REPRODUCTIVE BIOLOGY OF THE EGYPTIAN FREE-TAILED BAT, *TADARIDA AEGYPTIACA* .

THESIS

Submitted in Fulfilment of the

Requirements for the Degree of

MASTER OF SCIENCE

of Rhodes University

by

JOHANNES NGOAKO TSITA

November 1993

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ACKNOWLEDGEMENTS

I am very grateful to the following people for their help during the project:

My supervisor, Prof. Ric. Bernard, for his help and encouragement throughout the study.

The Open Society Scholars Fund for financial assistance.

N. Cannon, R. Cross and S. Pinchuck for helping me with the photography.

Tim Bouwer of the computer centre for helping me with computers.

The Apostolic Faith Mission, Alexandria Congregation and Mrs J. Villet, of Grahamstown, for allowing me to sample on their premises.

Tina Bojarski for proofreading the manuscript.

My parents and family for their continued support during my study.

DEDICATED TO MY PARENTS

ABSTRACT

The reproductive biology of *Tadarida aegyptiaca* was studied using specimens collected in the Cape Province of South Africa. The morphology of the reproductive tract of the species was generally similar to that of other molossids, however, the absence of Cowpers glands was unusual. Spermatogenesis began in February and spermatozoa were released to the cauda epididymis during August and September. Follicular development started in March and culminated with the appearance of Graafian follicles in July. Ovulation probably occurred in August and specimens were pregnant by September. Gestation length was estimated to be four to five months and a single young was born in December. The data suggest that *T. aegyptiaca* is monotocous and monoestrous.

CHAPTER 1. GENERAL INTRODUCTION

Reproduction within the Chiroptera is highly variable both within and between taxa, and within a single species (see Carter, 1970; Gustafson, 1979; Jerrett, 1979; Krutzsch, 1979; Oxberry, 1979 for reviews). While most species are monoestrous and monotocous, some are polyoestrous, monotocous or polytocous, while others include a variety of reproductive adaptations and delays. Within a single species, reproduction may vary both at one locality (e.g. the gestation length of *Pipistrellus*, Racey, 1969; the timing of parturition in *Hipposideros caffer*, Brosset, 1968) and with latitude (e.g. *Myotis adversus*, Dwyer, 1970; *Chalinolobus gouldii*, Kitchener, 1975; *Miniopterus schreibersii*, Bernard, 1980a,c). Many insectivorous bats from temperate latitudes hibernate during winter and this has a profound effect on reproduction which becomes limited to the warm summer months (Gustafson, 1979; Oxberry, 1979 for reviews)

In general bats are noteworthy in that anatomical and/or morphological functional asymmetry is more frequent and profound than recorded for any other mammalian order (Wimsatt, 1979). Dextral dominance is the most frequent, but cases of sinistral dominance have been recorded.

Although the family Molossidae has a worldwide distribution (Koopman and Jones, 1970), their ability to fly high and fast and their habit of occupying small cracks and crevices during the day has resulted in the family being poorly studied. The limited available data suggest that they display unusual morphological stability in the arrangement of the reproductive tract (Wimsatt, 1979).

The reproductive cycle of molossid bats that reside in temperate environments is characterised by a single, brief gametogenic phase in late winter that is followed shortly by insemination and conception, with parturition in summer (Krutzsch and Crichton, 1985; Crichton and Krutzsch, 1987). Tropical and subtropical species, on the other hand, may have strikingly different reproductive cyclicity showing patterns ranging from a single to many annual breeding cycles (Krutzsch and Crichton, 1985; Van der Merwe *et al.*, 1986).

Tadarida aegyptiaca occurs extralimitally to the African continent from southwestern Arabia to India, in North Africa from Algeria and Egypt to the coast of the Mediterranean, widely south of the Sahara and throughout the Southern African subregion (Skinner and Smithers, 1990). Despite having such a wide distribution, its breeding biology is little known. Kasyap (1980) and Gopalakrishna *et al.* (1991) reported on its reproductive cycle and early development respectively in India, but no detailed reproductive studies have been done on the species in Africa in general. Therefore, the aims of the present study were threefold:

1) To describe the gross morphology of the reproductive organs of male and female *Tadarida aegyptiaca* from the Cape Province of South Africa.

2) To describe gametogenesis in *T. aegyptiaca.*

3) To describe the reproductive cycle of *T. aegyptiaca* from the Cape Province of South Africa.

CHAPTER 2. MATERIALS AND METHODS

2.1. SAMPLING

Specimens of male and female *Tadarida aegyptiaca* were collected as they emerged from roosts in the Grahamstown ($33^{0}20'$ S, $26^{0}30'$ E) and Alexandria ($33^{0}40'$ S, $26^{0}25'$ E) regions of the Eastern Cape Province of South Africa. Specimens were collected for all months except June and August when females could not be obtained. Additional specimens that had been collected from various roosts in the Cape Province were supplied by the Kaffrarian Museum (Table 1).

The live caught bats were brought back to the laboratory where they were killed by asphyxiation with carbon dioxide and weighed to the nearest 0.01g. The reproductive tracts were removed, weighed to the nearest 0.0001g, fixed in Bouin's fluid and later transferred to 70% alcohol for storage.

2.2. LIGHT MICROSCOPY

Tissues were embedded in Paraplast, sectioned at 5 µm, and stained with Ehrlich's haematoxylin and counterstained in eosin using standard techniques.

2.3. QUANTIFICATION

Changes in the seminiferous tubule diameters were quantified by measuring two diameters at right angles in cross-sections of twenty tubules per specimen. Changes in the height of the seminiferous epithelium were obtained by measuring two epithelial heights of twenty tubules per testis, always including maximum and minimum measurements.

Ovarian activity was quantified by plotting mean monthly diameters for secondary and

Graafian follicles. The follicular diameters were calculated from two measurements taken at right angles.

Thickness of the wall of the uterine horns was measured at 10 positions per specimen from the lumen to the outer layer of cells.

2.4. GRAPHICAL DISPLAY OF DATA

Data are displayed graphically. Throughout this report, the points plotted on the graphs are mean values, vertical lines are plus or minus one standard deviation from the mean and the numbers above the vertical lines are sample sizes.

2.5. STATISTICAL ANALYSIS

Where appropriate, ANOVA (Kruskal-Wallis) and Student t-test have been used to test for significant differences between mean values. Regression analysis was carried out between oocyte and follicular diameters, using statgraphics.

Table 1. Monthly sample sizes of adult *T. aegyptiaca* specimens used in the study.

MONTH	SAMPLE SIZE	
	MALE	FEMALE
JAN	3	3
FEB	3	2
MAR	7	4
APR	3	1
MAY	4	4
JUN	1	0
JUL	1	1
AUG	4	0
SEP	5	4
OCT	4	5
NOV	4	4
DEC	1	4

CHAPTER 3. THE GROSS MORPHOLOGY OF THE MALE AND FEMALE REPRODUCTIVE TRACTS.

3.1. INTRODUCTION.

The reproductive tract of male mammals generally consists of the testes and associated epididymes, vasa deferentia, accessory gland complex, urethra and penis. The accessory gland complex contributes various substances to the ejaculate, such as fructose, citric acid, sialic acid (Rajalakshmi and Prasad, 1970; Krutzsch *et al.*, 1976; Mokkapati and Dominic, 1976; Gonzales, 1989) and may consist of ampullary, prostate, urethral and Cowper's glands and paired seminal vesicles (Krutzsch, 1979). However not all glands are present in every mammalian order (Setchell, 1978) and in the Chiroptera this is revealed in studies by Bernard (1985; 1986) on *Rhinolophus capensis*, Paton (1988) and Bernard *et al.* (1991) on *Miniopterus schreibersii*, Matthews (1941) on *M. dasythrix (=schreibersii)*, *Taphozous* sp., *Nycteris luteola* and *N. hispida* and see Krutzsch (1979) for review.

The available data for molossids suggest that variation beyond size in the morphological details of the primary and accessory sex organs are not great (Krutzsch,1979; Krutzsch and Crichton, 1987). Differences that do exist are in small details of size and shape of the accessory organs and in the presence of a variety of secondary sexual characteristics, such as facial, anal and chest glands. Thus molossids as a group show great homogeneity in the arrangement of the male reproductive tract (Krutzsch, 1979).

The female reproductive anatomy of molossids has, like the male, been little studied. Available information for this family shows the members to have a bicornuate uterus with

the right ovary being morphologically larger and functionally more active than the left (Sherman, 1937; Harrison, 1958; Mutere, 1973a, b; Jerrett, 1979; Wimsatt, 1979; Kitchener and Hudson, 1982; Krutzsch and Crichton, 1985, Crichton and Krutzsch, 1987; Van der Merwe *et al.*, 1986; Van der Merwe *et al.*, 1987; Gopalakrishna *et al.*, 1991). In all the above studies except Sherman (1937; *Tadarida brasiliensis);* Van der Merwe *et al.* (1986) and Van der Merwe *et al.*, (1987) on *T. pumila* the right uterine horn has been found to be morphologically larger than the left and to be the site of implantation. In young of the year prior to their first oestrus, such as in *Tadarida condylura* and *T. pumila* (Mutere, 1973a); *Molossus fortis* (Krutzsch and Crichton, 1985) and *Mormopterus planiceps* (Crichton and Krutzsch, 1987) the uterine horns were reported to be bilaterally symmetrical. The left ovary of molossids has been shown to be an interstitial organ, with the follicles never developing beyond the secondary stage (Jerrett, 1979). Graafian follicles and corpora lutea have been found only in the right ovary (Krutzsch and Crichton,1985, Crichton and Krutzsch, 1987). Reproductive asymmetry is not restricted to the family Molossidae and both sinistral and dextral dominance occur in other bats (Wimsatt, 1979 for review). However, in the Molossidae dextral dominance is the most widely encountered (Wimsatt, 1979).

No published account of the reproductive anatomy of *Tadarida aegyptiaca* exist for the southern African subregion, thus the aim of this chapter is to give a description of male and female reproductive anatomy of the species.

3.2. RESULTS

3.2.1. MALE REPRODUCTIVE ANATOMY.

The male reproductive tract (Figs 1a & 1b) consists of paired abdominal testes, the accessory gland complex and the penis. At the terminal end of the vas deferens is a specialised secretory gland, the ampullary gland. Paired bilobed seminal vesicles surround the ampullary glands, and their ducts join the ejaculatory duct just prior to its entrance into the urethra. The epithelium of both the ampullary gland and seminal vesicle consists of columnar secretory cells whose secretion into the lumen is of uniform appearance during periods of activity (Fig. 2a). The epithelium is low columnar during secretory inactivity (Figs 2b & 2c). A compound prostate gland is situated ventral to the bladder, with the most caudal part of the gland encircling the prostatic urethra. The lumen of this gland is lined by columnar epithelial cells whose secretion is in the form of spherical droplets (Fig. 2a). The posterior end of the complex consists of a bilobed urethral gland, which is tubular with a columnar secretory epithelium. The urethra terminates into the penis.

Para-anal glands, which are situated approximately 2mm from the anus are enclosed by the anal sphincter muscle. The epithelial cells lining the lumen of these glands are columnar.

3.2.2. FEMALE REPRODUCTIVE ANATOMY.

The female reproductive tract (Fig. 3) is approximately Y-shaped, consisting of the vagina, the uterine corpus, the bicornuate uterine horns, oviducts and the ovaries.

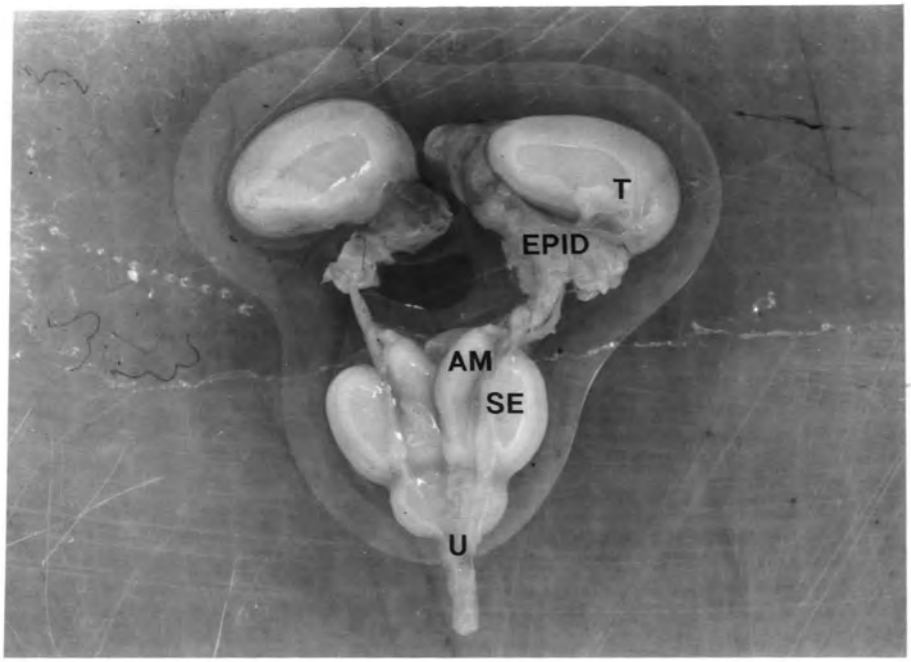
The vagina, which opens externally through a transverse vulva, is lined by a stratified epithelium (Fig. 4). The vagina is separated from the uterus by a cervix. Proximal to the cervix is the uterine corpus (Fig. 5) which is lined by a cuboidal epithelium.

The uterus is bicornuate with the right uterine horn larger than the left (Fig. 3). Both cornua comprise an inner glandular endometrium and an outer myometrium which consists of connective tissue and smooth muscle (Fig. 6). The anterior ends of the horns terminate in the oviducts which are lined with a ciliated cuboidal epithelium (Fig. 7).

The ovaries are ovoid structures enclosed in an ovarian bursa and partly enclosed by the oviducts. In cross-section the ovaries are divided into an outer cortex and an inner medulla. The left ovary is smaller than the right and has primordial, primary and secondary follicles, and large amounts of interstitial tissue whilst the larger right ovary has, in addition to these, Graafian follicles and the corpus luteum. The diameter of the right ovary ($X = 18.45 \pm 1.94$ mm) is significantly larger than that of the left ovary ($X = 14.35 \pm 1.99$ mm), ($P < 0.05$ for both).

Fig. 1a. Dorsal view of male reproductive system from a specimen of *Tadarida aegyptiaca* collected in September showing testis (T), epididymis (EPID), ampullary gland (AM), seminal vesicle (SE) and urethra (U). (8X).

Fig 1b. Ventral view of the reproductive organs from the specimen in Fig. 1a showing, in addition to the strutures mentioned above, the bladder (BL) and prostate gland (P). (8X).


T
EPID
AM
SE
U

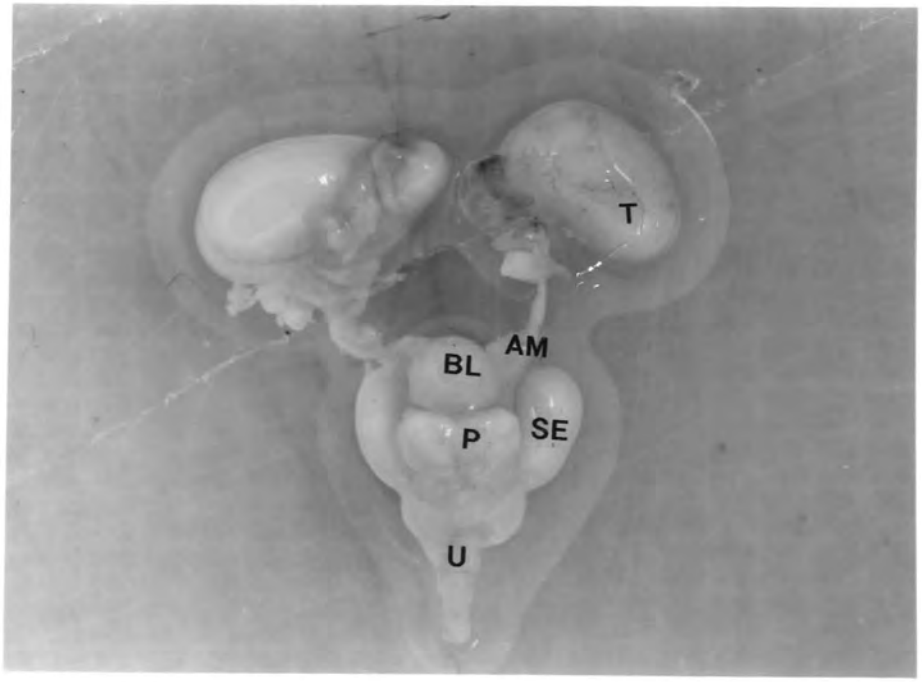
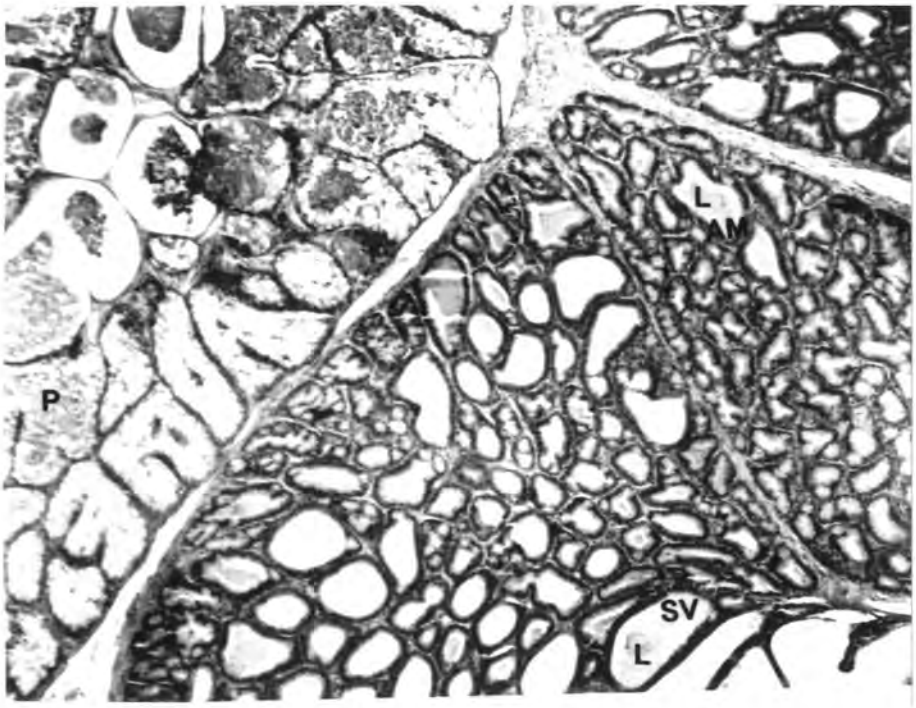
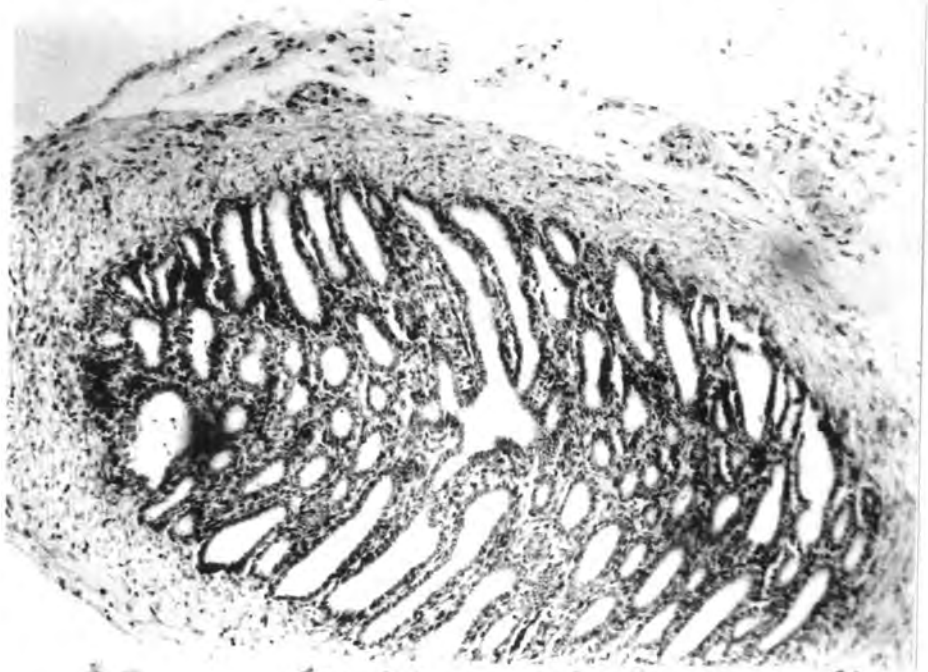

T
AM
BL
P
SE
U

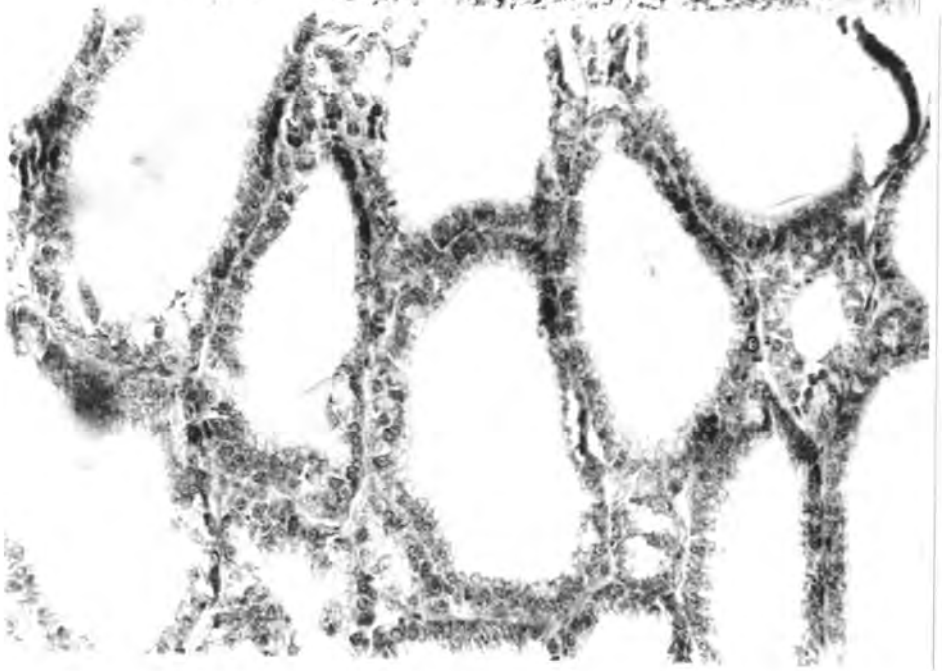
Fig. 2a. Section through the accessory gland complex during period of activity (September) showing secretion into the lumen (L) of the ampullary gland (AM), prostate (P) and seminal vesicle (SV) (35X).

Fig. 2b. Section through an inactive ampullary gland (April) (35X).

Fig. 2c. Section through an inactive prostate gland (April). (87.5X).


L
AM
P
SV
L





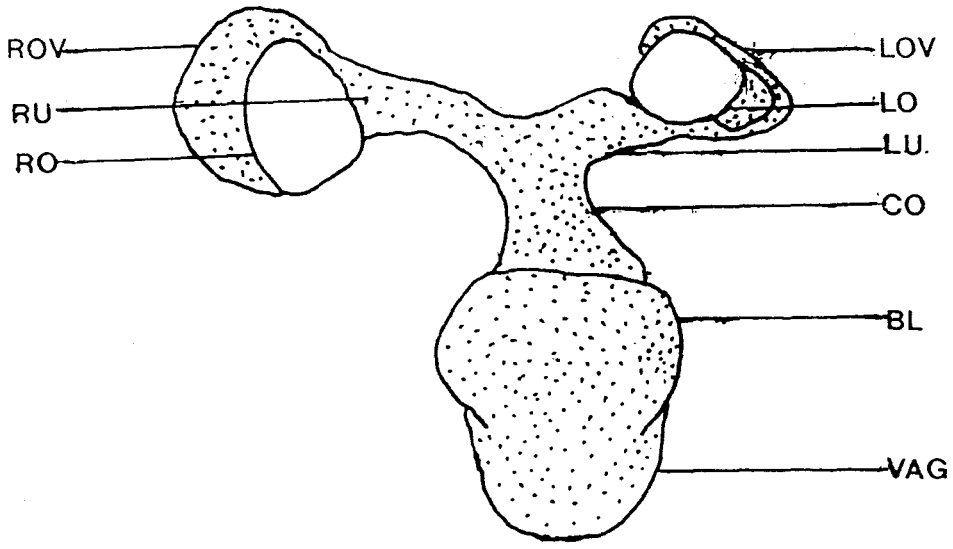
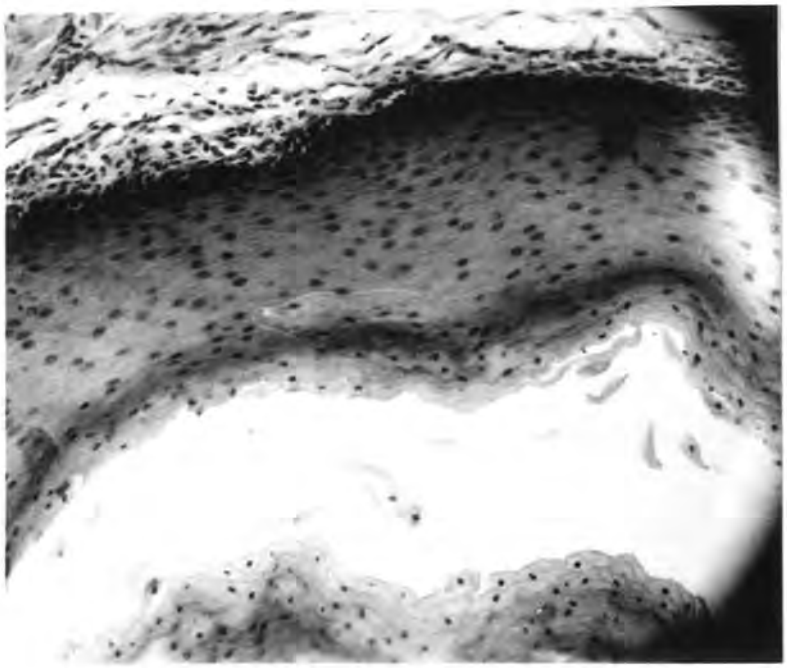


Fig. 3. Ventral view of the female reproductive organs of *T. aegyptiaca* collected in September showing bladder (BL), vagina (VAG), corpus uteri (CO), left uterine horn (LU), right uterine horn (RU), right oviduct (ROV), left oviduct (LOV) left ovary (LO) and the right ovary (RO). (8X).

Fig. 4. Section through the wall of the vagina of a specimen just after oestrus showing sloughing off of the epithelium. (87.5X).

Fig. 5. The typical appearance of the lining of the uterine corpus from a specimen collected in February. (140X).



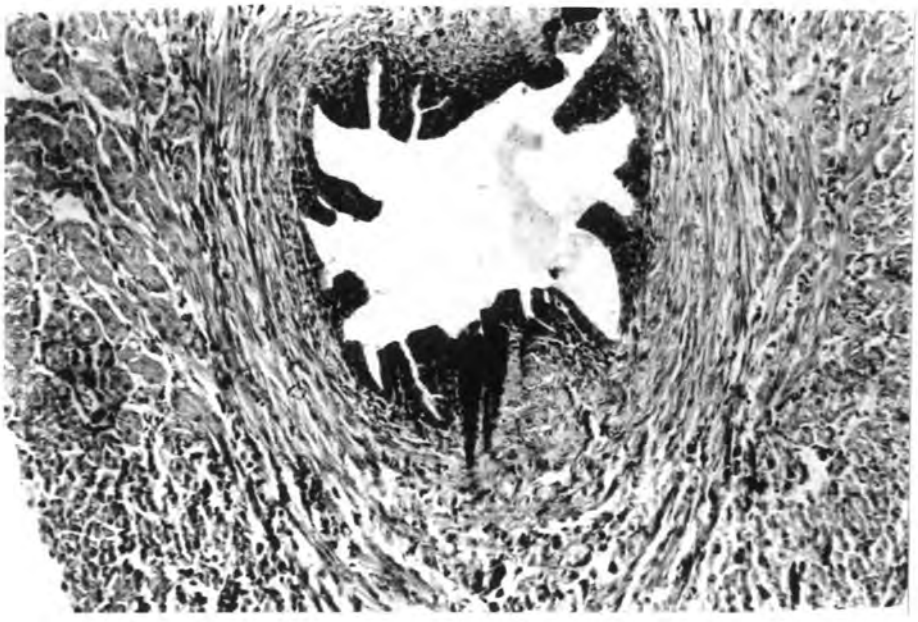
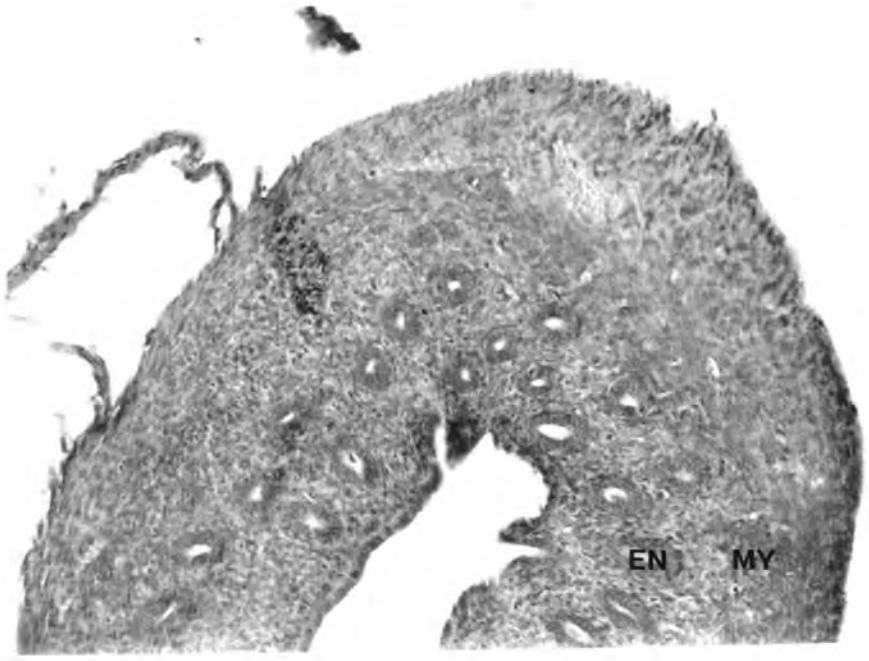
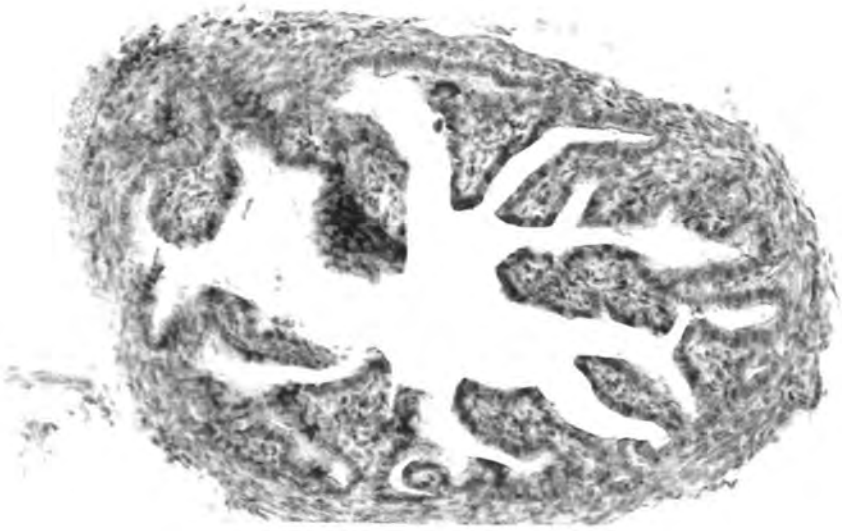


Fig. 6. Section through the wall of the right uterine horn indicating the outer myometrium (MY) and the inner endometrium (EN). (September) (140X).

Fig. 7. Section through the ampullary region of the oviduct showing the irregularly folded mucosa and the thin muscular wall (September). (140X).


EN
MY



3.3. DISCUSSION

The gross anatomy of the male reproductive tract of the Egyptian free-tailed bat shows some similarity to that of other molossids that have been studied to date (e.g. Krutzsch, 1979; Krutzsch and Crichton, 1987) in that ampullary, prostate, urethral and para-anal glands are present. However the Egyptian free-tailed bat differs from other molossids in that Cowper's gland is missing. The position of the testes in the family Molossidae varies from abdominal to inguinal and extraabdominal such as in *Tadarida brasiliensis* (Krutzsch, 1955a); *Chaerephon hindei* (Marshall and Corbet, 1959) and *Mormopterus planiceps* (Krutzsch and Crichton, 1987). By contrast, in *Tadarida aegyptiaca* the testes were always abdominal and scrotal sacs did not occur. Krutzsch and Crichton (1987) suggest that variation in position of the testes seems related to season and therefore presumably to reproductive activity. It is suggested that the scrotum serves to reduce testicular temperature to levels below that of body temperature (Wislocki, 1933; Cowles, 1965), possibly to reduce spontaneous mutation rate (Ehrenberg *et al.*, 1957). However, evidence from Setchell (1978) point to the contrary, since mammals with abdominal testes have been shown to have body temperatures as high as those without.

While Cowper's glands occur commonly in the Chiroptera in general and in several molossids (Krutzsch, 1979), there is ample evidence to show substantial variation in the presence of specific accessory glands from one species to another. It is therefore not surprising that Cowper's gland does not occur in *Tadarida aegyptiaca.*

The para-anal glands have previously only been reported in *Mormopterus planiceps* where they probably function in sexual attraction and marking of roosts with their distinct odour

(Krutzsch and Crichton, 1987). The latter feature is characteristic of molossid bats in general and *Tadarida aegyptiaca* in particular.

The anatomy of the female reproductive organs of *Tadarida aegyptiaca* conforms to the dextral functional asymmetry reported for other members of the family, such as *Tadarida brasiliensis* (Jerrett, 1977, 1979); *Tadarida australis* (Kitchener and Hudson, 1982); *Molossus fortis* (Krutzsch and Crichton, 1985); *Mormopterus planiceps* (Crichton and Krutzsch, 1987); *Tadarida pumila* (Van der Merwe *et al.*, 1986). This confirms earlier reports that the molossids show great homogeneity in the organisation of this organ system. Dextral functional asymmetry is not confined to this family and has been reported for the Rhinolophidae (Matthews, 1937; Gaisler, 1965, 1966; Gopalakrishna and Ramakrishna, 1977; Bernard, 1983, 1985); Hipposideridae (Gopalakrishna and Moghe, 1960); Emballonuridae (Kitchener, 1973, 1976; Harrison, 1958). Stephens (1962) reported that the uterine horns of *Tadarida brasiliensis* were of equal size but this could be due to the fact that young specimens were used in the study (Jerrett, 1979). It is, however, not possible to account for the observations of Van der Merwe *et al.* (1986) and Van der Merwe *et al.* (1987) that the horns were of equal size in *Tadarida pumila* since it is apparent from their study that adult specimens were used. *T. pumila* is a monotocous and polyoestrous species but only the right uterine horn is used. Finally it would appear that dextral dominance in the family Molossidae is not absolute since conceptuses have been found in the left uterine horn of *T. midas* (Smithers, 1971).

The ovarian morphology provides additional evidence that dextral ovarian asymmetry is universal in the family. The lack of growth above secondary follicle stage and the interstitial, steroidogenic nature of the left ovary have been reported for other molossids (Kayanja and

Mutere, 1975; Jerrett, 1979). It is not possible to conclude from the observation of Smithers (1971) whether the conceptuses found in the left uterine horn of *T. midas* resulted from an ovulation in the adjacent ovary since a histological examination was not undertaken. Furthermore, transuterine migration, even though not reported in the Molossidae, is known to occur in other bats (Wimsatt, 1979).

A local pathway for the ovarian stimulation of the ipsilateral uterine horn has been suggested in some mammals (Ginther, 1967, 1974, 1976) and bats (Bernard, 1988, *Roussetus aegyptiacus*; Marshall, 1953, *Pteropus giganteus*; Rasweiller, 1978, *Noctilio albiventris).* The actual mechanisms underlying the phenomenon in the Chiroptera have not been investigated.

CHAPTER 4. SPERMATOGENESIS

4.1. INTRODUCTION

The process of spermatogenesis comprises a series of sequential mechanisms reflecting cell proliferation, maturation and differentiation (Delhon and von Lawzewitsch, 1987). The process has not been adequately described in the Chiroptera in general, and the Molossidae in particular.

In a number of mammalian species for which detailed information on spermatogenesis is available the process has been divided into stages based on germ cell associations (Foote *et al.*, 1972, dog; Grocock and Clarke, 1975, vole; Tait and Johnson, 1982, squirrel; Delhon and von Lawzewitsch, 1987, llama). A number of methods for describing the spermatogenic cycle have been proposed but two of them are in common use. Clermont and Leblond (1955, in Dehlon and von Lawzewitsch, 1987) classify the germ cell associations according to fourteen groups based on the acrosomic characteristics of the spermatids. The other method is based on the morphological associations of the germ cell nuclei. Both these methods do not deal with spermatogenesis prior to the stage when spermatids have been formed. Furthermore, the process is described over a relatively short period of time.

The duration of spermatogenesis appears to be specific for each species (de Kretser and Kerr, 1988). In non-microchiropteran mammals de Kretser and Kerr (1988) report the length (in days) to be 34 to 35 in the mouse, 35 to 36 in the hamster, 34 to 35 in the boar, 48 to 53 in the rat, 49 in the ram, 54 in the bull, 48 to 51 in the rabbit 45 in the monkey (*Macaca speciosa*), 70 in the monkey *(Macaca mulatta)* and 64 in humans. In chiropterans other than

molossids the length has been reported as about four months in *Macrotus waterhousii* (Krutzsch *et al.*, 1976), *Pipistrellus pipistrellus* (Racey and Tam, 1974); about seven months in *Rhinolophus capensis* (Bernard, 1985) and *Nyctalus noctula* (Racey, 1974) and about five months in *Rhinolophus clivosus* (Bernard, 1983). Information on the Molossidae suggest a period of about seven months in *Tadarida brasiliensis* (Sherman, 1937) and *Mormopterus planiceps* (Krutzsch and Crichton, 1987).

The only Old World temperate molossid for which information on spermatogenesis is available is *Mormopterus planiceps* (Krutzsch and Crichton, 1987). The purpose of the present study is to describe the process in the Egyptian free-tailed bat over a period of a year.

4.2.RESULTS.

Spermatogenically inactive testes were found in the period October to January. In these specimens the seminiferous epithelium consisted of a single and sometimes double layer of spermatogonia and Sertoli cells (Fig. 8a).

Spermatogenesis commenced in February with the appearance of primary spermatocytes (Fig. 8b) and spermiogenesis began in April and was characterised by the appearance of both round and elongated spermatids (Fig. 8c).

Spermatozoa were first released into the lumen of the seminiferous tubule in July and were present in the cauda epididymis during August and September (Fig. 8d).

A few specimens collected in April, June and July during the period of reproductive activity were spermatogenically inactive.

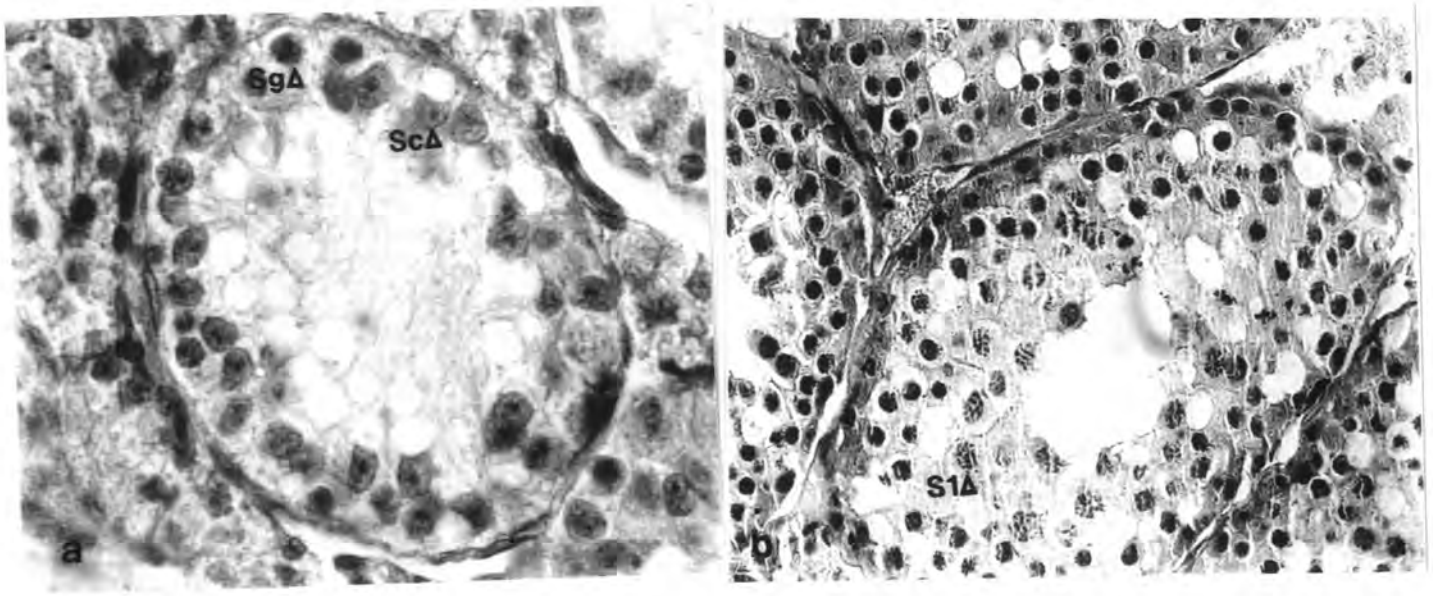
Fig. 8. Light micrographs illustrating some of the stages of spermatogenesis in *T. aegyptiaca.* Open triangles point towards the cell types.

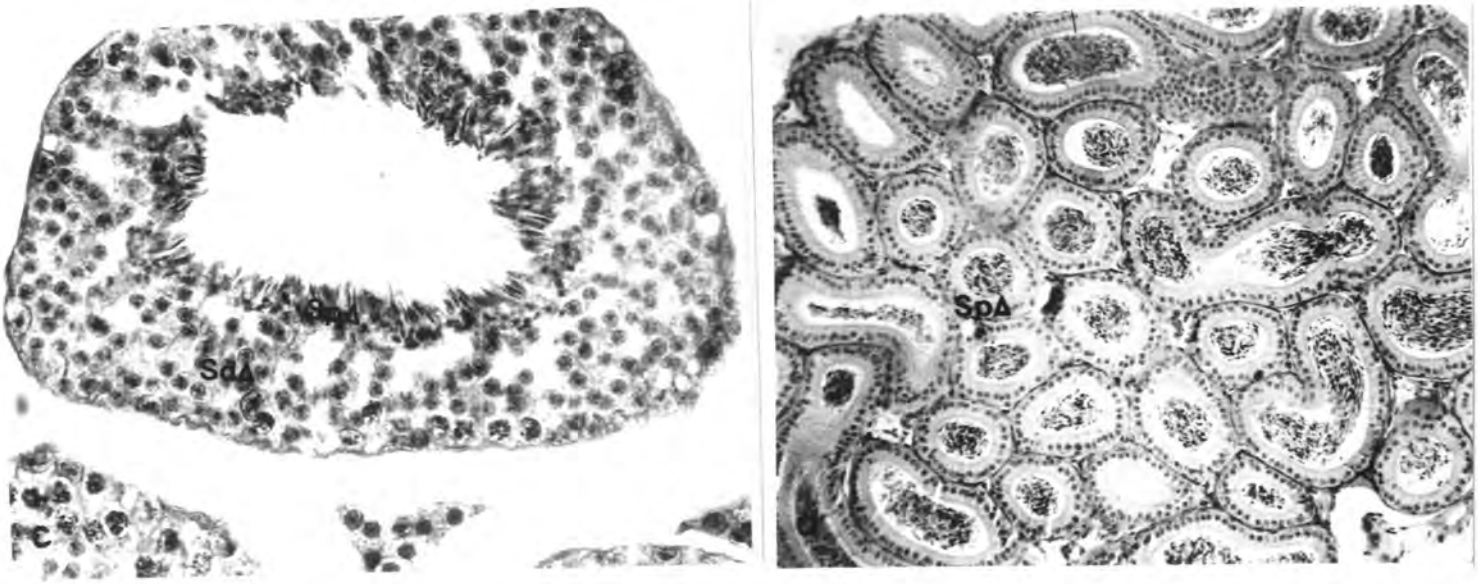
a. Section through an inactive seminiferous tubule showing Sertoli cells (Sc) and spermatogonia (Sg). (March) (350X).

b. Section through seminiferous tubule from a specimen in early spermatogenesis collected during February showing primary spermatocytes (S1) and other cell types. (350X).

c. Section through a seminiferous tubule from a specimen in late spermatogenesis collected in August. Spermatids (Sd) and spermatozoa (Sp) can be seen. (140X).

d. Section through the cauda epididymis of a specimen from August showing the spermatozoa (Sp) in the lumen. (87.5X).


SgΔ
ScΔ
a
S1Δ
b


SdΔ
SpΔ

4.3. DISCUSSION.

The spermatogenic cycle observed in the present study is similar to that described for other mammals such as, dog (Foote *et al.*, 1972); vole (Grocock and Clarke, 1975) and llama (Delhon and Lawzewitsch, 1987). However the approximately seven month duration of spermatogenesis observed in the present study is greater than the longest duration (70 days in *Macaca mulatta*) in the non-chiropteran mammals for which there is information (de Kretser and Kerr, 1988), but similar to some non-molossid bats such as *Rhinolophus capensis* (Bernard, 1983) and *Nyctalus noctula* (Racey, 1974) and to all members of the family Molossidae reported (*Tadarida brasiliensis*, Sherman, 1937 and *Mormopterus planiceps*, Krutzsch and Crichton, 1987). It appears as if a relatively long duration of spermatogenesis is characteristic of the Chiroptera in general and the Molossidae in particular, and the exact reason for this remains obscure.

In the Molossidae studied to date, reproductive activity has not been shown in animals supposedly in their first year (Short, 1961 and Davis *et al.*, 1962 in *Tadarida brasiliensis* and Krutzsch and Crichton, 1987 in *Mormopterus planiceps*). The occurrence of spermatogenically inactive males in April, June and July probably indicates that *Tadarida aegyptiaca* does not reach sexual maturity in its first year. All of the spermatogenically inactive animals were of adult size but it is established that bats reach full adult size within six months of birth, and size alone is not a satisfactory indicator of sexual maturity.

Krutzsch and Crichton (1987) reported epididymal sperm storage in *Mormopterus planiceps* over the winter months while Sherman (1937) and Davies *et al.* (1962) did not report sperm storage in *Tadarida brasiliensis*. It appears as if the spermatogenic activity in the Egyptian

free-tailed bat is similar to that of *Tadarida brasiliensis* of Sherman (1937) and Davies *et al.* (1962). In these species production and utilisation of spermatozoa occurs within a short period after which testicular and accessory gland functions wane, epididymal sperm reserves disappear, and the animal is no longer fertile.

CHAPTER 5. FOLLICULAR DEVELOPMENT

5.1. INTRODUCTION.

Accounts of folliculogenesis in molossids, even though fragmentary, have shown the process to be typically mammalian and to proceed from primordial to Graafian follicle (e.g.Krutzsch and Crichton, 1985, *Molossus fortis;* Crichton and Krutzsch, 1987, *Mormopterus planiceps*; Kitchener and Hudson, 1982, *Tadarida australis*). However, a common feature is that only follicles in the right ovary complete the whole process, while those in the left do not normally develop beyond the secondary follicle stage (see Jerrett, 1979 for review; Krutzsch and Crichton, 1985, *Molossus fortis*; Crichton and Krutzsch, 1987, *Mormopterus planiceps*). Follicular development and atresia have not been reported in the non-hibernating molossid bat, *Tadarida aegyptiaca*. A characteristic of Graafian follicles in hibernating bats is a hypertrophy of the cumulus oophorus cells, an adaptation for survival of the primary oocyte over winter (Wimsatt, 1944; Wimsatt and Kallen, 1957). In the molossids, which do not hibernate, no hypertrophy occurs (Kitchener and Hudson, 1982, *Tadarida australis*; Crichton and Krutzsch, 1987, *Mormopterus planiceps*).

Growth of ovarian follicles has been widely reported as occurring in two stages. Brambell (1928) was the first to show the two stage growth relationship between follicular and oocyte diameters in the mouse. This has been confirmed by later authors for nearly all mammalian species (Rowlands and Weir, 1984). During the first phase both the oocyte and follicle growth rates are high while only the follicle continues to grow in the second phase. Bernard (1980a, b, c, d) reported a three phase growth characterised by highest rate of oocyte growth in primordial and primary follicles which becomes progressively lower for secondary and lowest

for the Graafian follicles.

Although a large number of follicles begin growth at the start of each cycle of follicular development, most of them undergo atresia (Guthrie and Jeffers, 1938a), defined as a process by which oocytes are lost from the ovary other than by ovulation (Ingram, 1962, in Van der Merwe, 1979). Atresia occurs more often in the advanced stages of follicular growth, even though there are more follicles in the early stages of development at any one time (Arai, 1920 and Himmelstein-Braw *et al.*, 1976, in Peters and McNatty, 1980). The process has been reported throughout the mammals and widely described in the microchiroptera in general (Matthews, 1937; Guthrie and Jeffers 1938a; Van der Merwe, 1979) and in the Molossidae in particular (Kitchener and Hudson, 1982; Krutzsch and Crichton, 1985; Crichton and Krutzsch, 1987; Rasweiler, 1988).

Due to the fragmentary information on the process of folliculogenesis in the Molossidae, the aim of the present study was to describe follicular development, growth and atresia in *Tadarida aegyptiaca.*

5.2.RESULTS

Follicular development in the right ovary was typically mammalian and four stages were recognised: primordial, primary, secondary and Graafian follicles. In the left ovary no Graafian follicles were present, therefore the description of the latter will be based on follicles from the right ovary only.

5.2.1. PRIMORDIAL FOLLICLES.

These were situated at the periphery of the ovarian cortex and the primary oocytes were surrounded by a single, investing layer of about 3-5 fusiform cells (Fig. 9a). The mean diameter of such follicles was 21.2 ± 4.32 μm (n= 15). The oocytes were ovoid structures with eccentric nuclei. Most of the oocytes were tightly packed together and the covering layer of fusiform cells was not continuous around them.

5.2.2. PRIMARY FOLLICLES.

The primary follicles (Fig. 9a) had a single layer of typically cuboidal cells which, with increasing hypertrophy, became more columnar. The mean diameter of such follicles was 78.8 ± 19.2 μm (n= 60). Some of the follicles were ovoid because many primary oocytes were oval and the follicular cells at the apices were more columnar than those at the sides (Fig. 9a). Furthermore cells at the poles had usually divided forming a second layer in the stratum granulosum. The zona pellucida and theca folliculi first appeared in large primary follicles, the former as a thin acidophilically stained band around the oocyte and the latter as a thin layer of fusiform cells around the follicles.

5.2.3. SECONDARY FOLLICLES.

These follicles were characterized by having two or more layers of follicular cells in the stratum granulosum (Fig. 9b). The mean diameter for follicles with two layers was 111.3 ± 1.65 µm (n= 10) and for those with five layers was 213.8 ± 43.5 µm (n= 10). More cell divisions had occurred at the poles as mentioned earlier for primary follicles, thus the two layered condition was not always as obvious in the oval follicles as in the round follicles which were rarely observed. The primary oocyte was surrounded by a zona pellucida and thin theca folliculi surrounded the entire follicle. The theca is composed of mostly cuboidal and a few fusiform cells with their long axis parallel to the stratum granulosum (Fig. 9b).

5.2.4. GRAAFIAN FOLLICLES

The Graafian follicles were formed when small antral cavities developed between the follicular cells. These central cavities later enlarged and coalesced to form the single antrum that characterises large Graafian follicles (Fig. 9c). The diameters of the smallest and the largest Graafian follicles were 212.5 µm and 381 µm respectively. As the antral cavity increased in size, the primary oocyte was pushed to one side so that the cumulus oophorus was attached to the surrounding stratum granulosum by a column of cells. The primary oocyte was surrounded by a zona pellucida which in turn was surrounded by the cells of the cumulus oophorus, the latter never showing any sign of hypertrophy. The antrum was filled with an acidophilically stained coagulum, described as the secondary liquor folliculi in the ferret (Robinson, 1918).

5.2.5. FOLLICULAR GROWTH.

Growth of ovarian follicles occurred in three stages (Fig. 10). In the first stage, representing growth of the primary and primordial follicles, both the follicle and the oocyte grew at approximately the same rate ($Y= B + AX = 6.08 + 3.10X$; where Y= primary oocyte diameter, B= intercept of the regression line, A= the slope of the regression line and X= Follicular diameter, $r= 0.89$; $P< 0.001$). In the second stage, representing secondary follicle growth, the follicle grew faster than the oocyte compared to the first stage ($Y= B + AX= 32.5 + 1.53X$; symbols as above; $r= 0.61$; $P< 0.001$). The second stage was characterised by the addition of second and more layers of follicular cells. In the third stage, representing Graafian follicle growth the follicle continued to grow while there was relatively little growth of the oocyte ($Y= B + AX= 76.2 + 0.08X$, symbols as above; $r= 0.11$; $P> 0.1$). Some late primary follicles were larger than early secondary follicles and the same situation prevailed between late secondary and early Graafian follicle growth stages.

5.2.6. CORPUS LUTEUM.

Cells of the corpus luteum were distinguished from interstitial cells by being larger in size and less intensely eosinophilic (Fig. 11a). The smallest corpus luteum had a diameter of (493.8 μm) and the largest a diameter of (862.5 μm). All specimens had one corpus luteum except on one occasion were a specimen with two corpora lutea was observed (Fig. 11b). The corpus luteum was first observed in a specimen collected in late September and was present in all specimens until December, after which the structure was absent. The mean diameter (631.3 ± 59.6 μm; $n= 4$) of corpora lutea collected during September was the highest of all months for which they were observed. The size subsequently decreased to a mean of 518 ± 35.4 μm ($n= 4$) in December and no corpora lutea were observed in specimens collected from

January onwards.

5.2.7. FOLLICULAR ATRESIA.

Two types of atresia were observed in the ovaries of the Egyptian free-tailed bat: In Type I atresia, which occurred commonly in secondary and Graafian follicles follicular cells began to degenerate before the oocyte. In Type II, which was more common in primordial and primary follicles, the oocyte degenerated before the follicular cells (Fig. 12).

Type I atresia was characterised by pyknosis (polymerisation and condensation of nuclear fragments) and karyorrheksis (fragmentation of the nucleus) of the follicular cells which resulted in the appearance of cells with pyknotic nuclei and nuclear fragments in the antra of Graafian follicles. Disruption of the follicular cells was followed by deformation of the primary oocyte leaving a collapsed zona pellucida lying in a small cavity (Fig. 9c). Formation of corpora atretica by hypertrophy of thecal cells was not observed.

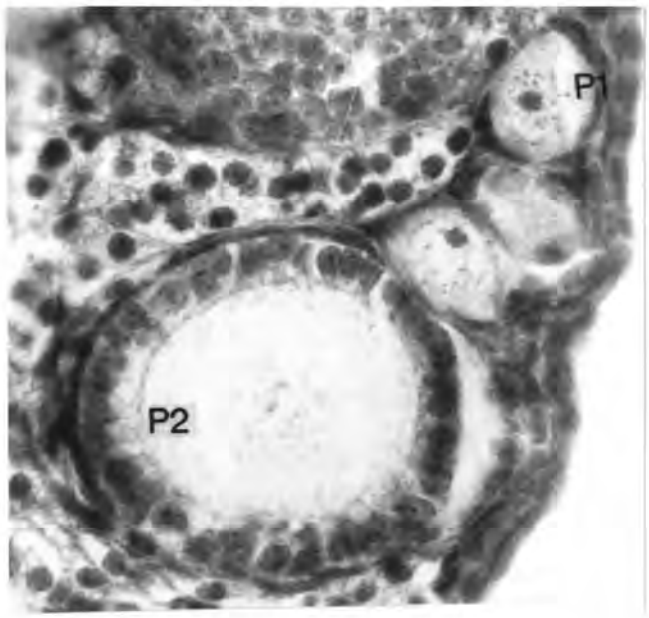
In type II atresia the oocyte initially appeared deformed, while the theca and stratum granulosum remained intact.

5.2.8. POLYOVULAR FOLLICLES.

Two polyovular follicles were seen in one specimen in the present study. In such follicles two primary oocytes were enclosed in one follicle (Fig. 13).

Fig. 9a. Section through the periphery of the right ovary showing primordial follicles (P1) and a single primary follicle (P2). (May). (350X).

Fig. 9b. Section through an ovary showing a secondary follicle with two layers of follicular cells in the stratum granulosum. (May). (350X)


P1
P2

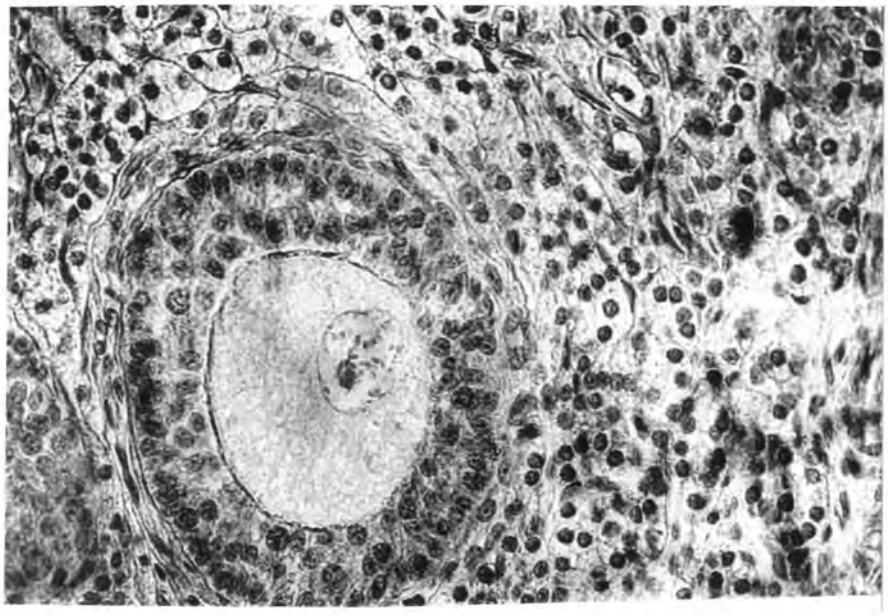
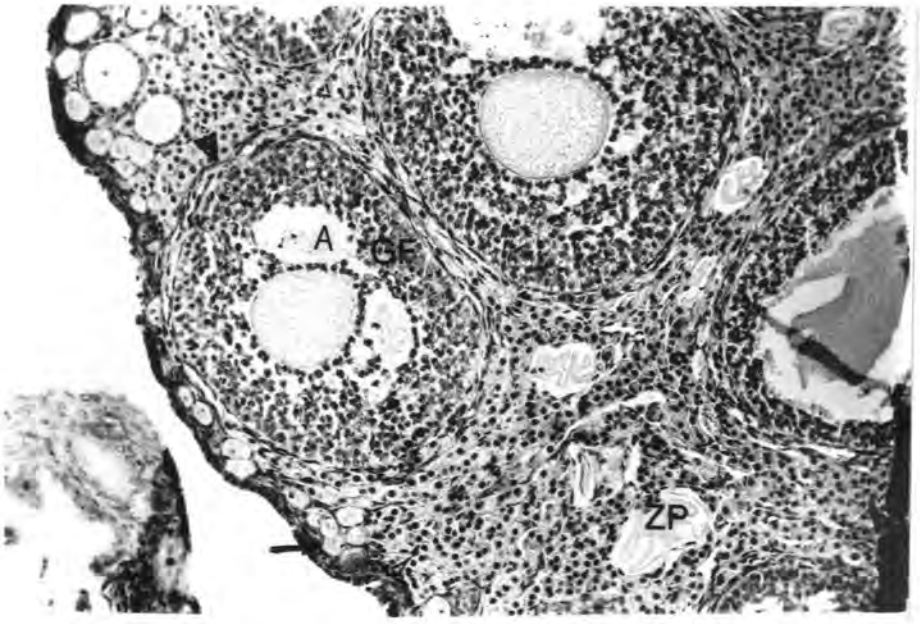


Fig. 9c. Section through the right ovary showing the mode of formation of an antrum (A) from an early Graafian follicle (GF). A collapsed zona pellucida (zp) and a theca folliculi (▾) are also indicated. Note the packed primordial follicles (↑) at the periphery. (May). (140X).


A
GF
ZP

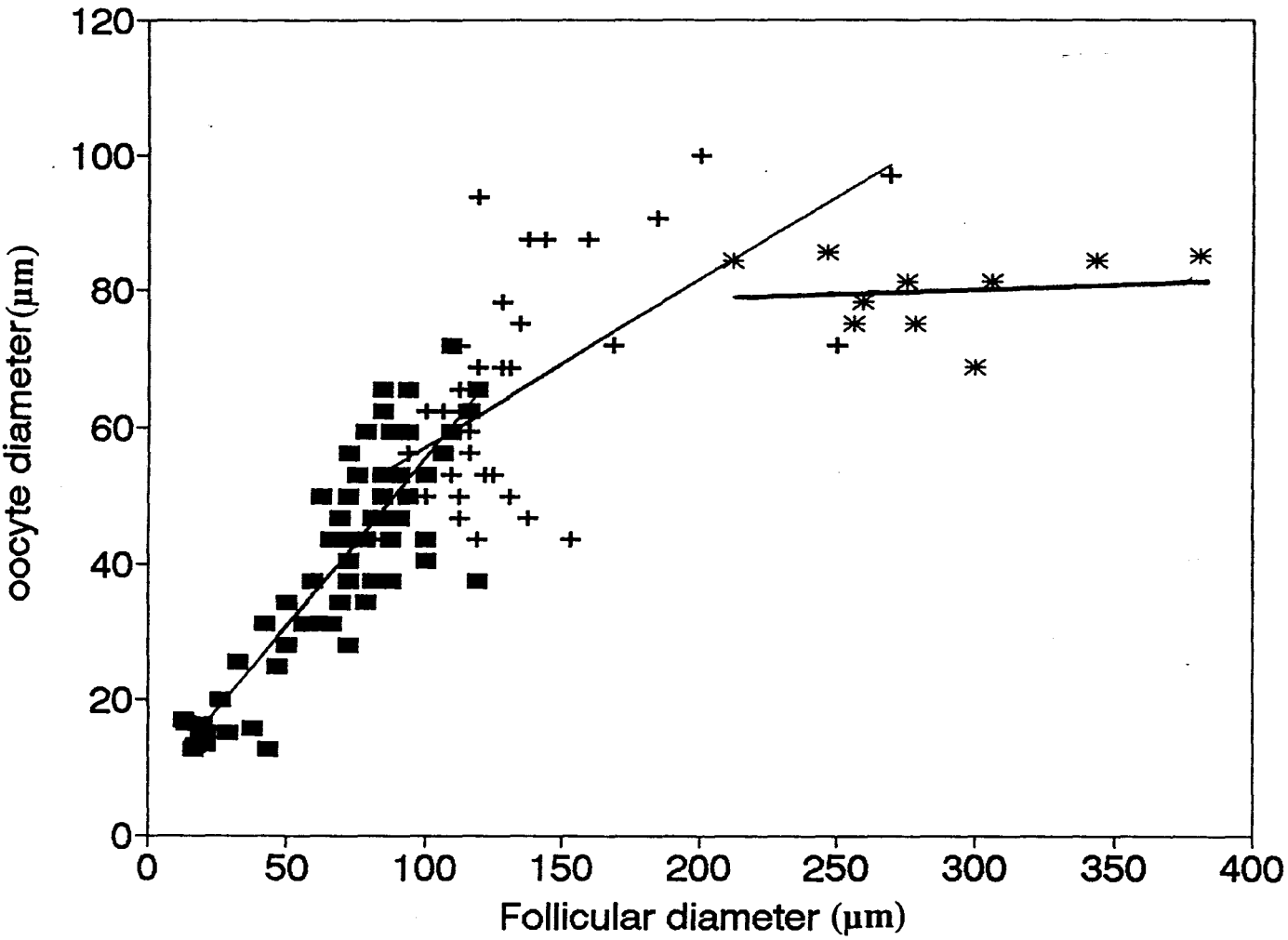
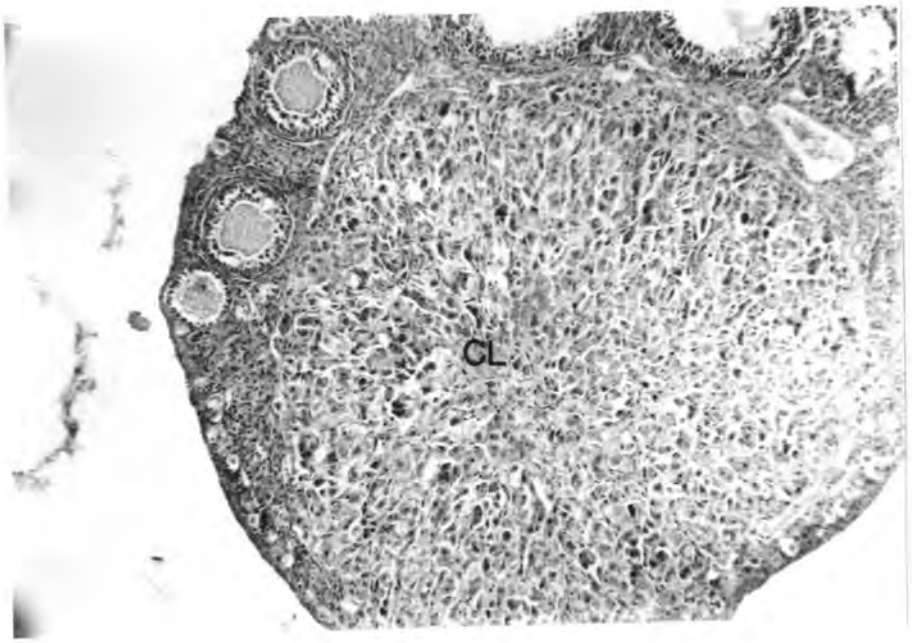


Fig. 10. Relative growth rates of oocyte and follicle during follicular development for primordial and primary (■), secondary (+) and Graafian follicles (*).

Fig 11a. Section through the right ovary of a specimen collected in October showing a fully developed corpus luteum (CL). (87.5X).

Fig. 11b. Section through the right ovary a specimen with two corpora lutea (CL 1 & 2). (October). (140X).


CL

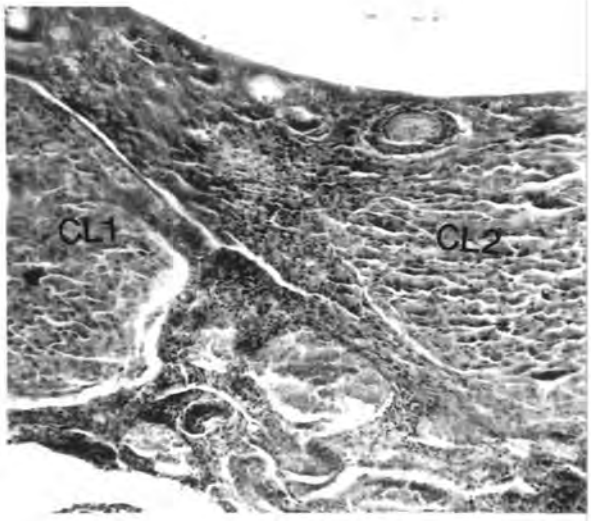
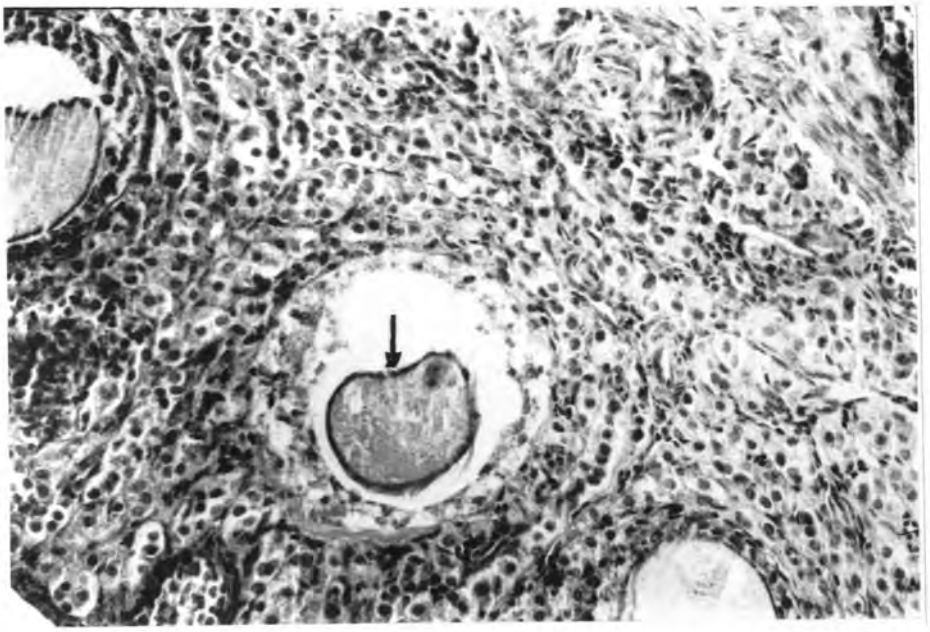
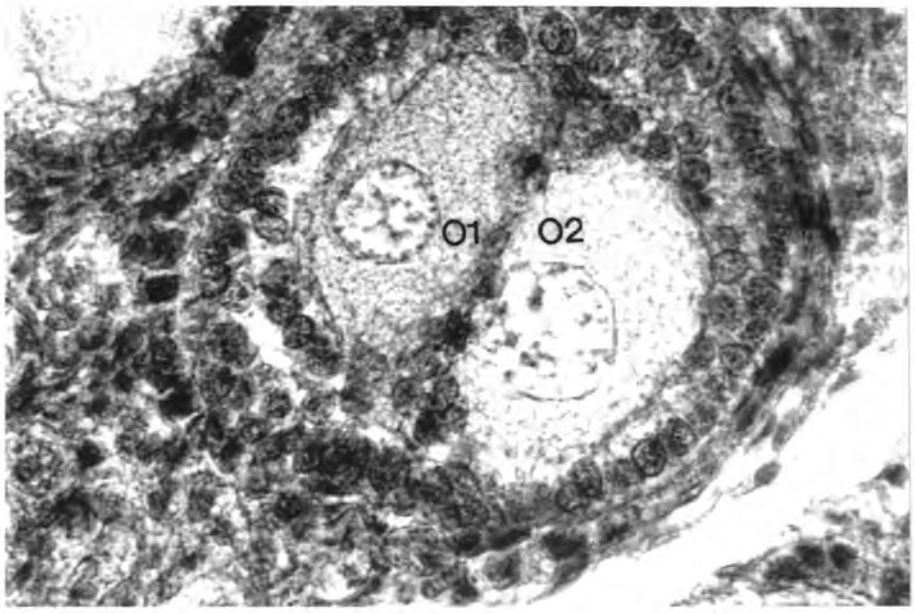

CL1
CL2

Fig. 12. Section through the ovary showing a follicle in Type II atresia. Follicular cells have degenerated and the oocyte (arrow) is starting to show signs of deformation. (September) (350X).

Fig. 13. A biovular follicle from the right ovary showing the two oocytes (O1 & O2). (October). (350X).




O1
O2

5.3.DISCUSSION

The development of ovarian follicles from the primordial via primary and secondary follicles to Graafian follicles, as observed in the present study is characteristic of all eutherian mammals.

The lack of hypertrophy of the cumulus oophorus cells of *Tadarida aegyptiaca* has been reported for other members of the family Molossidae (*Tadarida australis*, Kitchener and Hudson, 1982; *Mormopterus planiceps*, Crichton and Krutzsch, 1987). This is not unexpected since *Tadarida aegyptiaca* and other molossids do not hibernate and hypertrophy of the cumulus oophorus cells is a characteristic of hibernating vespertilionids (Kitchener, 1975; Kitchener and Halse, 1978; Kitchener and Coster 1981). Wimsatt (1944) noted that hypertrophy of the cumulus cells in hibernating vespertilionids may have some functional significance correlated with prolonged winter hibernation, probably supplying the nutritional requirements to the developing oocyte during this period.

The theca folliculi, which has been reported in all mammals for which information is available (Mossman and Duke, 1973), originates from the surrounding ovarian connective tissue and in some species occurs in two layers. The inner, cellular theca interna is present in all mammals and the outer, fibrous theca externa occurs in some species, especially the larger ones. The theca externa is rare in the microchiroptera and has only been reported in a few species, such as *Rhinolophus ferrumequinum* (Matthews, 1937) and *Myotis lucifugus* (Guthrie and Jeffers, 1938b). The theca interna has been described for many Microchiroptera including *Taphozous longimanus* (Gopalakrishna, 1955) and *Miniopterus schreibersii* (Van der Merwe, 1979). In the Molossidae, Crichton and Krutzsch (1987), described both thecal

layers in *Mormopterus planiceps* while in *Tadarida australis* (Kitchener and Hudson, 1982) only the theca interna is present. The results of the present study indicate that in *T. aegyptiaca* the theca interna comprises mostly cuboidal cells with a few fusiform cells. Apart from their capillaries providing nourishment to the developing oocyte, follicles and corpus luteum, cells of the theca folliculi may give rise to endocrine tissue either by formation of thecal glands or by formation of corpora atretica (Kayanja and Mutere, 1975; Jerrett, 1979).

The zona pellucida, a thick non-cellular coat that surrounds all mammalian eggs (Wassarman, 1988) appeared around the oocyte of late primary follicles. There has been some controversy over the origin of the zona pellucida. Guthrie and Jeffers (1938a) and Sotello and Porter (1959) suggested that the zona pellucida of *Myotis lucifugus* is formed by the oocyte while Kingsbury (1939) and Chiquoine (1960) have suggested that it is the follicular cells that produce the zona pellucida. Stegner and Wartenberg (1961, in Peters and McNatty, 1980) report that the zona is produced by both the follicular cells and the oocyte. More recently it has been established that the protein component of the zona is secreted by the growing oocytes (Wassarman, 1988).

The decrease in the relative size of oocyte in relation to follicular size during follicular growth in the present study shows similarities with that described for other eutherian mammals (Parkes, 1931 and Pincus, 1936 for reviews for the mouse, rat, ferret, rabbit, baboon and pig) and microchiropterans (Wimsatt, 1944, Myotis lucifugus; Gopalakrishna, 1955, *Taphozous longimanus*; Van der Merwe, 1979, *Miniopterus schreibersii*). However, in contrast to these authors, the present study reveals a three stage follicular growth similar to that reported by Bernard (1980a) for *Miniopterus schreibersii*, *Miniopterus fraterculus*, *Myotis*

tricolor, *Hipposideros caffer* and *Nycteris thebaica*. The reasons suggested for this apparent pattern of growth is that the first stage, represented by growth of primary and primordial follicles is by cellular hypertrophy, the growth of the secondary follicles is by cell division of the follicular cells whilst the growth of Graafian follicles occurs by the addition of the liquor folliculi in the antrum. In the latter stage the Graafian follicle is growing but the oocyte has reached full size. The insignificant but positive correlation observed in this study could be due to a possible absence of material for the preovulatory Graafian follicle stage.

Gopalakrishna and Badwaik (1988) recognise three types of corpora lutea in bats, namely included, pedunculated and extrovert. The corpora lutea in the present study correspond to the included corpora lutea of Gopalakrishna and Badwaik (1988). The occurrence of two corpora lutea of the same histological age and status is surprising since multiple births have not been observed in *Tadarida aegyptiaca*. However, observations of simultaneous ovulations, although rare, have been made in other members of the family (Sherman, 1937, *Tadarida cynocephala*, Kitchener and Hudson, 1982, *Tadarida australis*; Van der Merwe *et al.*, 1987, *T. pumila*; Rasweiler, 1988, *Molossus atter*). Such ovulations could provide extra eggs to compensate for occasional failures of fertilisation or early embryonic development (Rasweiler, 1988). This would be of advantage to a seasonal breeder with a low reproductive potential. However, it is unlikely that multiple births will occur in this species since Gopalakrishna *et al.* (1991) have shown that the blastocyst expands after entering the uterus and implants superficially. Implantation takes place at the cranial end of the uterine horn and the already implanted blastocyst may exclude additional ones, thus precluding their advanced development.

Gopalakrishna and Badwaik (1988) measured the growth of the corpora lutea in relation to pregnancy in seven species of Indian bats and noted a marked difference in the growth rate between stages. However, in all species the maximum size was reached at about implantation, after which the placenta takes over progesterone secretion from the corpus luteum for the maintenance of pregnancy. A number of other studies have confirmed this, indicating that the corpus luteum reaches maximum size at implantation (Myers, 1977; Bernard, 1980a, b; and Ramakrishna *et al.*, 1981). It appears from studies of Karim (1973), Bernard (1980a, 1982) and Gopalakrishna and Badwaik (1988) that the corpus luteum degenerates before parturition in species where it is extruded from the ovary. In species where it is included in the ovary (*Mormopterus planiceps*, Crichton and Krutzsch, 1987 and *Tadarida aegyptiaca*, present study) the corpus luteum survives to term. Gopalakrishna *et al.* (1986) report on a corpus luteum in *Rousettus leschenaulti* that survives for a relatively long time after birth, which had not been known to exist in any mammal. They suggest that this is a unique adaptation to enable the species to bring about physiological alternation of the ovaries in successive reproductive cycles.

The two types of atresia that occurred in the present study are similar to those described in other Microchiroptera (Matthews, 1937, *Rhinolophus ferrumequinum*; Guthrie and Jeffers, 1938a, *Myotis lucifugus*; Van der Merwe, 1979, *Miniopterus schreibersii*; Bernard, 1980a, *M. schreibersii, M. tricolor, M. fraterculus, Hipposideros caffer* and *Nycteris thebaica*; Bernard, 1985, *Rhinolophus capensis*). The significance of atresia is unclear but thecal cells arising from atresia of multilaminar follicles may give rise to interstitial gland tissue (Guthrie and Jeffers, 1938a). Since interstitial gland tissue may secrete steroid hormones (Mossman and Duke, 1973), atresia is probably a significant event, not just a way of removing excess

follicles.

Polyovular follicles are rare in bats (Guthrie and Jeffers, 1938a) and have only been observed once in the present study. They occur more often in fetal and juvenile ovaries than in adult bats (Mossman and Duke, 1973), although in the present study the specimen was an adult. The origin, fate and significance of polyovular follicles is not clearly understood. Leach and Conaway (1963, in Mossman and Duke, 1973) stated that such follicles arise in primary sex cords by the incorporation of two or more oocytes in a common follicular envelope. The follicles seem of little significance since, in species which ovulate a large number of eggs, they do so through uniovular follicles (Mossman and Duke, 1973). It is unlikely that polyovular follicles increase litter size since multiple births have never been observed in Egyptian free-tailed bats. However their possible role in providing extra eggs for reasons similar to multiple ovulations discussed above should not be discounted. Furthermore, polyovular follicles would provide less thecal and luteal gland tissue in direct proportion to the number of eggs released.

CHAPTER 6. SEASONALITY OF REPRODUCTION.

6.1. INTRODUCTION.

Nonhibernating bats display a variety of reproductive patterns even when environmental cues seem subtle and seasonality poorly marked (Krutzsch, 1979). The female may be aseasonally polyoestrous, seasonally polyoestrous or seasonally monoestrous and may include specialisations such as menstruation, prolonged embryonic development, delayed implantation and ovarian-uterine asymmetry (Jerrett, 1979). All patterns excluding the specialisations have been reported in the family Molossidae (Braestrup, 1933; Harrison, 1958; Marshall and Corbett, 1959; Mutere, 1973a, b; Krutzsch and Crichton, 1985; Van der Merwe *et al.*, 1986; Van der Merwe *et al.*, 1987)

In molossids from the temperate regions of the New World a single annual reproductive cycle appears to be the rule (Crichton and Krutzsch, 1987), with oestrus and breeding occurring in late winter/early spring, and male and female cycles reportedly or presumably being synchronised (Sherman, 1937; Hamlett, 1947; Krutzsch, 1955a,b, 1959 ; Davis *et al.*, 1962; Jerrett, 1979; Kitchener and Hudson, 1982; Krutzsch and Crichton, 1987). The scanty information relative to Old World temperate molossids concerns female *Tadarida australis* (Kitchener and Hudson, 1982) and both sexes in *Mormopterus planiceps* (Krutzsch and Crichton, 1987; Crichton and Krutzsch, 1987). These studies confirmed Krutzsch and Crichton's (1985) contention that New and Old World temperate molossids behave similarly.

The ultimate factors controlling seasonal breeding in mammals are climate, caloric availability and/or nutrient quality of an animal's food (Baker, 1938 in Bronson, 1985, Sadlier, 1969,

Chapman, 1982). However, few studies have measured climatic variables and food supply at the same time as investigating bat reproductive cycles (Racey, 1982). Births occur just before the onset of peak rains in *Eidolon helvum* (Mutere, 1967) and *Hipposideros caffer* (Mutere, 1970), and during peak rains in *Otomops martiensenii* (Mutere, 1973a). Marshall and Corbett (1959) and Mutere (1973b) observed that at $0^{0}26'$ and $0^{0}6'$ north respectively, *Tadarida pumila* breeds throughout the year. In both studies there were two peaks in the occurrence of pregnant females, with maximum precipitation coinciding with lactation. In the latter study peaks in insect abundance coincided with the period of maximum rainfall.

In some male mammals reproduction is seasonal and reproductive organs, accessory glands and secondary sexual structures undergo seasonal changes in size, histology and secretions (Chapman, 1982), while others are able to breed throughout the year, such as man, the laboratory rat and the bull. Although a species may reproduce aseasonaly throughout most of its distribution some populations may reproduce seasonaly. Cowgill (1966a,b) reported seasonal reproduction in the !Kung of the Kalahari, where birth appeared to be related to rainfall. The timing of reproduction varies with latitude in many species of seasonally breeding mammals and in several bats (Racey, 1982). At higher latitudes reproduction tends to be seasonal.

In most nonhibernating bat species, spermatozoa are produced and accessory sex glands are secretorily active at a time which coincides with oestrus in the female and copulation, ovulation and fertilisation are contemporary events (Krutzsch, 1979). However, exceptions have been reported in nonhibernating *Pipistrellus ceylonicus* (Gopalakrishna and Madhavan, 1971); *Tylonycteris robustula* and *T. pachypus* (Racey *et al.*, 1973) and *Myotis albescens* and

Myotis nigricans (Myers, 1977). In these species sperm are stored in the same way as in the hibernating *Myotis lucifugus* (Wimsatt *et al.*, 1966). In the Molossidae sperm storage has only been reported in the epididymis of *Mormopterus planiceps* (Krutzsch and Crichton, 1987).

Knowledge concerning the seasonality of reproduction in the Molossidae in Southern Africa is fragmentary. Rautenbach (1982) and Smithers (1983) collected pregnant *Tadarida pumila* between August and February and lactating specimens in February respectively in the subregion. Van der Merwe *et al.* (1986) concluded that this species is polyoestrous and undergoes a post-partum oestrus in the eastern Transvaal. The few studies concerning reproduction in *Tadarida aegyptiaca* are from India (Kasyap, 1980 and Gopalakrishna *et al.*, 1991) and both show the species to be a seasonal breeder giving birth in summer. The only relevant data concerning the species in the Southern Africa subregion is that pregnant and lactating females have been collected between September and November and December respectively in the Transvaal (Skinner and Smithers, 1990) and Hersellman and Norton (1985) found heavily pregnant specimens during November in the Cape Province. The aim of this chapter is therefore to describe the seasonality of reproduction in *T. aegyptiaca* from the Cape Province of South Africa.

6.2. RESULTS

6.2.1. MONTHLY CHANGES IN BODY MASS AND REPRODUCTIVE ORGANS OF MALE *T. AEGYPTIACA*

Spermatogenically active animals were found between February and October and copulation, as evidenced by the occurrence of early pregnancy in females, occurred between August and September.

There was a statistically significant change in body mass throughout the year ($p < 0.05$) with lowest masses during June (winter) and highest masses in December (summer) (Fig. 14). Mean body mass of spermatogenically active animals was significantly higher than inactive specimens ($P < 0.05$).

Monthly changes in testis mass (Fig. 15), seminiferous tubule diameter (Fig. 16) and thickness of the seminiferous epithelium (Fig. 17) were all statistically significant ($p < 0.05$ for all). In all cases values were low during spermatogenic inactivity, began to increase in February and reached a peak in August, at the probable time of copulation (Figs 15-17).

The mass of the accessory glands and the para-anal glands showed a significant seasonal variation with a peak in August/September (Figs 18 & 19). The glands were non-secretory from November to July.

6.2.2. MONTHLY CHANGES IN BODY MASS AND REPRODUCTIVE ORGANS OF FEMALE *T. AEGYPTIACA*.

Female body mass differed significantly between the months ($p < 0.05$) with a peak in late pregnancy (December) and was lowest during winter (July) (Fig. 20).

Since ovulation and implantation were restricted to the right ovary and uterine horn, only changes in these organs were quantified. The change in the diameter of the secondary follicles in the right ovary during the annual reproductive cycle was statistically significant ($p < 0.05$) with maximum values obtained during January/February and minimum values during October/November (Fig. 21).

There were two periods of Graafian follicle production in the present study (Fig. 21). The first period was between May and July and resulted in an ovulation. The second period was between October and December and did not result in an ovulation.

There was a significant increase ($p < 0.05$) in thickness of the uterine wall between July and October coinciding with early pregnancy (Fig. 22). During November and December little uterine tissue was left after removing the foetus so the uterine material from these months was not sectioned. Between January and March the thickness of the wall of the right uterine horn decreased (Fig. 22).

The vaginal epithelium was thin (one to three layers thick) from September to June and just before copulation in August developed an outer stratified layer which was shed by September.

6.2.3. THE OESTROUS CYCLE.

The pro-oestrous condition occurred from March to July when developing Graafian follicles were present in the right ovary. The oestrous condition was not observed but must have occurred in August since by September corpora lutea were present and specimens were pregnant. Pregnancy lasted from September to December with births occuring in December after a gestation period of about four to five months.

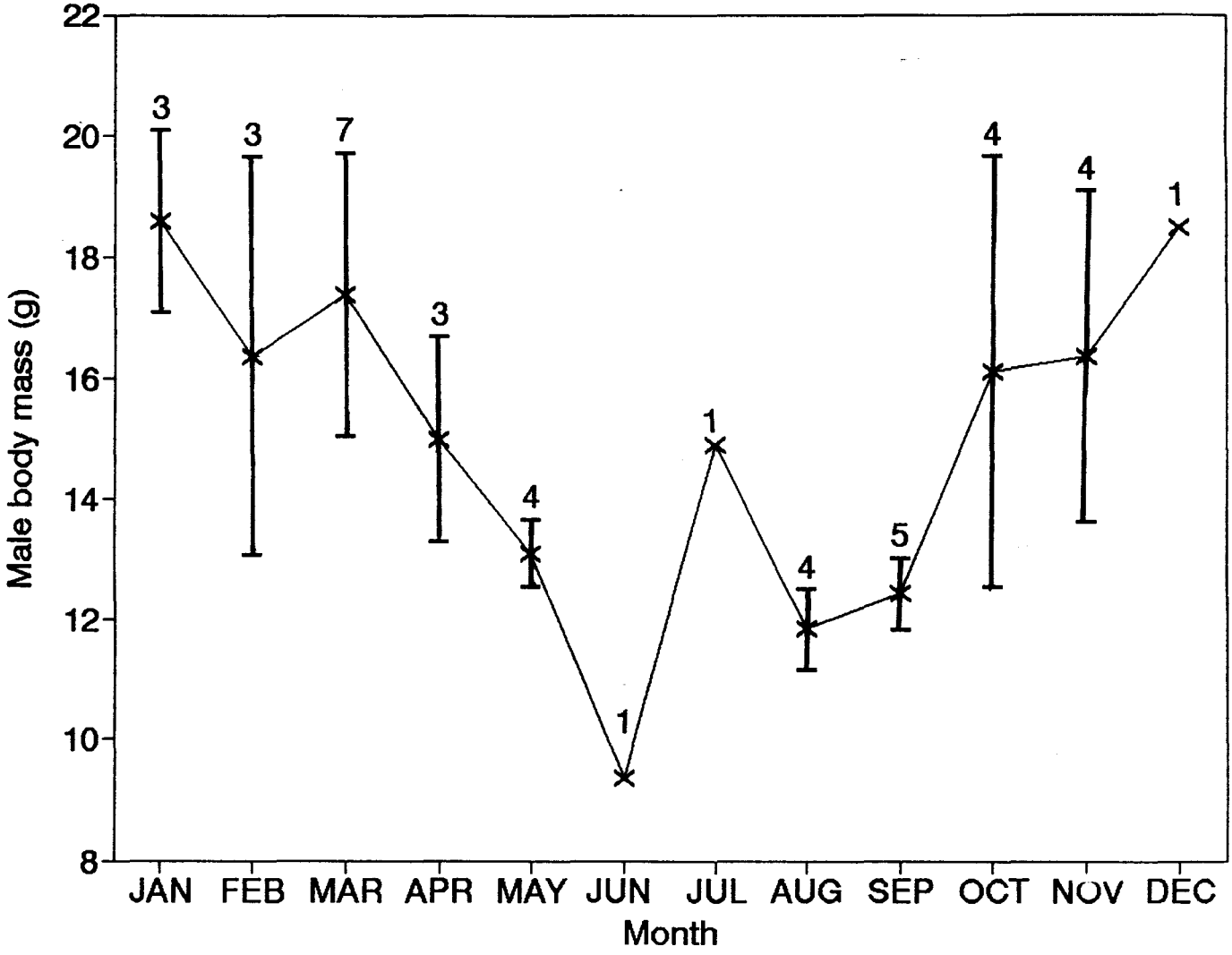


Fig. 14. Monthly changes in the body mass of male *T. aegyptiaca*. Note the increase in mass during the summer months and decrease in mass in winter.

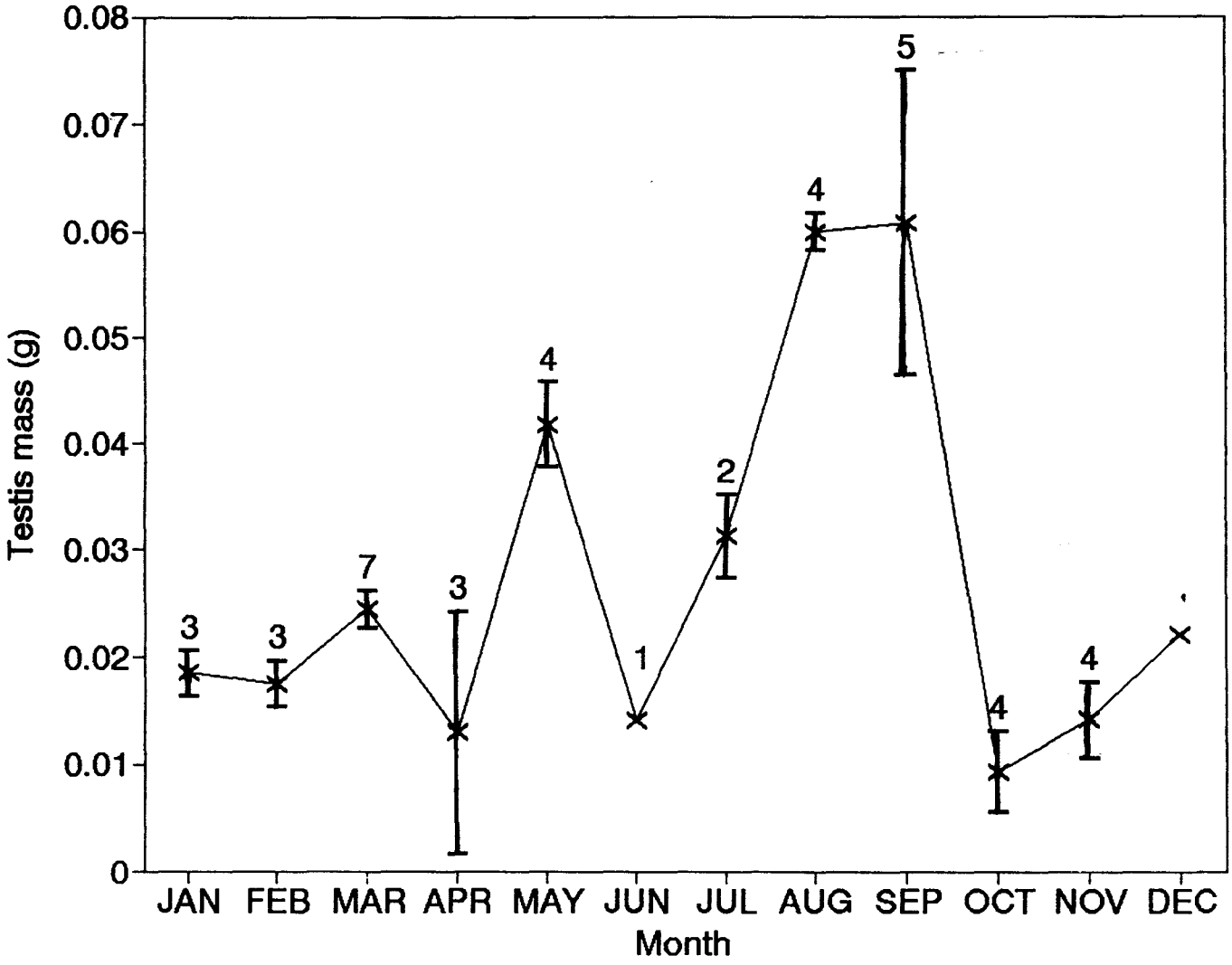


Fig. 15. Monthly changes in the testis mass of the Egyptian free-tailed bat.

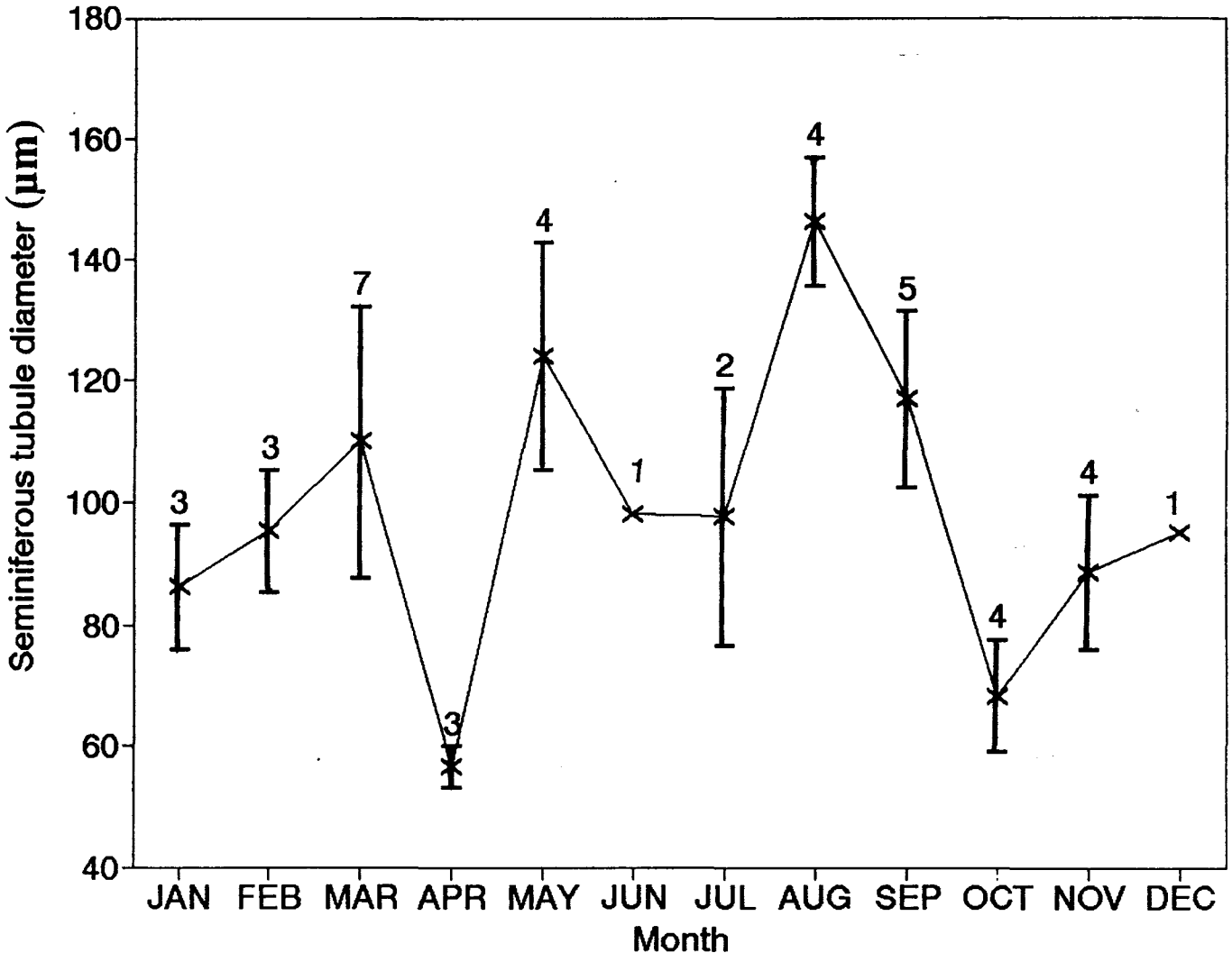


Fig. 16. Monthly changes in the seminiferous tubule diameter.

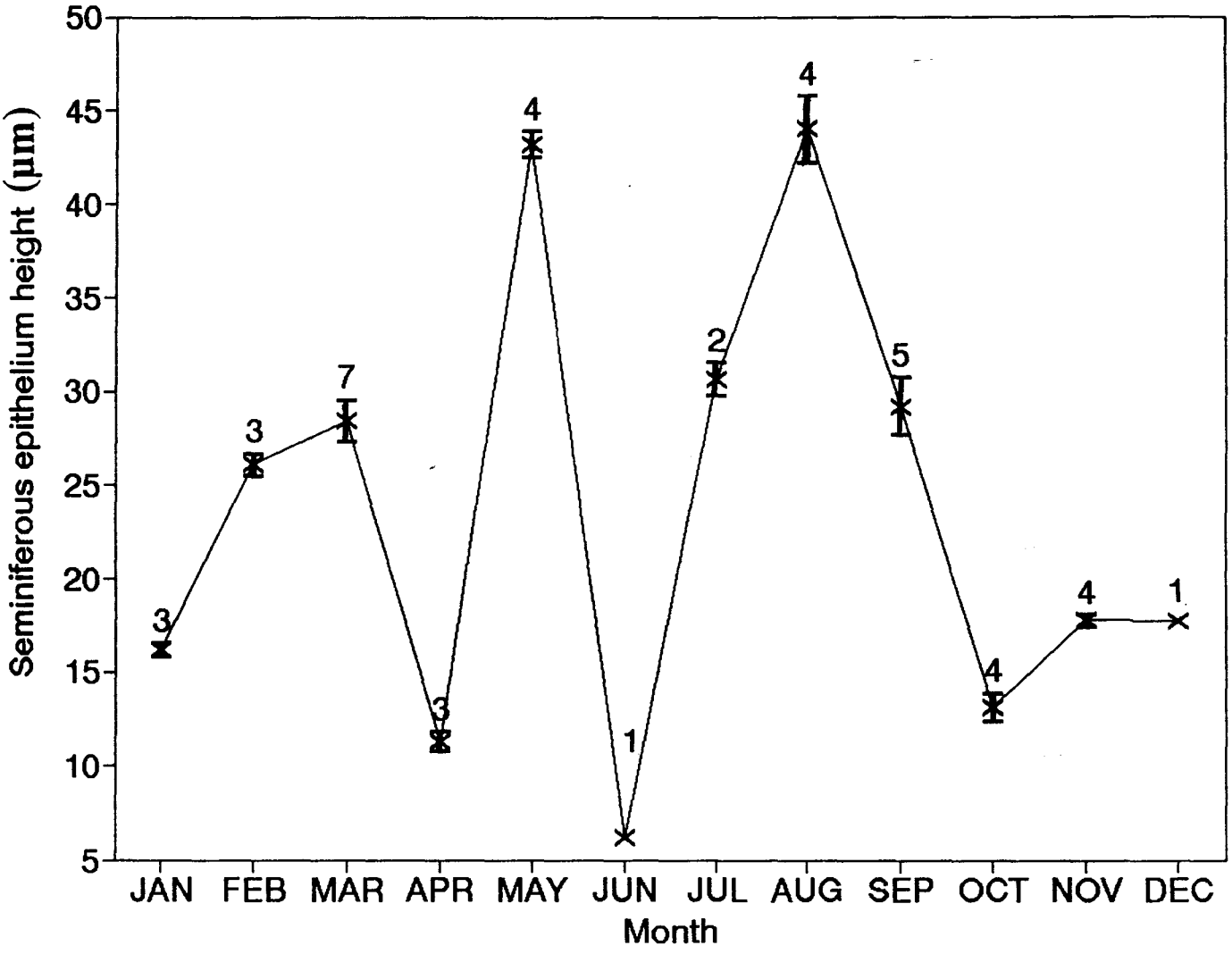


Fig. 17. Monthly changes in the seminiferous epithelium height.

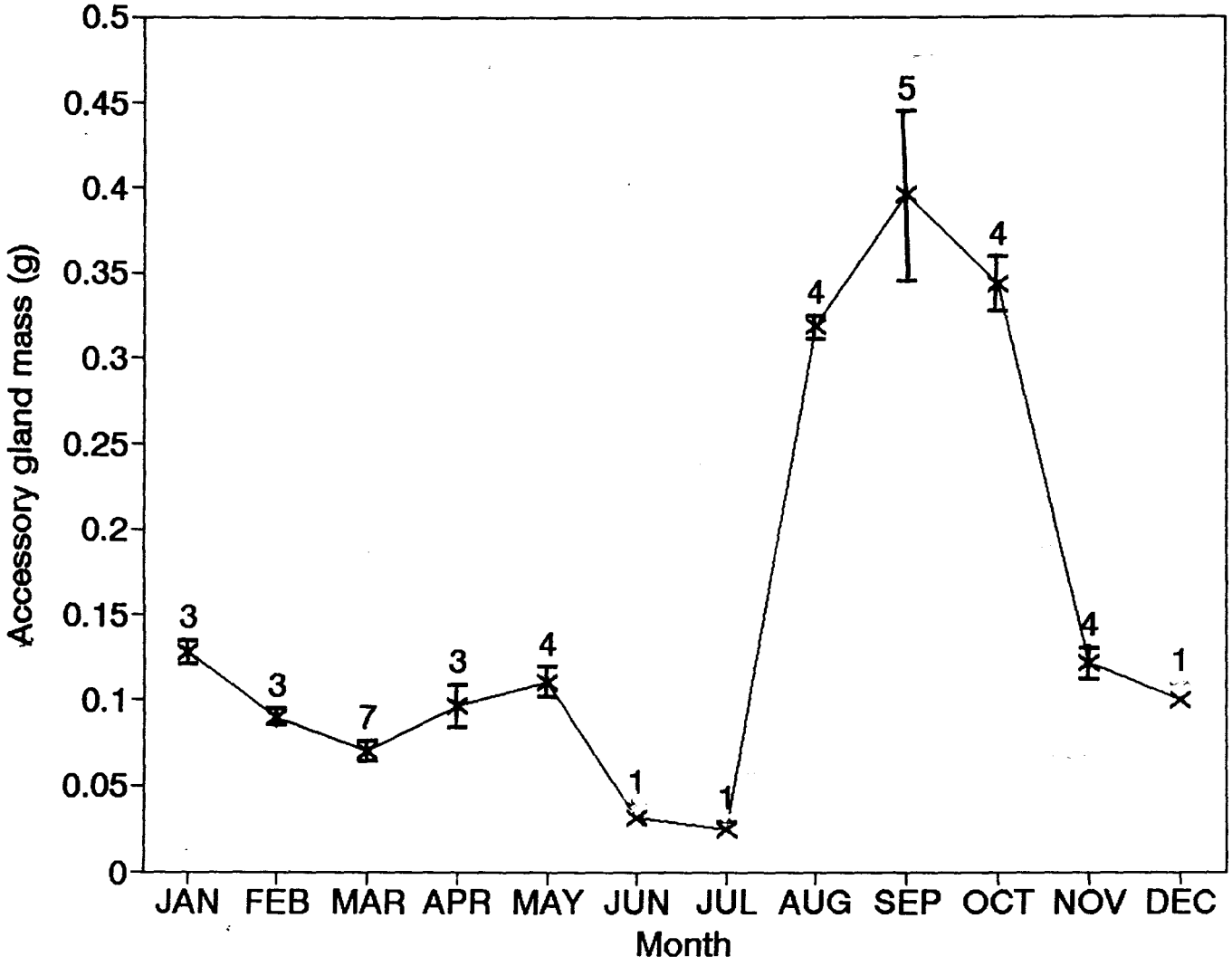


Fig. 18. Mean monthly changes in the mass of the accessory gland complex showing the peak in August.

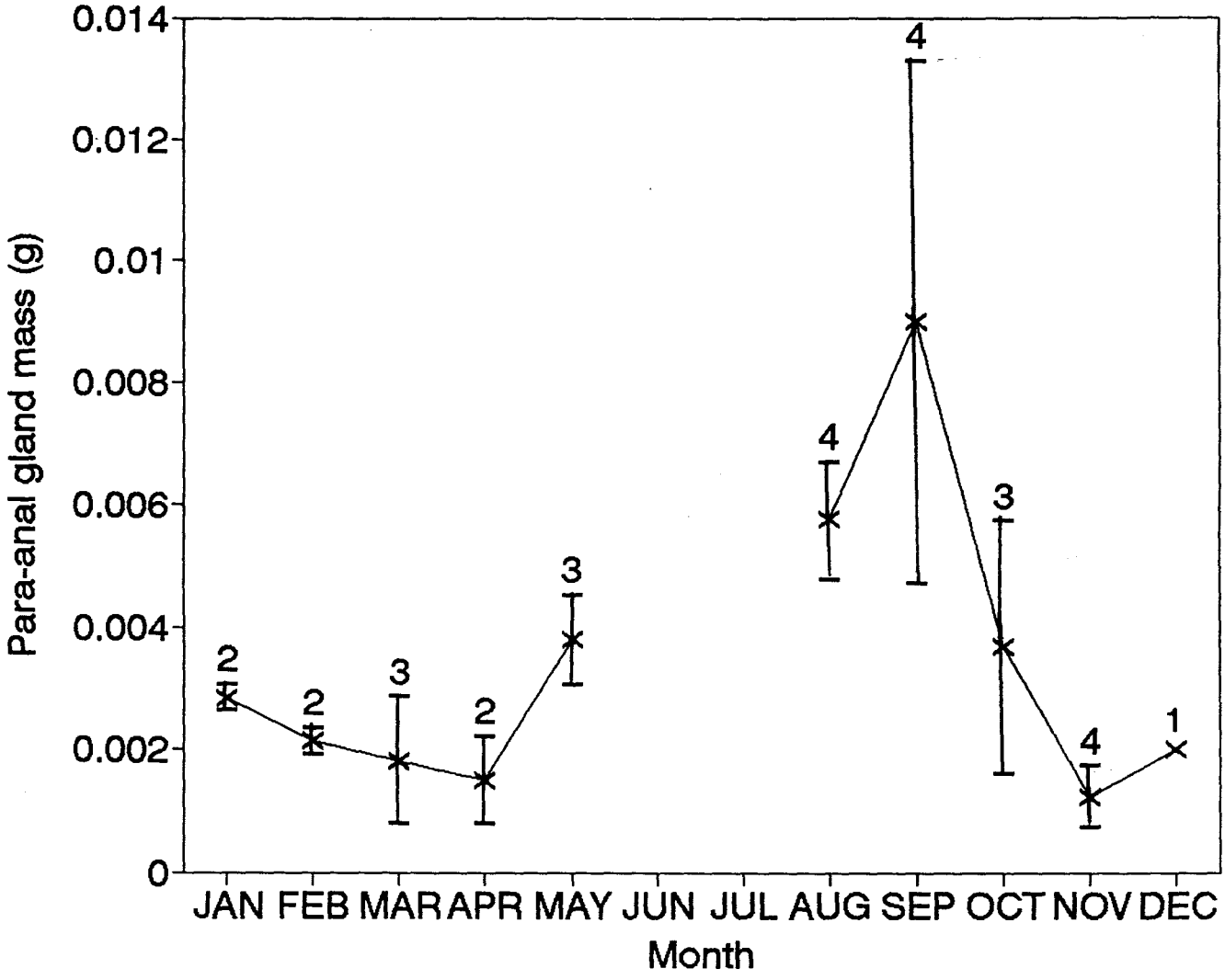


Fig. 19. Monthly changes in the weight of the para-anal glands throughout the annual cycle. Note the high values during the period August/September.

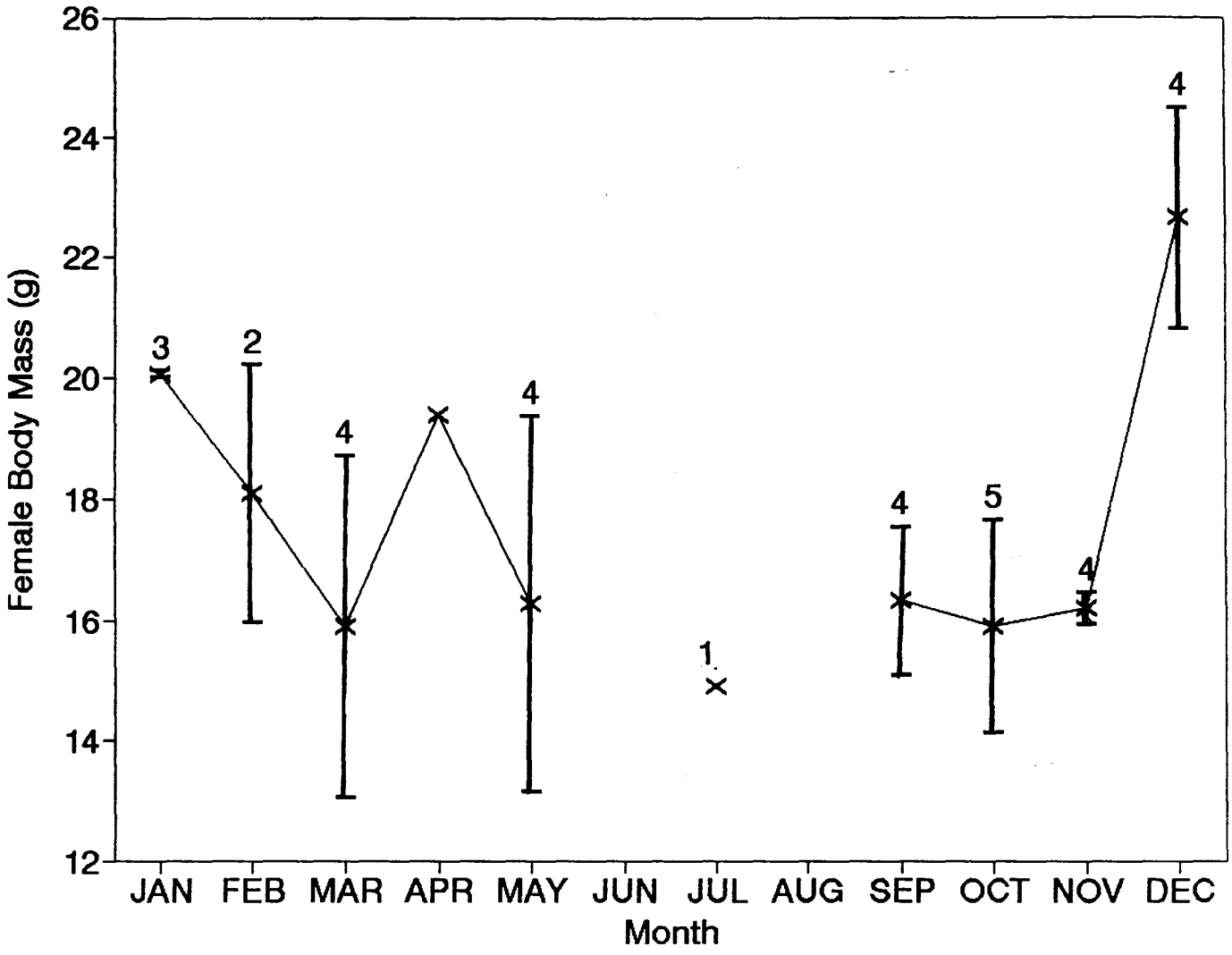


Fig. 20. Monthly changes in the body mass of adult females.

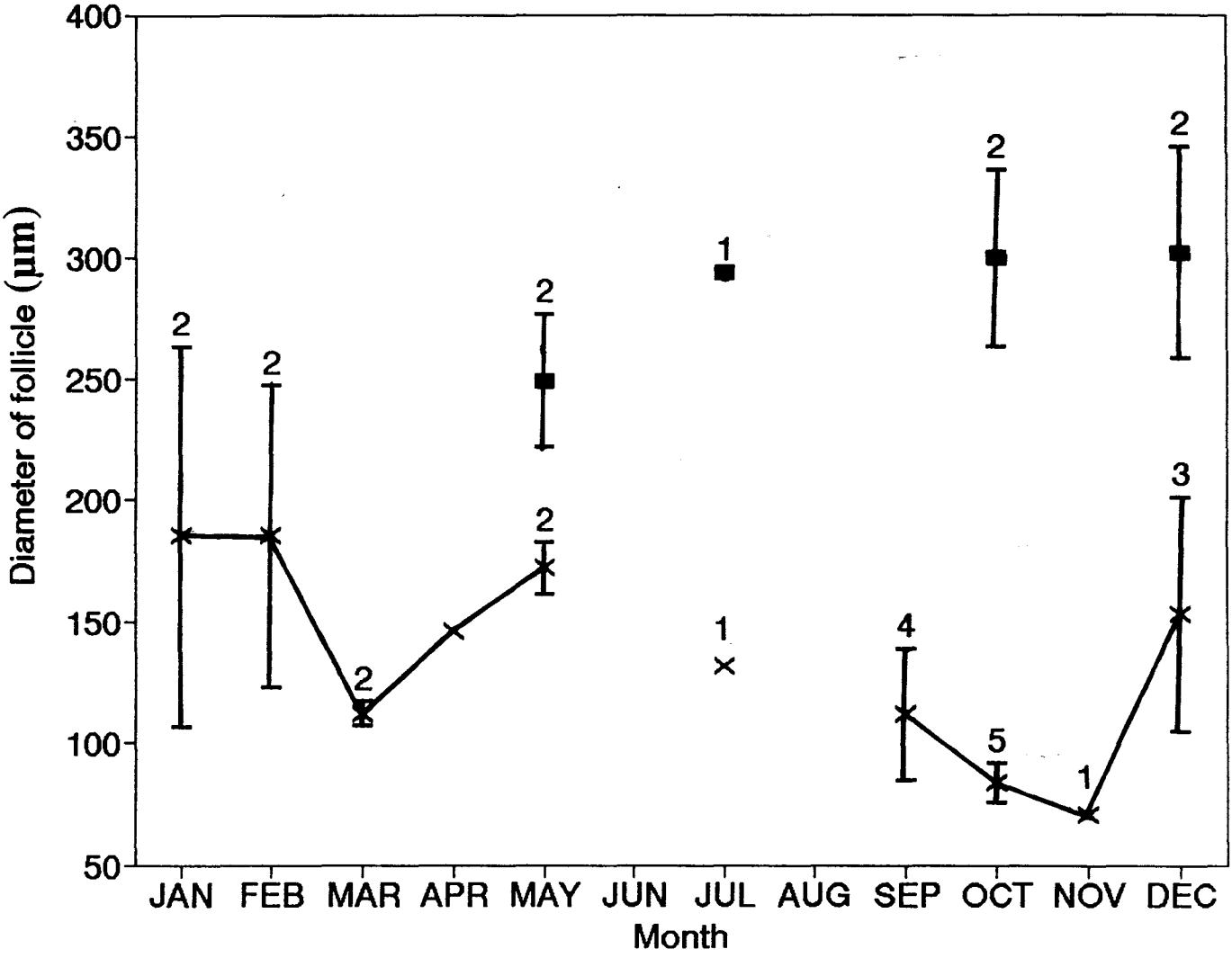


Fig. 21. Monthly changes in diameters of secondary (x) and Graafian follicles (■) from the right ovary of the Egyptian free-tailed bat.

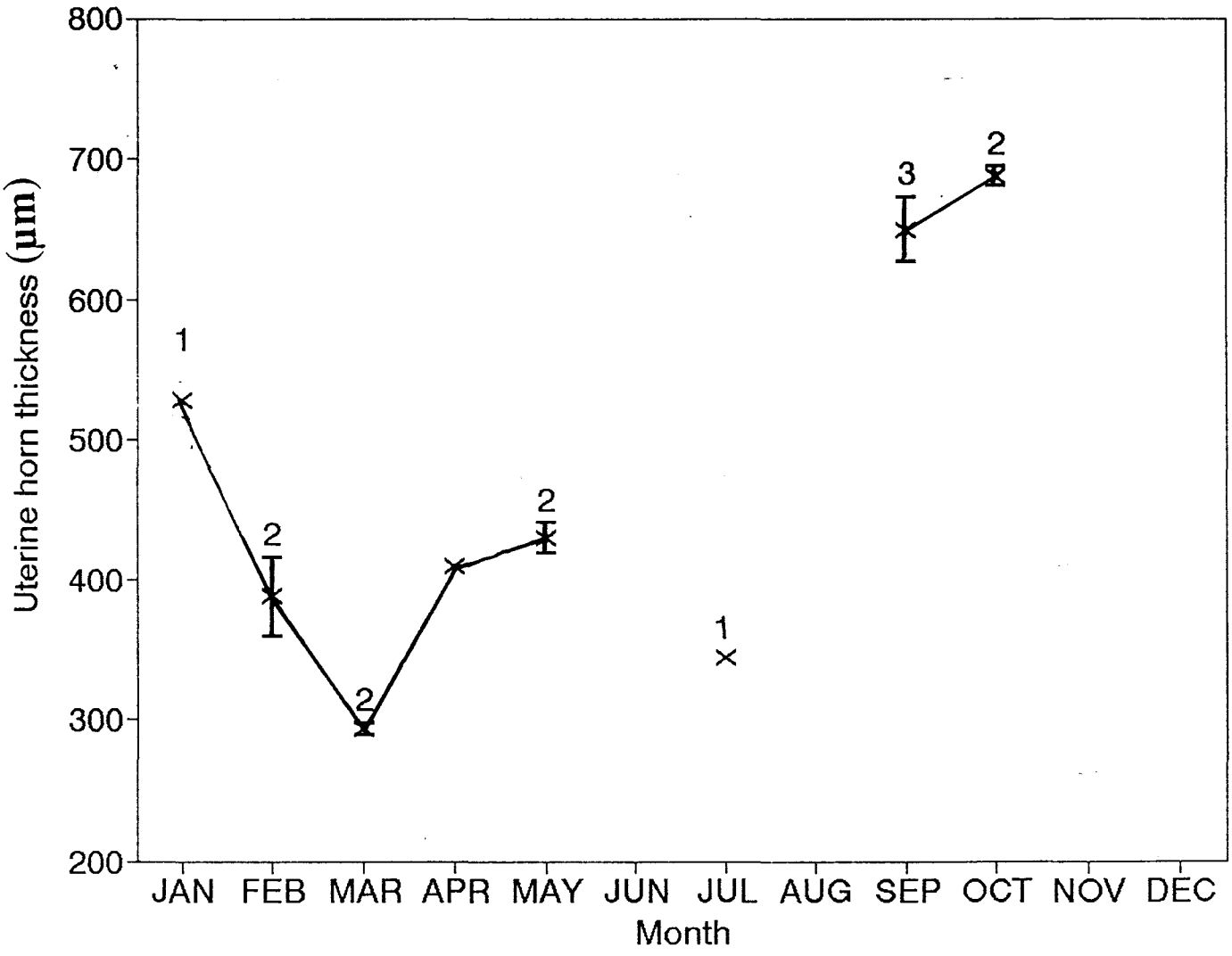


Fig. 22. Monthly changes in the thickness of the wall of the right uterine horn of the Egyptian free-tailed bat.

6.3. DISCUSSION.

The seasonal changes in mean male and female body mass observed in the present study are not unexpected since maximum body mass coincides with maximum summer rainfall and this climatic factor has been shown to affect insect abundance (Mcwilliam, 1988).

The results indicate that reproduction in the Egyptian free-tailed bat is seasonal and similar to other temperate members of the family in having a single annual reproductive cycle (Kitchener and Hudson 1982; Sherman, 1937; Davies *et al.*, 1962; Krutzsch, 1959). Seasonal monoestry is not restricted to temperate molossids and has been observed in the relatively stable environment of the tropics (Mutere, 1973a, *Otomops martienseni*). However, even in the tropics molossids feed high above ground where insect abundance may be highly seasonal (McNab, 1976) and this could explain the reproductive pattern observed in *Otomops martiensenii* at a tropical latitude.

In the tropical Molossidae polyoestry appears to be the norm and females have more than one period of Graafian follicle growth (Krutzsch and Crichton, 1985, *Molossus fortis*; Van der Merwe *et al.*, 1986 and Van der Merwe *et al.*, 1987, *Tadarida pumila*). Polyoestry has not been reported in purely temperate species and *Tadarida aegyptiaca* seems to follow this temperate pattern in being monoestrous. Van der Merwe *et al.* (1986) and Van der Merwe *et al.* (1987) report that *Tadarida pumila* is polyoestrous in the southern temperate latitudes (about 24^0S). However, their study area is close to the tropics and it may be more appropiate to consider it as being subtropical. The adaptive significance of the second period of Graafian follicle development observed in the present study remains obscure. It is possible that *T. aegyptiaca* may have originated in the tropics and that the second period of Graafian follicle

growth is a relict of the past.

Contrary to *Mormopterus planiceps* (Krutzsch and Crichton, 1987) the para-anal glands of *Tadarida aegyptiaca* showed seasonal fluctuation in secretory activity in synchrony with spermatogenesis and this suggests the glands play a role in reproduction.

Kasyap (1980) and Gopalakrishna *et al.* (1991) reported the gestation period of *Tadarida aegyptiaca* to be between 77 and 90 days in India. The gestation of about four to five months observed in the present study is considerably longer than for the same species in India and for some other molossid bats where the gestation is approximately three months (Sherman, 1937; Krutzsch, 1955a; Davies *et al.*, 1962), but very similar to that of *Mormopterus planiceps* (Krutzsch and Crichton, 1987). Such comparisons may be misleading since gestation length is positively correlated with body mass (Kihlström, 1972; Sacher and Staffeldt, 1974). It is suggested that the longer gestation of *T. aegyptiaca* at about 33^{0}S (present study) is a consequence of the effect of lower temperature on the foetal growth rate. This type of effect of temperature on the gestation length is well documented for bats (Racey and Swift, 1981). Tropical and subtropical species appear to have a relatively short gestation period, for example, about two months in *Tadarida pumila* (Van der Merwe *et al.*, 1986) and *Tadarida condylura* (Happold and Happold, 1989). It is apparent from Kitchener and Hudson (1982) that the gestation period in *Tadarida australis* from Australia is about four to six months. However, it seems likely that this variation is an experimental artefact since samples were collected from the whole of Australia and over a very long period of time (88 years).

In India *T. aegyptiaca* has a strictly defined breeding cycle (Kasyap, 1980; Gopalakrishna *et*

al. 1991). Copulation, followed by fertilisation and pregnancy occurs in spring and parturition takes place in summer. In tropical and subtropical Africa most molossids are polyoestrous and have an extended breeding season (*T. pumila*, Van der Merwe *et al.*, 1986; *T. condylura* and *T. pumila*, Mutere, 1973b; *Chaerephon hindei*, Marshall and Corbett, 1959). Of these species, births in *T. pumila* (Van der Merwe *et al.*, 1986) are less synchronous. *T. aegyptiaca* (present study) appears to follow the same breeding strategy as the members of the species from India.

Although many mammals (including bats) have evolved various mechanisms to distribute the energy cost of pregnancy over a longer period of time, lactation, once initiated, does not lend itself to interruption or modulation, so that adequate food supply during lactation and weaning is the most important selection pressure in the timing of mammalian reproductive cycles (Racey, 1982). It is therefore not surprising that in *Tadarida aegyptiaca* reproduction is timed so that lactation coincides with the summer rainy season.

CHAPTER 7. GENERAL DISCUSSION AND CONCLUSIONS.

Results of the present study indicate that *T. aegyptiaca* is a monoestrous, monotocous, seasonal breeder and therefore similar to other molossids from temperate latitudes e.g. *Tadarida brasiliensis* (Sherman, 1937; Jerrett, 1979), *Mormopterus planiceps* (Krutzsch and Crichton, 1987; Crichton and Krutzsch, 1987) and *Tadarida australis* (Kitchener and Hudson, 1982).

Slight differences exist in the male accessory glands in that in *T. aegyptiaca* the absence of Cowper's gland is reported for the first time in the family and the para-anal glands that are present in *T. aegyptiaca* have only been reported in *Mormopterus planiceps* (Krutzsch and Crichton, 1987).

The dextral functional reproductive asymmetry observed in *Tadarida aegyptiaca* supports the contention of Wimsatt (1979) that this pattern is the most profound and widely encountered type of asymmetry in the Chiroptera. Asymmetry in bats is always associated with the monotocous habit and in the relatively few known polytocous species the female organs are bilaterally symmetrical (Wimsatt, 1979). The reasons and origin of the observed asymmetry are, however, still unknown. Wimsatt (1979) suggests an early origin, but could not explain the non-systematic distribution of the various patterns among taxa because these create problems in identifying a probable ancestral type. The selective pressures and adaptive significance of asymmetry observed in bats in general remains to be investigated.

The spermatogenic cycle observed in the present study is typically mammalian and similar

to that of other members of the family (*Tadarida brasiliensis*, Sherman, 1937 and *Mormopterus planiceps*, Krutzsch and Crichton, 1987). However, the significance of the relatively long period of spermatogenesis remains unclear.

Follicular development appears to be the same as in other molossids (*Tadarida australis*, Kitchener and Hudson, 1982; *Molossus fortis*, Krutzsch and Crichton, 1985; *Mormopterus planiceps*, Crichton and Krutzsch, 1987; *Tadarida pumila*, Van der Merwe *et al.*, 1986). However, more than one period of Graafian follicle production has only been shown in polyoestrous species (Krutzsch and Crichton, 1985, *Molossus fortis*; Van der Merwe et al., 1986 and Van der Merwe *et al.*, 1987, *Tadarida pumila*) and *T. aegyptiaca* is monoestrous. It is suggested that the second period of Graafian follicle production may be a relict from a tropical ancestor.

As mentioned previously, the ultimate factors that control the timing of mammalian reproduction are climate, caloric availability and/or the nutrient quality of the animals food (see Bronson, 1985; 1988). In *T. aegyptiaca* from the Cape Province reproduction is timed so that births occur in mid-summer (December). Most other bats from South Africa for which data are available give birth in summer (Skinner and Smithers, 1990 for review) and it is generally assumed that insects are abundant during this time. Since *T. aegyptiaca* is insectivorous (Advani, 1982; Skinner and Smithers, 1990) it seems likely that births are timed to coincide with a period of increased insect abundance.

REFERENCES.

ADVANI, R. (1982). Feeding biology of *Tadarida aegyptiaca thomasi*. Z. Saügtierk. **47**: 18-22.

BERNARD, R. T. F. (1980a). Female reproduction in five species of Natal cave-dwelling Microchiroptera. Phd Thesis. University of Natal. Pietermaritzburg, South Africa.

BERNARD, R. T. F. (1980b). Female reproductive anatomy and development of ovarian follicles in *Miniopterus schreibersii*. S.Afr. J. Zool. **15**: 111-116.

BERNARD, R. T. F. (1980c). Reproductive cycles of *Miniopterus schreibersii* (Kuhl, 1819) and *Miniopterus fraterculus* Thomas and Schwann, 1906. Ann. Trans. Mus. **32**: 56-64.

BERNARD, R. T. F. (1980d). Monthly changes in the reproductive organs of female *Miniopterus shreibersii natalensis* (A. Smith, 1834). Z. Saügetierk. **45**: 217-224.

BERNARD,R. T. F. (1982). Female reproduction and the female reproductive system in *Hipposideros caffer* (Sundeval, 1846). Ann. Trans. Mus. **33**(8): 131-134.

BERNARD, R. T. F. (1983). Reproduction in *Rhinolophus clivosus* (Microchiroptera) in Natal, South Africa. Z. Saügetierk. **48**: 321-329.

BERNARD, R.T.F. (1985). Reproduction in the Cape horseshoe bat (*Rhinolophus capensis*) in South Africa. S. Afr. J. Zool. **20**:129-135.

BERNARD, R. T. F. (1986). Seasonal changes in plasma testosterone and Leydig cell activity in the Cape horseshoe bat (*Rhinolophus capensis*). J. Reprod. Fert. **78**: 413-422.

BERNARD, R. T. F. (1988). An ovarian rete arteriosum may facilitate reproductive asymmetry in the fruit bat *Rousettus aegyptiacus*. S. Afr. J. Sci. **84**: 856-857.

BERNARD, R. T. F., BOJARSKI, C. and MILLAR, R. P. (1991). Patterns of Leydig cell and LH gonadotroph activity, and plasma testosterone concentrations in the seasonally reproducing Schreibers' long-fingered bat (*Miniopterus schreibersii*). J. Repr. Fert. **91**: 479-492.

BRAESTRUP, F. W. (1933). On the taxonomic value of the genus *Lophomops* (Nyctinomine Bats), with remarks on the breeding times of African bats. Ann. & Mag. Nat. Hist. (Ser 10) **6**: 269-274.

BRAMBELL, F. W. R. (1928). The development and morphology of the gonads of the mouse. Part III.- The growth of follicles. Proc. Roy. Soc . Lond. **103B**: 258-272.

BRONSON, F. H. (1985). Mammalian Reproduction: An Ecological perspective. In: Biol. Reprod. **32**(1): 1-26.

BRONSON, F. H.(1988). Seasonal Regulation of Reproduction in Mammals. In: The Physiology of Reproduction. Knobil, E and Neill, J *et al.* (Eds). Raven Press, Ltd., New York.

BROSSET, A. (1968). La permutation de cycle sexuel saisonnier chez le Chiroptère *Hipposideros caffer*, au voisinage de l'èquateur. Biol. Gabon. **43**: 325-341.

CARTER, D. C. (1970). Chiropteran Reproduction. In: About Bats. Slaughter, B. H. and Walton D. W. (Eds). Southern Methodist University Press. Dallas, Texas.

CHAPMAN, D. I. (1982). Seasonal changes in the gonads and accessory glands of male mammals. Mammal Review **1** (7/8): 231-248.

CHIQUOINE, A. D. (1960). The development of the zona pellucida of the mammalian ovum. Am. J. Anat. **106**: 149-169.

COWGILL, U. M. (1966a). The seasons of birth in man. Contemporary situation with special reference to Europe and the southern hemisphere. Ecology **42**: 699.

COWGILL, U. M. (1966b). Historical study of the season of birth in the city of York, England. Nature **209**: 1067-1070.

COWLES, R. B. (1965). Hyperthermia, aspermia, mutation rates and evolution. Quart. Rev. Biol. **40**: 341-367.

CRICHTON, E. G. and KRUTZSCH, P. H. (1987). Reproductive biology of the female Little Mastiff bat, *Mormopterus planiceps* (Chiroptera: Molossidae). Am. J. Anat. **78**: 269-386.

DAVIS, R. B., HERREID II, C. F. and SHORT, H. L. (1962). Mexican free-tailed bats in Texas. Ecol. Monograph **32**(4): 311-346.

DELHON, G. A. and von LAWZEWITSCH, I. (1987). Reproduction in the Male Llama (*Llama llama*), a South American Camelid. Acta anat. **129**: 59-66.

DE KRETSER, D. M. and KERR, J. B. (1988). The Cytology of the testis. In: The Physiology of Reproduction. Knobil, E and Neill, J. *et al.* (Eds). Raven Press, Ltd., New York.

DWYER, P. D. (1970). Latitude and breeding season in polyoestrus species of *Myotis*. J. Mammal. **51**: 405-410.

EHRENBERG, L., HEDGRAN, A. AND von EHRENSTEIN, G. (1957). Gonad Temperature and Spontaneous Mutation- rate in Man. Nature **180**: 1433-1434.

FOOTE, R.H., SWIERSTRA, E. E. AND HUNT, W. L. (1972). Spermatogenesis in the dog. Anat. Rec. **173**: 341-352.

GAISLER, J. (1965). The female sexual cycle and reproduction in the lesser horseshoe bat (*Rhinolophus hipposideros hipposideros*, Bechstein, 1800). Acta Soc. Zool. Bohemoslar. **29**: 336-352.

GAISLER, J. (1966). Reproduction in the lesser horseshoe bat (*Rhinolophus hipposideros*

hipposideros Bechstein, 1800). Bijdr. Dierkd. **36**: 45-64.

GINTHER, O. J. (1967). Local utero-ovarian relationship. J. Anim. Sci. **26**: 578-585.

GINTHER, O. J. (1974). Internal regulation of physiological processes through veno-atrial pathways: a review. J. Anim. Sci. **39**: 550-564.

GINTHER, O. J. (1976). Comparative anatomy of utero-ovarian musculature. Vet. Scope. **20**: 1-17.

GONZALES, G. F. (1989). Functional structure and ultrastructure of seminal vesicles. Archives of Andrology **22**: 1-13.

GOPALAKRISHNA, A. (1955). Observation on the breeding habits and the ovarian cycle in the Indian sheath-tailed bat, *Taphozous longimanus* (Hardwicke). Proc. Nat. Inst. Sci. India **21**: 29-41.

GOPALAKRISHNA, A. and BADWAIK, N. (1988). Growth of the corpus luteum in relation to gestation in some Indian bats. Curr. Sci. **57**(16): 883-886.

GOPALAKRISHNA, A, BADWAIK, N. and NAGARAJAN, R. (1986). The corpus luteum of the Indian fruit bat, *Rousettus leschenaulti* Desmarest. Curr. Sci. **55**(24): 1227-1231.

GOPALAKRISHNA, A and MADHAVAN, A. (1971). Parturition in the Indian Vespertilionid

bat, *Pipistrellus ceylonicus chrysothrix*. J. Bombay Nat. Hist. Soc. **68**: 66-670.

GOPALAKRISHNA, A. and MOGHE, M. A. (1960). Development of foetal membranes of the Indian leaf-nosed bat, *Hipposideros bicolor pallidus*. Z. Anat. EntGesh. **122**: 137-149.

GOPALAKRISHNA, A. and RAMAKRISHNA, P. A. (1977). Some reproductive anomalies in the Indian Rufus horseshoe bat, *Rhinolophus rouxi* (Temminck). Curr. Sci. **46**(20): 767-770.

GOPALAKRISHNA, A., SANDHU, S. K., BADWAIK, N. AND PENDHAKAR, Y. (1991) Early development, Implantation of the blastocyst and amniogenesis in the Indian Molossid bat, *Tadarida aegyptiaca* (Geoffrey). Proc. Indian natn. Sci. Acad. **57**(1): 47-58.

GROCOCK, A. N. AND CLARKE, J. R. (1975). Spermatogenesis in mature and regressed testes of the vole (*Microtus agrestis*). J. Reprod. Fert. **43**: 461-470.

GUSTAFSON, A. W. (1979). Male reproductive patterns in hibernating bats. J. Reprod. Fert. **56**: 317-331.

GUTHRIE, M. J. and JEFFERS, K. R. (1938a).A cytological study of the ovaries of the bats *Myotis lucifugus lucifugus* and *Myotis grisescens* J. Morph. **62**: 523-557.

GUTHRIE, M. J. and JEFFERS, K. R. (1938b). Growth of follicles in the ovaries of the bat *Myotis lucifugus lucifugus*. Anat. Rec. **11**: 477-496.

HAMLETT, G. W. D. (1947). Embryology of the molossid bat *Eumops*. Anat. Rec. **97**: 340-341.

HAPPOLD, D. C. D. and HAPPOLD, M. (1989). Reproduction in Angola free-tailed bats (*Tadarida condylura)* and little free-tailed bats (*Tadarida pumila*) in Malawi (Central Africa) and elsewhere in Africa. J. Reprod. Fert. **85**: 133- 149.

HARRISON, D. L.(1958). A note on successive pregnancies in an African bat. Mammalia **22**: 592-594.

HERSELLMAN, J. C. and NORTON, P. M. (1985). The distribution and conservation status of bats in the Cape Province. Ann. Cape Prov. Mus. Nat. Hist. **16**(4): 73-127.

JERRETT, D. P. (1977). Structural-functional differences in the right and left ovaries in the Mexican free-tailed bat *Tadarida brasiliensis mexicana*. Anat. Rec. **187**: 615.

JERRETT, D. P. (1979). Female reproduction in nonhibernating bats. J. Reprod. Fert. **56**: 369-378.

KARIM, K. B. (1973). A rare case of twinning in the bat *Hipposideros fulvus fulvus* (Gray). Curr. Sci. **42**: 62-63.

KASYUP, S. K. (1980). Reproductive cycle of the Indian molossid bat *Tadarida aegyptiaca*. Curr. Sci. **49**: 252-253.

KAYANJA, F. I. B. AND MUTERE, F. A. (1975). The ovary of the insectivorous bat *Otomops martienseni*. Anat. Anz. Bd. **137**: 166-175.

KIHLSTRÖM, J. E. (1972). Period of gestation and body weight in some placental mammals. Comp. Biochem. Physiol. A, **43**:673-384.

KINGSBURY, B. F. (1939). Atresia and the interstitial cells of the ovary. Am. J. Anat. **65**(2): 309-331.

KITCHENER, D. J. (1973). Reproduction in the common sheath-tailed bat *Taphozous georgianus* (Thomas) (Microchiroptera: Emballonuridae)), in Western Australia. Austr. J. Zool. **21**: 375-379.

KITCHENER, D. J. (1975). Reproduction in female Gould's Wattle Bat, *Chalinolobus gouldii* (Gray) (Vespertiolionidae) in Western Australia. Aust. J. Zool. **23**: 29-42.

KITCHENER, D. J. (1976). Further observations on reproduction in the common sheath-tailed bat, *Taphozous georgianus* Thomas, 1915 in western Australia, with notes on the gular pouch. Rec. West. Aust. Mus. 1976, **4**(4): 335-347

KITCHENER, D. J. AND COSTER, P. (1981). Reproduction in female *Chalinolobus morio* (Gray) (Vespertilionidae) in South-Western Australia. Aust. J. Zool. **29**: 305-320.

KITCHENER, D. J. AND HALSE, S. A. (1978). Reproduction in female *Eptesicus regulus*

(Thomas) (Vespertilionidae) in South-Western Australia. Austr. J. Zool. **26**: 257-267.

KITCHENER D. J. and HUDSON, C. J. (1982). Reproduction in the Female White Striped Mastiff Bat, *Tadarida australis* (Gray) (Molossidae). Aust. J. Zool. **30**: 1-14.

KOOPMAN, K. F. and JONES, J. K. (1970). Classification of bats. In: About Bats. Slaughter, B. H. and Walton, D. W. (Eds), Southern Methodist University Press, Dallas.

KRUTZSCH, P. H. (1955a). Observation on the mexican free-tailed bat, *Tadarida mexicana*. J. Mamm. **36**(2): 236-242.

KRUTZSCH, P. H. (1955b). Observation on the California mastiff bat. J. Mamm. **36**: 407-414.

KRUTZSCH, P. H. (1959). The reproductive cycle of the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Anat. Rec. **133**: 400.

KRUTZSCH, P. H. (1979). Male reproductive patterns in nonhibernating bats. J. Reprod. Fert. **56**: 333-344.

KRUTZSCH, P. H. and CRICHTON, E. G. (1985). Observation on the reproductive cycle of female *Molossus fortis* (Chiroptera: Molossidae) in Puerto Rico. J. Zool., Lond. **207**: 137-150.

KRUTZSCH, P. H. AND CRICHTON, E. G. (1987). Reproductive biology of the male little mastiff bat, *Mormopterus planiceps* (Chiroptera: Molossidae) in Southeast Australia. Am. J. Anat. **178**: 352-368.

KRUTZSCH, P. H., WATSON, R. H. and LOX, C. D. (1976). Reproductive biology of the male leaf-nosed bat *Macrotus waterhousii* in Southwestern United States. Anat. Rec. **184**: 611-636.

MARSHALL, A. J. (1953). The unilateral endometrial reaction in the giant fruit bat (*Pteropus giganteus* Brunnich) J. Endocrin. **9**. 42-43.

MARSHALL, A. J. AND CORBETT, P. S. (1959). The breeding biology of equatorial vertebrates: Reproduction in the bat *Chaerephon hindei* Thomas at latitude $0^{0}26'$. Proc. Zool. Soc. Lond. **132**: 607-616.

MATTHEWS, L. H. (1937). The female sexual cycle in the British horseshoe bats, *Rhinolophus ferrumequinum* Barrett-Hamilton and *R. hipposideros minutus* Montagu. Trans. Zool. Soc. Lond. **23**: 224-267.

MATTHEWS, L.H. (1941). Notes on the genitalia and reproduction of some African bats. Proc. Zool. Soc. Lond. **3B**: 289-342.

MCNAB, B. K. (1976). Seasonal fat reserves in two tropical environments. Ecology **57**: 332-338.

MCWILLIAM, A. N. (1988). The reproductive cycle of long-fingered bat, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae) in a seasonal environment of the African trpics. J. Zool., Lond. **216**: 119-129.

MOKKAPATI, S AND DOMINIC, C. J. (1976). Sites of formation of fructose and citric acid in the accessory reproductive glands of three species of male chiropterans. Biol. Reprod. **14**: 627-629.

MOSSMAN, H. W. AND DUKE, K. L. (1973). Comparative morphology of the mammalian ovary. The University of Wisconsin Press, Madison.

MUTERE, F. A. (1967). The breeding biology of equatorial vertebrates: reproduction in the fruit bat, *Eidolon helvum*, at latitude $0^0 20'$ N. J. Zool., Lond. **153**: 153-161.

MUTERE, F. A. (1970). The breeding biology of equatorial vertebrates: Reproduction in the insectivorous bat *Hipposideros caffer,* living at $0^0 27'$ N Bijdr. Dierkd. **40**: 56-58.

MUTERE, F. A. (1973a). A comparative study of reproduction in two populations of the insectivorous bats, *Otomops martienseni* at latitudes $1^0 5'$ N and $2^0 30'$ S . J. Zool. Lond. **171**: 79-92.

MUTERE, F. A. (1973b) Reproduction in two species of equatorial free-tailed bats (Molossidae) E. Afr. Wildl. J. **11**: 271-280.

MYERS, P. (1977). Patterns of reproduction in four species of vespertilionid bats in Paraguay. Univ. Calif. Publ. Zool. **107**: 1-41.

OXBERRY, B. A. (1979). Female reproductive patterns in hibernating bats. J. Reprod. Fert. **56**: 359-367.

PARKES, A. S. (1931). The Reproductive Processes of certain Mammals. II.- The Size of the Graafian Follicles at Ovulation. Proc. Roy. Soc. Lond. **B109**: 185-196.

PATON, J. C. (1988). Seasonal changes in the physiological and hormonal aspects of reproduction in the male long fingered bat *Miniopterus schreibesii* (Mammalia: Chiroptera). MSc Thesis. Rhodes University. Grahamstown, South Africa.

PETERS, H. and MCNATTY, K. P. (1980). The Ovary. Granada Publishing Ltd.

PINCUS, G. (1936). The Eggs of Mammals. Expt. Biol. Monographs. Macmillan Co., New York.

RACEY, P. A. (1969). Diagnosis of pregnancy and experimental extension of gestation in *Pipistrellus pipistrellus*. J. Reprod. Fert. **19**: 465-474.

RACEY, P. A. (1974). The reproductive cycle in male noctule bats, *Nyctalus noctula* . J. Reprod. Fert. **41**: 169-182.

RACEY, P. A. (1982). Ecology of reproduction. In: Ecology of Bats. Kunz, T. H. (Ed). New York. Plenum Press.

RACEY, P. A., SUZUKI, F, AND MEDWAY, LORD (1973). The relationship between stored spermatozoa and the oviductal epithelium in bats of the genus *Tylonycteris*. In: The Biology of the spermatozoa. Hafex, E. S. E. and Thibault, C. G. (Eds). Karger. Basal.

RACEY, P. A. AND SWIFT, S. M. (1981). Variations in gestation length in a colony of pipistrelle bats (*Pipistrellus pipistrellus*) from year to year. J. Reprod. Fert. **61**: 123-129.

RACEY, P, A. and TAM, W. H. (1974). Reproduction in male *Pipistrellus pipistrellus* . J. Zool., Lond. **172**:101-122.

RAJALAKSHMI, M and PRASAD, M. R. N. (1970). Sites of formation of fructose, citric acid and sialic acid in the accessory glands of the giant fruit bat, *Pteropus giganteus giganteus* (Brünnich). J. Endocr. **46**: 413-416.

RAMAKRISHNA, P. A., BHATIA, D. and GOPALAKRISHNA, A. (1981). Development of the corpus luteum in the Indian leaf-nosed bat, *Hipposideros speoris* (Schneider). Curr. Sci. **50**(6): 264-268.

RASWEILER, J. J. (1978). Unilateral oviductal and uterine reactions in the little bulldog bat, *Noctilio albiventris*. Biol. Reprod. **19**. 467-492.

RASWEILER, J. J. (1988) Ovarian function in the black mastiff bat, *Molossus atter*. J. Reprod. Fert. **82**: 97-111.

RAUTENBACH, I. L. (1982). Mammals of the Transvaal. Ecoplan Monograph No. 1. Pretoria.

ROBINSON, A. (1918). The formation, rupture and closure of ovarian follicles in ferrets and ferret-polecat hybrids, and associated phenomenona. Trans. Roy. Soc. Edinb. **52**: 302-362.

ROWLANDS, I. W. AND WEIR, B. J. (1984). Mammals. NonPrimate Eutherians. In: Marshall's Physiology of Reproduction. Vol. 1. Lamming G. E. (Ed). Longman Group Ltd.

SACHER, G. A. and STAFFELDT, E. F. (1974). Relation of gestation and brain weight in placental mammals: implications for the theory of vertebrate growth. Am. Nat. **108**(963): 593-615.

SADLIER, R. M. F. S. (1969). The ecology of reproduction in wild and domestic animals. Methuen. London.

SETCHELL, B. P. (1978). The Mammalian Testis. Cornell University Press. Ithaca., New York.

SHERMAN, H.B. (1937). Breeding habits of free-tailed bats. J. Mamm. **18**: 176-187.

SHORT, H. L. (1961).Age at sexual maturity of Mexican free-tailed bats. J. Mamm. **42**(4): 533-536.

SKINNER, J. D. and SMITHERS, R. H. N. AND (1990). Mammals of the Southern African Subregion. University of Pretoria, Pretoria

SMITHERS, R. H. N. (1971). The Mammals of Botswana. Mem. Nat. Mus. Rhod. **4**: 1-340.

SMITHERS, R. H. N. (1983). Mammals of the Southern African subregion. University of Pretoria, Pretoria.

SOTELLO, J. R. and PORTER, K. R. (1959). An electron microscope study of the rat ovum. J. Biophys. Biochem. Cytol. **5**: 327-341

STEPHENS, R. J. (1962). Histology and histochemistry of the placenta and fetal membranes in the bat, *Tadarida brasiliensis cynocephala* (with notes on maintaining pregnant bats in captivity). Am. J. Anat. **111**: 259-275.

TAIT, A. J. and JOHNSON, E. (1982). Spermatogenesis in the grey squirrel (*Sciuris carolinenses*). J. Reprod. Fert. **65**: 53-58.

VAN DER MERWE, M. (1979). Growth of ovarian follicles in the Natal clinging bat. S. Afr. J. Zool. **14**: 111-117.

VAN DER MERWE, M., RAUTENBACH, I. L. and VAN DER COLF, W. J. (1986). Reproduction in females of the little free-tailed bat, *Tadarida (Chaerephon) pumila*, in the eastern Transvaal, South Africa. J. Reprod. Fert. **77**: 355-364.

VAN DER MERWE, M., GIDDINGS, S. R. and RAUTENBACH, I. L. (1987). Post-partum oestrus in the free-tailed bat, *Tadarida (Chaerephon) pumila* (Microchiroptera: Molossidae at 240°S. J. Zool., Lond. **213**: 317-326.

WASSARMAN, P. I. (1988). The Mammalian Ovum. In: The Physiology of Reproduction. Vol. 1. Knobil, E and Neill, J. *et al* (Eds). Raven Press, Ltd, New York.

WIMSATT, W. A. (1944). Growth of the ovarian follicle and ovulation in *Myotis lucifugus lucifugus*. Am. J. Anat. **74**(2). 129-173.

WIMSATT, W. A. (1979). Reproductive asymmetry and unilateral pregnancy in the Chiroptera. J. Reprod. Fert. **56**: 345-357.

WIMSATT, W. A. and KALLEN, F. C. (1957). The unique maturation response of the Graafian follicle of hibernating vespertilionid bats and the question of its significance. Anat. Rec. **129**: 115-131.

WIMSATT, W. A., KRUTZSCH, P. H. and NAPOLITANO, L. (1966). Studies on sperm survival mechanisms in the female reproductive tract of hibernating bats. Am. J. Anat. **91**: 415-445.

WISLOCKI, G. B. (1933). Seasonal changes in the testes, epididymides and seminal vesicles of deer investigated by histochemical methods. Endocrinology **44**: 167- 189.