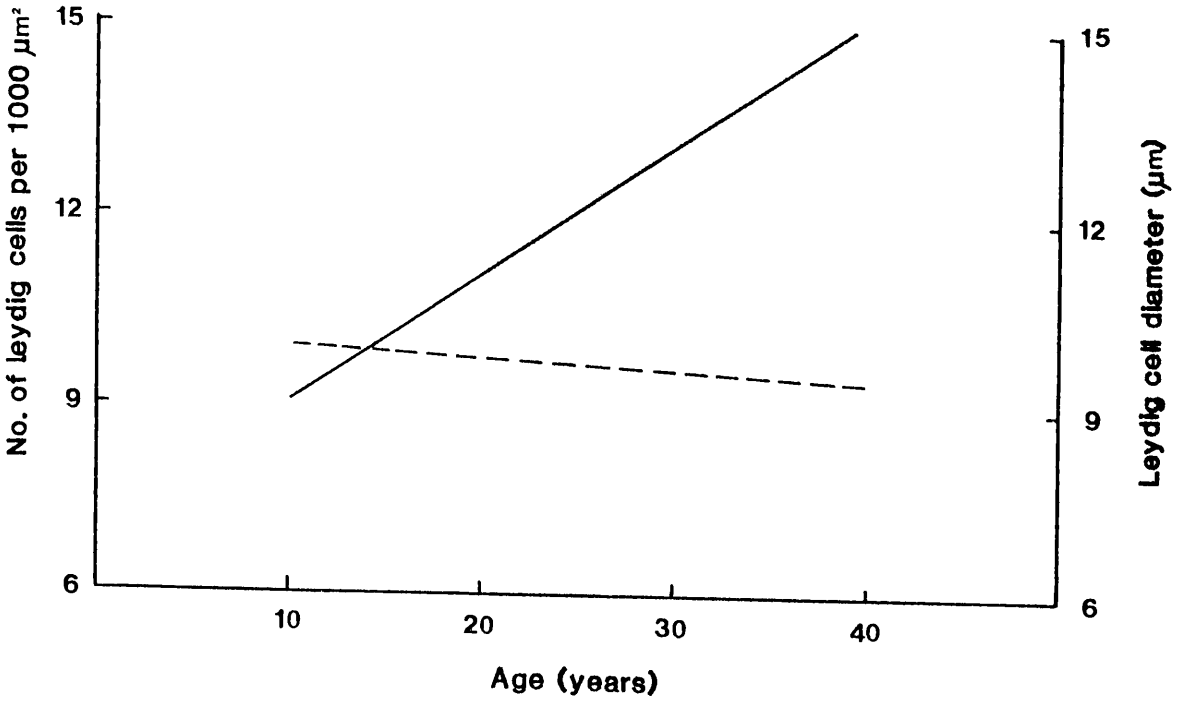

Figure 13. The relationship between Leydig cell number (--) per 1 000 μm^2 of interstitium and diameter (—) with age. Lines fitted through linear regression analysis, are described by the equations $y = 10,17 - 0,02x$ ($r^2 = 0,01$) and $y = 7,10 + 0,20x$ ($r^2 = 0,67$) respectively.

Table VIII. A comparison between some histological features of testes in mature (> 18 years) elephant bulls with active and less active temporal glands.

Histological feature	Active TG's (> 75 % activity)	Inactive TG's (50 - 74,9 %)	t-test value
Paired testes mass (g)	4 095 ± 970 (13)	3 260 ± 690 (29)	t = 1,59
Seminiferous tubule diam(μm)	199,1 ± 13,4 (15)	190,0 ± 13,2 (15)	t = 2,16*
Phase analysis % below phase 5	23,9 ± 19,0 (15)	25,2 ± 17,9 (35)	t = 0,23
Phase 8 (%)	6,3 ± 5,5 (15)	6,5 ± 5,6 (35)	t = 0,12

* significantly different (p > 0,05)

No. of animals in parenthesis.

Discussion

None of the criteria, namely, spermatogenic activity, mass of the testes and seminiferous tubule diameter, used for investigating a possibility of seasonal sexual cycles in elephant bulls provided substantial evidence that this occurs. Testes mass and diameter of seminiferous tubules are probably not good criteria to use since testes as well as tubules continue to grow throughout life (Johnson & Buss 1967 b , Short et al. 1967).

A phase analysis procedure was employed to obtain quantitative information about various cell associations of both immature and mature seminiferous epithelium in elephant testes. Johnson & Buss (1967 b) found that relatively slow testicular growth characterizes the first six years of life. Following this period , gonad development is rapid and by 9 - 11 years of age the testicular components reach histological levels comparable to those of mature elephants. Spermatids and spermatozoa are first produced by the seminiferous epithelium during this rapid development phase.

Older elephants frequently possessed a quantity of immature tubules which is in agreement with results of Johnson & Buss (1967 a). Information on the rate of spermatogenesis in elephants is unavailable.

In the rock hyrax (Procavia habessinica) Leydig cell size increased during their breeding season (Neaves 1973). There was no increase in Leydig cell size for any month in elephants during the present study. Cell size does not seem to be associated with relative secretory activity of testosterone. Leydig cell volume also showed a significantly positive correlation with plasma testosterone in the seasonally breeding rock hyrax (Neaves 1973). This was not the case for elephants, where cell size gave no index of secretory activity.

The cytoplasmic characteristics of Leydig cells recorded corresponds with findings of Buss & Johnson (1967) where both vacuolated and non-vacuolated cells were found. Functional significance of the varied cytoplasm in elephant Leydig cells is uncertain. There was no explanation that vacuolated cell cytoplasm characterized active cells and non-vacuolated cell cytoplasm indicating declining activity. The question whether Leydig cells are active is to a large extent a problem in histo- or cytochemistry.

There are no age related differences in Leydig cell densities in the testes, only diameter. The actual number of Leydig cells would, however, increase with age due to the testes itself that increases in size with age.

Low plasma testosterone concentrations were measured in elephants with active spermatogenesis. The wide fluctuation

in testosterone concentration in the present study does not represent cyclical changes in reproductive activity, but rather inhibition of lower ranking individuals by dominant bulls in the hierarchy which could have the effect of low levels of circulating androgens. (See Chapter 3 on testosterone concentration and temporal gland histology). Furthermore, only single blood samples were collected from animals during the present study and true androgen concentrations could have been obscured due to pulsatile secretion of testosterone.

Results indicated no marked differences, other than seminiferous tubule diameter, when testicular features of bulls with active temporal glands, including musth bulls, were compared with bulls with less active glands. This suggests that temporal gland activity in bulls is not related to a period of increased sexual activity. Howard, Bush, De Vos & Wildt (1984) furthermore examined ejaculate quality, sperm characteristics and testosterone concentration during electroejaculation procedures. They found field secretion from temporal glands and ejaculate quality were independent and that high quality semen could be collected from mature African elephants regardless of season or temporal gland activity.

No distinct breeding season has been reported for the African elephant, conceptions have, however, been found to be related to rainfall (Laws 1969 , Laws, Parker & Johnson

1970 , Hanks 1972). In Kruger National Park on an average 70 % of all conceptions occur during the six wet months from November to April (Smuts 1975). The latter author used the Hugget and Widdas (1951) method for determining fetal ages.

Laws et al. (1970) suggested that rainfall and the resulting improvement in vegetation nutrititive quality is the proximate factor in controlling the seasonality of conceptions in elephant cows. Thus, if there is any reproductive cyclicity in the African elephant bulls, it must necessarily manifest itself on an individual basis. All the inconsistencies described in this section emphasize individual variation in male reproduction in the African elephant. For a population that does not breed seasonally it is what one would expect, that all mature bulls are apparently capable of fertile matings at any time.

CHAPTER 5

PROGESTERONE AND OVARIAN ANATOMY

Introduction

Studies have been carried out on corpora lutea of African elephants (Loxodonta africana) ; progesterone concentrations were found to be very low in corpora lutea and only minute quantities of progesterone could be measured in peripheral plasma by early investigators (Short & Buss 1965, Short 1966, Smith, Hanks & Short 1969, Hanks & Short 1972). Therefore corpora lutea were reported to have a significant but limited secretory capacity for progesterone (Smith et al. 1969, Ogle, Braach & Buss 1973).

Plotka, Seal, Schobert & Schmoller (1975) reported a modest increase in plasma progesterone concentrations during pregnancy. This was confirmed by McNeilly, Martin, Hodges & Smuts (1983) who detected significantly higher progesterone levels during pregnancy with considerable overlap in values between pregnant and non-pregnant elephants.

The data presented in the present paper examine in some detail the macroscopic anatomy of ovaries of pregnant and non-pregnant African elephants at different stages of reproduction. Relationships between ovarian structures and

circulating concentrations of progesterone in maternal and fetal plasma were investigated as well as fluctuations during the ovarian cycle.

Materials and methods

Animals. Plasma samples were collected between 13h00 and 16h00 from family groups of elephants culled in the Kruger National Park. Heparinized blood samples, taken peripherally after the elephants were killed, were centrifuged within two hours after collection, the plasma removed and then stored at -20 °C until assayed.

In the field the uteri and ovaries of elephant cows were examined and weights and measurements of embryos and fetuses recorded. Data were collected from elephants in different stages of reproduction and following Laws & Parker (1968), elephant cows were classified as : (i) immature - when their ovaries contained neither large follicles, corpora lutea, nor corpora albicantia; (ii) pubertal - no corpora lutea or corpora albicantia but at least one follicle >5 mm diameter, and (iii) mature - at least one corpus luteum or corpus albicans in the ovaries. Fetuses were weighed using an appropriate spring balance. Very small fetuses were returned to the laboratory for weighing the same day. Heparinized blood samples were collected from fetuses by cardiac puncture and fetal ages were determined by the revised method of Craig (1984).

Ovaries of both pregnant and non-pregnant elephants were trimmed of mesentery, weighed and the maximum diameter of all corpora lutea (Cl) measured to the nearest 0,5 mm with a vernier caliper. Each ovary was sliced into 3 mm sections and Graafian follicles, small Cl, including accessory Cl, and corpora albicantia were identified, counted and measured. Corpora lutea refers to the yellow structures which formed after ovulation (Smith & Buss 1975). Ovulation stigmata could be seen after recent ovulations. Volume of luteal tissue was estimated by means of the formulae for volumes of spherical and ovoid bodies, depending on their shape, respectively.

Elephants with only corpora lutea present in the ovaries were assigned to the luteal phase and those with various numbers of small follicles and at least one Graafian follicle (8 - 20 mm diameter), but no Cl, to the follicular phase of the cycle.

Age determination to the nearest year was based on the replacement of molar teeth in the lower jaw (Laws 1966).

Radioimmunoassay of progesterone. The procedure was similar, with minor modifications, to that of Haresign, Foster, Haynes, Crighton & Lamming (1975) and van Aarde (1985). Duplicate plasma aliquants (0,1 ml) were extracted with 2,0 ml petroleum ether (Saarchem.(Pty) Ltd., Krugersdorp, South Africa). Standards ranging from 3,9-1000 pg progesterone (Δ^4-Pregnene-3,20-dione, Sigma Chemical Co., Dorset, U.K.) in ethanol were prepared in duplicate and included in each assay. Antiserum (R.P. Millar, Department of Chemical Pathology, University of Cape Town, Cape Town, South Africa) in phosphate buffer (0,1 ml) at a dilution of 1:8000 was added to standards, reagent blanks and plasma extracts. The mixture in each tube was incubated at room temperature (23-25 °C) for 10 min and , after the addition of 0,1 ml (~10 000 c.p.m.) [1,2,6,7-^{3}H] progesterone (Radiochemical Centre, Amersham, Bucks, U.K.) in phosphate buffer, the contents of the tubes were mixed for one min on a vortex mixer and incubated at 4 °C for at least 12 h (usually overnight). The separation of antibody-bound and free steroid was carried out at 4 °C by adding 0,8 ml dextran-coated charcoal consisting of a suspension of charcoal (Activole, Merck, Darmstadt, F.R.G.) in phosphate buffer (0,156 g/100 ml) containing 0,0156 g Dextran T-40 (Pharmacia, Uppsala, Sweden) to the contents of each tube. Extraction efficiency and the original volume of plasma extracted were taken into account when calculating the concentrations of progesterone in plasma samples.

Validation. The antiserum specificity was described by the supplier and cross-reactions of other steroids were : Pregnenolene , 1,9% ; 17 hydroxy-progesterone , 2,8% ; 11 hydroxy-progesterone , 24,9% ; 5 Pregnane-3-20-dione , 21,7% ; 20 hydroxy-4 pregnane-3-one , 0,3% ; 11 Deoxycorticosterone , 2,1% ; 11 Deoxycortisol , 1,6% ; Cortisol , <0,1% ; Testosterone , 4-Androstenedione , 17 Estradiol and Estrone , <0,001. Sensitivity of the assays , defined as twice the standard deviation of blank values ranged from 4,2 - 16 pg/ml (mean 11,1 ± 5,0 pg/ml; n=4). Buffer blanks included in the assays contained less than 11,1 pg progesterone equiv./ml. Recovery of known amounts of unlabelled progesterone (250, 500 & 1000 pg/ml) did not differ significantly from expected values and dilution curves were parallel to the respective standards. Recovery of ^{3}H-progesterone from plasma varied from 80,2 - 91,9 % (mean 85,3 ± 4,3 % ; n=4). Intra- and interassay coefficients of variation were 9,8 and 9,7 % respectively.

Results

Progesterone

Circulating levels of progesterone (Fig. 14) in pregnant elephants ranged from 75 - 1331 pg/ml ($\bar{x}$ = 664,5 ± 341,6 pg/ml ; N = 44) and did not differ significantly (t = 1,41 ; d.f. = 65 ; p < 0,01) from the values of non-pregnant elephants (range 212 - 1283 pg/ml ; $\bar{x}$ = 771,3 ± 268,1 pg/ml ; N = 23).

During the first half of pregnancy progesterone concentration ($\bar{x}$ = 830,8 ± 299,1 pg/ml ; N = 22) did differ significantly (t = 3,71 ; d.f. = 42) from that during the second half ($\bar{x}$ = 497,7 ± 296,9 pg/ml ; N = 22), there being a gradual decline towards term (Fig. 14). No consistent relationship existed between luteal tissue volume and circulating plasma progesterone concentration (Fig. 15) in pregnant or non-pregnant cows.

Fetal plasma progesterone concentrations averaged 1148,8 ± 487,1 pg/ml (N = 12) and increased with gestation age, while maternal levels decreased (Fig. 16). During the first 14 - 15 months of gestation there was a significant positive correlation between maternal and fetal plasma progesterone concentrations (Pearson r = 0,96 ; Spearman r = 1,00 ; N = 5)(Walpole & Myers 1985). Values were not related during the last trimester of gestation (Pearson r

= -0,053 ; Spearman r = -0,288 ; N = 7). The correlation throughout gestation was not significant (Pearson r = -0,098 ; Spearman r = -0,137 ; n = 12).

In two of the four mature non-pregnant elephant cows with high plasma progesterone concentrations the ovaries had one developing follicle in the late follicular growth phase (~ 15 mm in diameter) and in another two cows the ovaries had 3 and 4 developing follicles (~ 8 mm in diameter) respectively. Progesterone concentration ($\bar{x}$ = 1083 ± 144 pg/ml ; N =4) was significantly higher than that for non-pregnant cows (t = 4,08 ; d.f. = 25)(Fig. 17). Progesterone concentration in non-pregnant females in the luteal phase (only corpora lutea present in the ovaries) was high ($\bar{x}$ = 705,5 ± 163,4 pg/ml ; N = 8) but did not differ significantly (t = 2,4 ; d.f. = 12) from that of mature cows with only corpora albicantia and small follicles present in the ovaries ($\bar{x}$ = 435 ± 216 pg/ml ; N = 5)(Fig. 17).

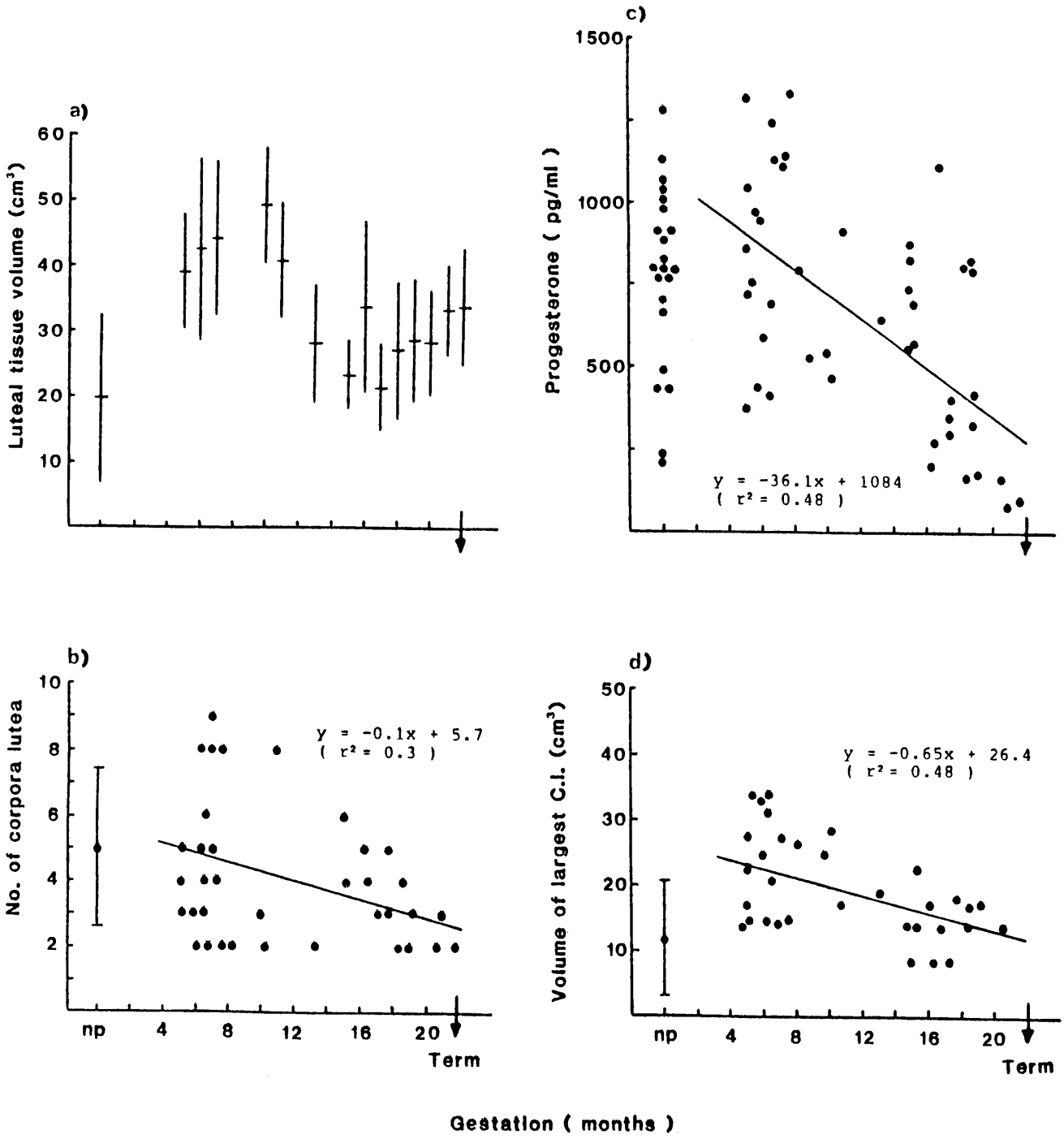

Figure 14. The relationships between (a) total luteal tissue volume ; (b) number of corpora lutea in maternal ovaries ; (c) circulating maternal plasma progesterone concentration ; and (d) volume of the largest single corpus luteum and gestation time. Values for non-pregnant (np) elephants are also presented.

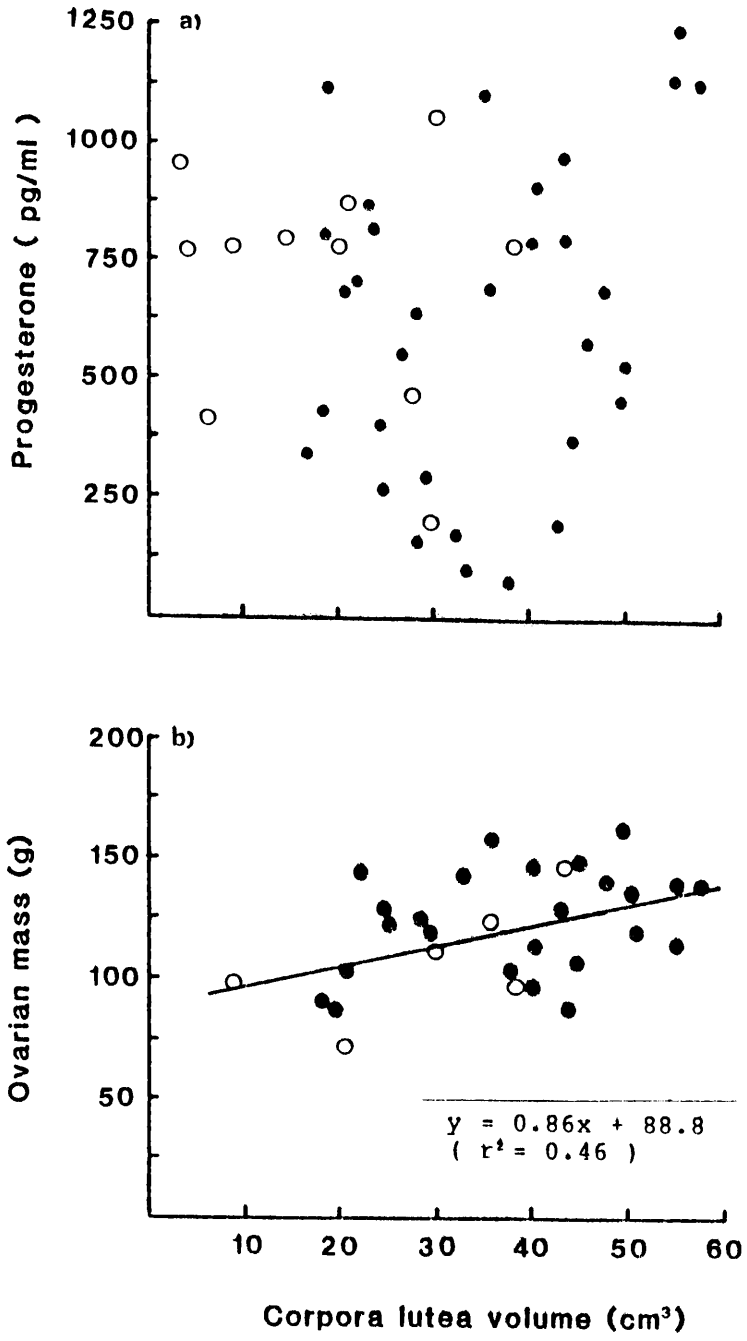

Figure 15. The relationship between (a) circulating concentrations of plasma progesterone and (b) ovarian mass in pregnant (•) and non-pregnant (o) elephants and total corpora lutea volume.

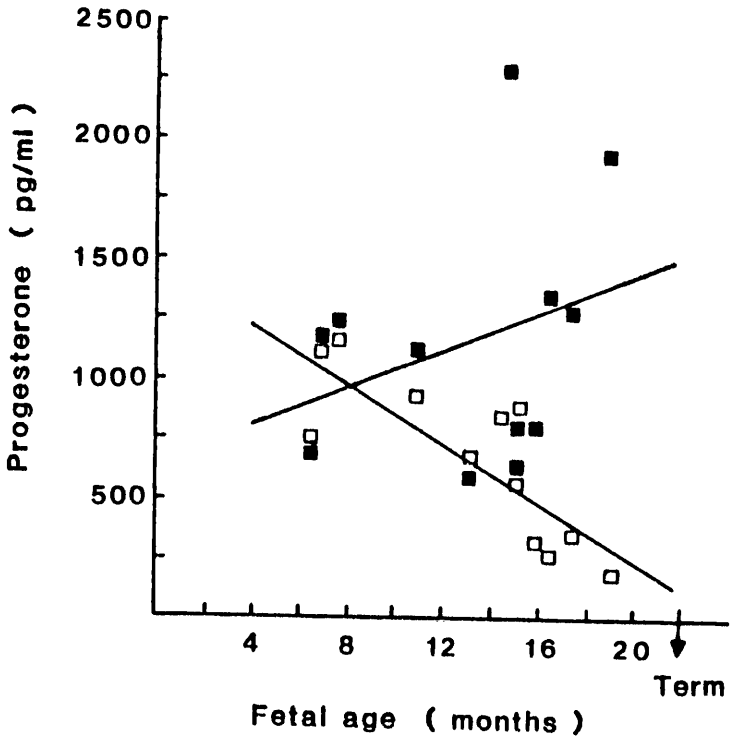

Figure 16. The relationship between maternal (□) and fetal (■) plasma progesterone concentration with fetal age. Lines were fitted through linear regression analysis for maternal ($y = -60.9x + 1458$; $r^2 = 0.79$) and fetal plasma ($y = 37.4x + 657$; $r^2 = 0.31$).

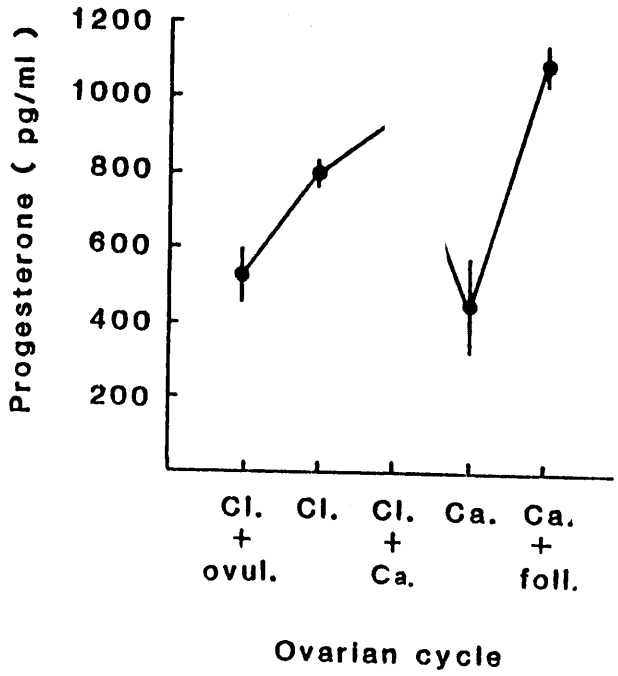

Figure 17. Plasma progesterone concentration (mean ± s.e.m.) in non-pregnant elephants in relation to the ovarian cycle where corpora lutea (Cl.), recent ovulation sites (ovul.) , corpora albicantia (Ca.) and large follicles (8-15 mm diameter) were present in the ovaries.

Ovarian morphology

The number of corpora lutea counted in young elephant cows (< 20 years ; N = 9) did not differ significantly from that in older cows (≥20 years ; N = 12)(t = 1,9). Volume of corpora lutea tissue in young (<20 years) pregnant cows ($\bar{X}$ = 36,3 ± 12,2 cm³ ; N = 9) did not differ significantly (t = 0,65) from that of cows over 20 years ($\bar{X}$ = 32,8 ± 11,9 cm³ ; N = 13).

The number of corpora lutea ($\bar{X}$ = 4,4 ± 2,3) was not related to luteal tissue volume ($\bar{X}$ = 36,7 ± 12,3 cm³ ; N = 40) in pregnant or in non-pregnant cows where the average number of corpora lutea was 4,8 ± 2,4 and corpora lutea volume 18,9 ± 13,4 cm³ (N = 16).

Corpora lutea were present throughout pregnancy and new ovulations did not occur during pregnancy. There was a slight decrease in total luteal tissue volume and number in relation to gestation period (Fig. 14). No mature non-pregnant cows with only corpora albicantia present in the ovaries were collected.

Corporà lutea volume increase was associated with increased ovarian mass in both pregnant and non-pregnant elephants (Fig. 15). The mean volume of corpora lutea on the side of implantation was significantly greater (t = 2,53 ; d.f. = 62)(Fig. 18). Left and right ovaries did not differ

significantly in weight (paired t = 0,06 ; d.f. = 78) nor did weight differ significantly between those from the side of implantation and the contralateral side (t = 2,4 ; d.f. = 48).

During the first half of gestation corpora lutea volume ($\bar{x}$ = 43,1 ± 10,9 cm³) was significantly greater (t = 4,88 ; d.f.= 37) than during the second half ($\bar{x}$ = 38,4 ± 7,9 cm³). For pregnant cows luteal volume ($\bar{x}$ = 36,7 ± 12,3 cm³) was significantly greater (t = 4,63 ; d.f. = 37) than for non-pregnant cows ($\bar{x}$ = 19,7 ± 12,8 cm³)(Fig. 14).

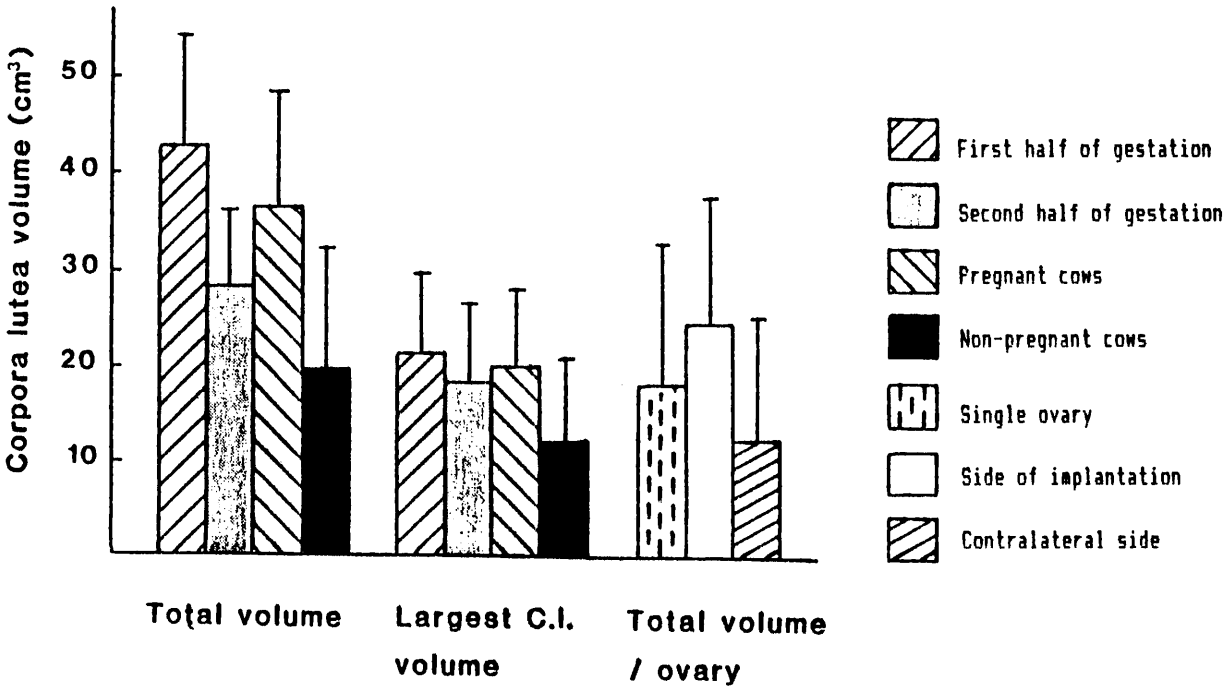

Figure 18. The variation in total and largest corpora lutea (Cl) volume in pregnant and non-pregnant elephants and total volume per ovary in relation to the side of implantation.

Discussion

In the present study no clear distinction between plasma progesterone concentrations in pregnant and non-pregnant elephant cows could be found due to variation, particularly for stage of pregnancy. Progesterone concentration increased during early pregnancy (5-8 months) and levels during the first half of gestation were significantly higher than during the second half. This is at variance with McNeilly et al. (1983) who found progesterone concentrations in pregnant elephant cows to be significantly greater than in non-pregnant cows, with maximum levels occurring at mid-pregnancy (9 - 12 months).

This difference is probably due to an error Craig (1984) points out in the published formula (Hugget & Widdas 1951) for calculating date of conception from fetal mass for elephants used by McNeilly et al. (1983). The accepted formula for the African elephant based on the Hugget and Widdas (1951) equation was first formulated by Perry (1953) and has been in use ever since. The revised method of Craig (1984) was used in the present study to determine fetal age, this implies a longer early phase of slow growth until followed by an increased exponential faster fetal growth rate.

Smuts (1975) reported that 70% of conceptions in the Kruger National Park occurred during the main rainfall months. In

the present study the formula, $t = 106w^{\frac{1}{3}} + 138$, proposed by Craig (1984) was used, where t is the fetal age and w the fetal mass. In this revised equation the estimate of t_o has been increased to 138 days from 66 days in the Hugget and Widdas equation. The initial growth rate of small elephant fetuses is slow and possibly deviates from a linear growth relationship (Craig 1984). To get small fetal ages of less than 140 days, weights of less than six gram are necessary. This equation shows the actual breeding cycle of elephants to be more clearly seasonal and breeding does appear to be associated with rainfall on a month to month basis (Craig 1984).

Corpora lutea were retained throughout gestation and ovaries in all pregnant females contained more than one luteal body (mean = 4,4 ± 2,3 ; N = 40). The decline in corpora lutea volume , decline in largest follicular size and no stigmata of recent ovulations seen in pregnant animals indicate that there is no replacement or augmentation of corpora lutea during pregnancy. Accessory corpora lutea were seen to form in one pregnant female with a 6,5 month old fetus where a watery follicle, 20 mm in diameter was found.

There is no increase in number of corpora albicantia with gestation (Smith & Buss 1975) and these stuctures persist for appoximately 77 months in the ovary, often into the next pregnancy (Buss & Smith 1966). Regression of corpora

lutea is very slow and explains why no significant decline in corpora lutea number was found but only in volume and largest follicle size. Regressing corpora lutea occurred in pregnant females and in 68,3 % of the ovaries examined corpora albicantia were present. No evidence for the formation of supplementary corpora lutea was found.

McNeilly et al. (1983) suggested a possible increase in progesterone concentration in relation to an increase in corpora lutea number in the ovaries of elephants. On the other hand , Hanks & Short (1972) suggested that a critical mass of luteal tissue needs to accumulate before conception can occur since in their study very little progesterone was secreted by corpora lutea. The present study indicated that neither number or total corpora lutea volume is critical to maintain progesterone secretion ; it is suggested that secretory activity of one or more corpora lutea play an important role in progesterone secretion.

Progesterone concentration appears to be reflected by lutein cell size in a general way ; corpora lutea with the largest lutein cells have higher progesterone concentrations and those with lower steroid levels contained the smallest lutein cells (Ogle et al. 1973). Lutein progesterone concentration and cell size increase during early pregnancy, peaking during the third month after which there is a decline, reaching a nadir during the last three months (Ogle et al. 1973). Buss and Smith (1966)

presented histological evidence which indicated a reduction in corpora lutea function with most steroidogenical activity between 2 and 14 months of gestation (Hugget & Widdas 1951 equation). This agrees with the present data where there is a decline in progesterone concentration and corpora lutea volume in the second half of gestation towards term.

The poor relationship between circulating plasma progesterone concentrations and luteal activity as suggested by volume and luteal cell size (Ogle et al. 1973, Buss & Smith 1975) may be indicative of extra ovarian sources of progesterone production. Care should be taken, however, when comparing endocrine activity with corpora lutea morphology (volume) or appearance only. The contribution of the placenta to circulating levels of progesterone to either maternal or fetal blood in elephants has never been assessed. During the first two-thirds (~ 14 months) of gestation a significant correlation was found between maternal and fetal plasma progesterone concentrations. Maternal progesterone levels decreased during the last trimester of gestation while the fetal levels increased and there was no significant correlation (Fig. 16). This indicates a possible role of the placenta in progesterone secretion during the second half of gestation, or it could be due to a fetal adrenal progesterone production

Zarembka, Plotka, Seal, Simmons, Teare, Phillips & Hinshaw (1987) suggested that the length of the ovarian cycle in African elephants, based upon changes in serum progesterone, averaged 15,0 ± 0,4 weeks ; luteal phase 9,2 ± 0,2 weeks and interluteal phase 5,8 ± 0,4 weeks. Serum progesterone concentration averaged 328 pg/ml and ranged from < 50 to 933 pg/ml during the luteal phase (Zarembka et al. 1987). Progesterone values were much higher during the luteal phase in the present study ($\bar{x}$ = 705,5 ± 163,4 pg/ml). Results indicate a decline in plasma progesterone levels towards the end of the luteal phase and an increase with follicular development (Fig. 17).

The four non-pregnant elephant cows with developing follicles in the late follicular growth phase, filled with follicular fluid, had high plasma progesterone concentrations. It is, however, not possible to estimate the relative contribution intrafollicular hormones make to those in peripheral plasma (McNatty, Hunter, McNeilly & Sawers 1975). Evidence suggests that intrafollicular steroids diffuse slowly out of follicular fluid into the ovarian vein (YoungLai & Short 1970). This seems to be the case for elephants, two of the cows had only a single follicle present and the other two cows had two and three corpora lutea with small volumes (mean = 2,00 ± 0,50 cm^3) respectively accompanied by high progesterone concentrations.

Little is known about the production and metabolism of progesterone and the relative contribution of the uterus and placenta in the elephant and the rates of secretion of this steroid are not necessarily reflected in the circulating levels.

CHAPTER 6

TEMPORAL GLAND SECRETION

Introduction

Temporal gland secretion of the African elephant has been analysed by gas chromatography and mass spectrometry (Adams, Garcia & Foote 1978 , Wheeler, Rasmussen, Ayorinde, Buss & Smuts 1982). Adams et al. (1978) indicated the presence of more than 40 volatile components in the secretion in contrast to the five volatile components detected by Wheeler et al. (1982). There were no clear and consistent differences in constituents of the secretion between the sexes (Wheeler et al. 1982).

Communication systems used by elephants are vocalization (Berg 1983) and infrasonic calls which have been reported for the Asian elephant (Payne, Langbauer & Thomas 1986). A possible role of temporal gland secretion in chemical communication has been suggested (Buss et al. 1976). Eyesight of elephants is poor and scent is a much more likely sense to be used in communication (Wyatt & Eltringham 1974).

The purpose of the present study was to compare the secretion from a full-musth bull with that of other bulls not in musth.

Material and methods.

Sample collection. All temporal gland secretion (TGS) samples were obtained from elephants immediately after immobilization with Scoline (Suxamethonium Chloride, Holpro. Chemical Co., Johannesburg, South Africa) during culling operations.

A blunt needle (19G) connected to a 20 ml plastic syringe was inserted into the external pore of a gland to collect secretion. Small volumes (0,5 - 1,5 ml) of secretion were obtained from non-musth bulls (N = 4) and about 6 ml from the musth bull. Immediately after collection, samples were sealed in plastic vials, kept cool and within two hours of collection stored at -20 °C until analysed.

A pooled TGS sample from four non-musth bulls (aged 17, 22, 27 & 44 years respectively) was compared with that of a 41 year old bull in full-musth.

Gas chromatography. Details of the technique, apparatus, its sensitivity and operation for high resolution gas-liquid chromatography, based on the dynamic solvent effect, was described by Apps, Pretorius, Lawson, Rohwer, Centner, Viljoen & Hulse (1987). The analysis was carried out at the Institute for Chromatography, University of Pretoria.

Results

Gas chromatograms of pooled temporal gland secretion of four non-musth elephant bulls is presented in Figure 19a and that of a full-musth bull in Figure 19b. The musth bull showed several components which did not occur in bulls which were not in musth.

Components of the secretion are presented in Table IX of which Alpha-pinene, n-Hexanal and the sesquiterpenes are possibly derived from the diet.

Table IX. Components of temporal gland secretion in mature elephant bulls.

Phenol
o-cresol
p-cresol
Alpha-pinene
n-Hexanal
Farnesol
4-methyl-3-pentenoic acid
Octanol
3,7-dimethyl-1,6-octadien-3-ol
6,10-dimethyl-(E)-5,9-undecadien-2-one
Sesquiterpenes

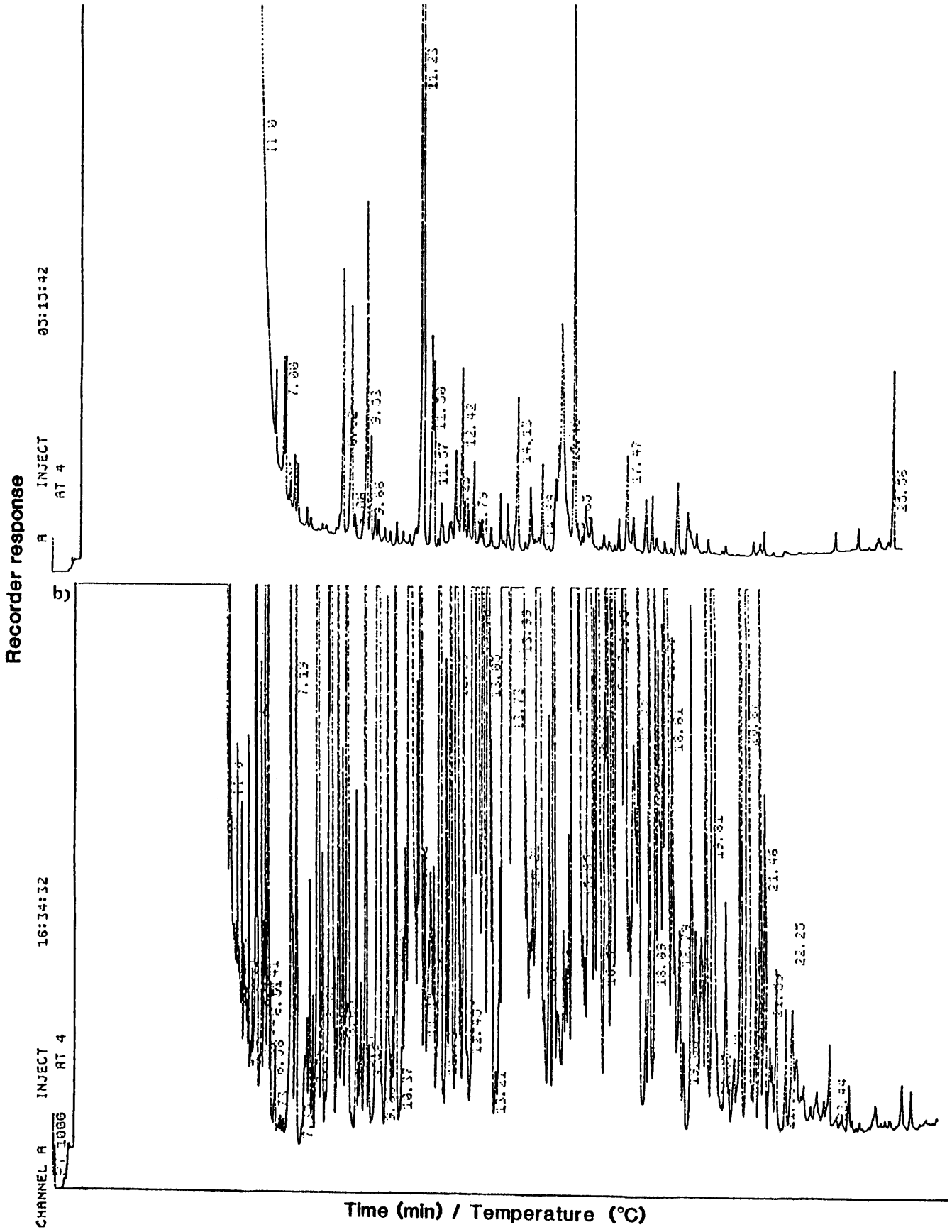

Figure 19. Gas chromatograms showing extracts of temporal gland secretion under standard conditions of (a) non-musth bulls and (b) one full-musth bull.

Discussion

Earlier reports (Wheeler *et al.* 1982) found no correlation between either sex or age and the composition of temporal gland secretion. The present study demonstrated that the odour of a full-musth bull is likely to be perceived as different from other conspecific odours due to the major differences in constituents of the secretion.

The hypothesis that temporal glands might function in herd member recognition has been proposed (Sikes 1971). Wyatt & Eltringham (1974) furthermore suggested that family groups can keep together through a sense of smell.

Some of the components in the secretion are possibly derived from the diet. A behavioural study (Buss 1961) showed that feeding habits and other behavioural patterns of individual elephants within groups, which are closely related, were similar. The assumption can be made that secretion from elephants in the same group will be roughly similar in some respects. Some degree of group member recognition may then be possible.

There are individual differences in chemical composition of the secretion. Bacterial decomposition of cresol in elephant TGS, especially within the temporal gland duct and external pore, may result in cresol differences among individuals and individual recognition (Wheeler *et al.*

1982). Halpin (1986) pointed out that it is not possible at present to state that individual recognition occurs. Individual discrimination, where an individual has the ability to distinguish one individual conspesific odour from another is possible (Halpin 1986).

The presence of o-cresol in TGS which has not been reported before (Adams et al. 1978 , Wheeler et al. 1982) was demonstrated.

Secretion from temporal glands in a group of captive elephants did not seem to affect behaviour of other individuals in the enclosure (Adams & Berg 1977). Gorman (1987) demonstrated that young African elephants showed no overt behavioural response, beyond a general interest, when they were exposed to volatiles present in TGS. The latter secretion was made up from synthetic chemical compounds. Whether volatiles play a role in chemical communication among African elephants remains to be ascertained by behavioural studies, together with detailed analysis of secretions from the temporal glands.

CONCLUSION

The present study investigated aspects of the temporal gland activity and physiology of the African elephant (Loxodonta africana). The prime objective was to obtain information on aspects of reproduction with emphasis on histologically determined temporal gland activity and endocrinology in elephants of both sexes.

Confirmation that temporal gland activity occurs in African elephants of both sexes from an early age was found. Elephants from breeding herds, including immature individuals, showed secretion of temporin trickling down the face. No relationship between temporal gland activity and the state of reproduction was evident. Evidence indicates individual cyclicity in temporal gland activity in bulls and cows with only mature bulls, over 24 years, experiencing musth periods. Younger bulls in bull associations which were physiologically sexually mature did not show musth. In a high percentage of bull groupings one of the bulls had temporal glands more active than the other bulls. This supports the hypothesis of Hall-Martin & Van der Walt (1984) that the temporal glands in African elephant bulls are used for the maintenance of hierarchical status in bull associations.

Increases in both plasma and temporal gland secretion testosterone concentrations are correlated with an increase in temporal gland activity in bulls. This is in agreement with results of recent studies for mature African elephant bulls. In the present study temporal gland activity was quantitatively determined through histological examination. During puberty there is an increase in testosterone concentration with no marked increase in temporal gland activity. Glandular activity is fairly constant for bulls from all age classes, only mature bulls experience musth, when they have fully active temporal glands. Within bull associations of different sizes there was usually one bull with an active temporal gland and a higher testosterone concentration than the other bulls.

Evidence showed that the production of sperm in mature bulls is independent of season. None of the criteria used to determine the possibility of seasonal sexual cycles in elephant bulls ; spermatogenic activity, mass of testes and seminiferous tubule diameter, provided substantial evidence that this occurs. Results indicated no marked differences, other than seminiferous tubule diameter, when testicular features of bulls with active temporal glands, including musth bulls, were compared with bulls with less active glands. This, in contrast to earlier reports and popular belief, indicates that temporal gland activity in bulls is not related to a period of increased sexual activity. Musth is definitely related to a number of behavioural changes

and the associated movements of bulls out of their home ranges as was described recently (Hall-Martin 1987, Poole 1987).

Mean plasma progesterone concentration measured in pregnant and non-pregnant elephant cows did not differ significantly from each other due to considerable variation, particularly for stage of pregnancy. Maximum progesterone concentrations were recorded during early pregnancy (5-8 months) and declined gradually towards term. The number of corpora lutea or total luteal tissue volume is not critical in maintaining progesterone secretion. An increase in plasma progesterone concentrations with the luteal phase of the ovarian cycle was evident. A possible role of the placenta in the second half of gestation is indicated by an increase in fetal progesterone concentrations towards term. No relationship between stage of pregnancy and temporal gland activity was apparent.

Gas chromatographic analysis manifested the existence of individual differences in chemical composition of temporal gland secretion between a full-musth bull and non-musth bulls. The odour from the temporin of a musth bull is likely to be perceived as different from conspecific odours. Some degree of group member recognition or individual discrimination amongst elephants may be possible.

Hall-Martin (1987) proposed the hypothesis that musth is an adaptation to maximize chances of breeding in some younger African elephant bulls. Based on data from all the parameters investigated in the present study, it is suggested that temporal glands in African elephant bulls are primarily used for signalling dominance in the hierarchical structure by means of olfactory signals.

GEVOLGTREKKING

In die huidige studie is aspekte van die temporaalklier-aktiwiteit en fisiologie van Afrika olifante (Loxodonta africana) ondersoek. Die vernaamste doel was om voortplantingsgegewens in olifante van beide geslagte te bepaal, met die klem op histologies vasgestelde temporaalklier-aktiwiteit en endokrinologie.

Bevestiging is verkry dat temporaalkliere reeds aktief raak in jong Afrika olifante van beide geslagte. Olifante in teeltroppe, insluitende onvolwassenes, het sekresie van die kliere getoon. Geen verwantskap tussen temporaalklier aktiwiteit en voortplantingstoestand was duidelik nie. Feite dui op 'n individuele siklus in die aktiwiteit van temporaalkliere in bulle en koeie, met slegs volwasse bulle ouer as 24 jaar wat musth periodes ondergaan. Jonger, fisiologies geslagsryp bulle in bulassosiasies, het geen musth periodes getoon nie. In 'n groot aantal bul-groeperings het een van die bulle meer aktiewe temporaalkliere as die ander getoon. Hierdie feit vind aansluiting by die hipotese van Hall-Martin & Van der Walt (1984) dat die temporaalklier in Afrika olifantbulle funksioneel is in die behoud van die hierargiese struktuur in bulassosiasies.

Toenames in beide plasma- en temporaalkliersekresie testosteroon konsentrasies in bulle, toon 'n korrelasie met 'n verhoging van temporaalklier-aktiwiteit. Laasgenoemde is in ooreenstemming met resultate van onlangse studies op volwasse Afrika olifant bulle. Temporaalklier aktiwiteit is in die huidige studie kwantitatief bepaal deur histologiese ondersoek. Tydens puberteit is daar 'n toename in plasma testosteroon konsentrasie sonder 'n merkbare toename in temporaalklier-aktiwiteit. Aktiwiteit van die kliere is redelik konstant vir bulle van alle ouderdomsgroepe, slegs volwasse bulle ondervind musth, wanneer die kliere uiters aktief is. In bulassosiasies van verskillende grootes wat gemonitor is, was daar meestal een bul met aktiewe temporaalkliere en 'n hoër plasma testosteroon konsentrasie as die ander.

Bewyse dui daarop dat spermproduksie in volwasse bulle onafhanklik van seisoen is. Die kriteria ondersoek ter aanduiding van 'n seisoenale seksuele siklus in olifant bulle; spermatogenese, testes massa en spermbuis deursnee, het geen bewyse daarvoor gelewer nie. Vergelyking van testikulêre kenmerke tussen bulle met aktiewe- (insluitende bulle in musth) en minder aktiewe temporaalkliere het geen duidelike verskille getoon nie. In teenstelling met vorige verslae en die algemeen aanvaarde idee, toon dit dat temporaalklier-aktiwitiet nie verband hou met 'n tydperk van intense seksuele aktiwiteit nie. Musth bulle toon verskeie gedragspatrone en daarmee verbonde, bewegings uit

hulle tuisgebiede uit, soos onlangs beskryf (Hall-Martin 1987, Poole 1987).

Die gemiddelde plasma progesteroon konsentrasie in dragtige en nie-dragtige olifant koeie het nie betekenisvol verskil nie weens aansienlike variasie, veral ten opsigte van die dragtigheidstadium. Hoë progesteroon konsentrasies was gedurende vroeër stadia van dragtigheid (5-8 maande) gemeet, met 'n afname nader aan die geboortedag. Die aantal corpora lutea of totale luteale weefsel volume, is nie noodsaaklik vir die handhawing van progesteroon afskeiding nie. Tydens die luteale fase van die estrussiklus was 'n toename in plasma progesteroon konsentrasie waarneembaar. Die plasenta is moontlik verantwoordelik vir progesteroon sekresie tydens die tweede helfte van dragtigheid as gevolg van 'n toename in fetale hormoonvlakke. Die stadium van dragtigheid en temporaalklier aktiwiteit het geen verwantskap getoon nie.

Gas chromatografiese analise het individuele verskille in chemiese samestelling van temporaalklier sekreet tussen 'n bul in volle musth en ander bulle duidelik uitgewys. Sekresie vanaf temporaalkliere in 'n bul in musth word waarskynlik anders as die reuke van spesiegenote waargeneem. 'n Mate van groepslid uitkenning of individuele onderskeiding tussen olifante is moontlik.

Hall-Martin (1987) het 'n hipotese voorgestel dat musth 'n aanpassing is om die kanse op paring in sommige jong Afrika olifant bulle te maksimaliseer. Gebaseer op data van parameters ondersoek in die huidige studie, word voorgestel dat die temporaalkliere in Afrika olifant bulle hoofsaaklik gebruik word om dominansie in die hierargiese tropstruktuur deur middel van reuk oor te dra.

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