Reproduction in the Angolan free-tailed bat *Tadarida condylura* in the Eastern Transvaal.

by

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ABSTRACT

The reproductive patterns of the male and female Angolan free-tailed bat, *T. condylura*, were investigated at two localities in the Eastern Transvaal. Reproductive tracts were examined histologically.

Female *T. condylura* were seasonal bimodally polyoestrus breeders with the breeding season extending from early spring (September) to late autumn (May). Parturition of the first offspring in early December was followed by a post-partum oestrus with females becoming pregnant within two weeks after first parturitions. Gestation and lactation periods were approximately 85 and 50 days, respectively. Ovarian follicle and endometrium development closely followed the breeding season, both displaying a bimodal pattern. The reproductive tract displayed dextral dominance typical of molossids. Prior to implantation, both uterine cornu were characterised by a decidual reaction. Implantation of the bilaminar blastocyst was mesometrial with the embryonic disc oriented antimesometrially. At the site of first attachment, a pre-placental pad of trophoblast cells was formed. Development of the placenta occurred in the abembryonic hemisphere.

Male *T. condylura* displayed a bimodally seasonal breeding pattern extending from early June to February which closely parallelled the female reproductive pattern. Spermatogenic and accessory gland activities were synchronised. The testes never regressed to prepubertal conditions, but maintained baseline spermatogenic activity during reproductive quiescence. Reproductive recrudescence began during June and maximum testes and epididymides size coincided with the onset of copulation and conception in females. Leydig cell morphology and nucleus diameter closely parallelled spermatogenic activity. The germinal epithelium displayed an eleven stage spermatogenic cycle.

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CHAPTER 1. GENERAL INTRODUCTION

Free-tailed bats of the family Molossidae (Chiroptera: Microchiroptera) are geographically widespread, occurring in temperate, subtropical and tropical regions almost worldwide. Only high latitude temperate regions and certain deserts are excluded from their distribution. The family Molossidae currently comprise eleven genera and more than eighty species (Koopman & Jones 1970), of which 31 occur in Africa south of the Sahara (Hayman & Hill 1971). Of the 76 bat species found in Southern Africa, thirteen are molossids (Skinner & Smithers 1990). Although molossids are thus relatively abundant, most have not been studied in any detail and the reproductive biology of most are almost unknown.

The subject of the present study, the Angolan free-tailed bat, *Tadarida* (Mops) *condylura* (A. Smith 1833), occurs in diverse habitats throughout most of sub-Saharan Africa. Its geographical range is one of the most extensive recorded for an Africa chiropteran (Mutere 1973b; Kingdon 1974; Skinner & Smithers 1990; Happold & Happold 1989) (Figure 1.1). *T. condylura* exploits clearings in rain-forest zones of West and central Africa, woodland savannas both north and south of the equator and in the Somali arid zone (Verschuren 1957). In the Southern African subregion it is widespread in northern Botswana, south-eastern Zimbabwe, Mozambique and South Africa. Within South Africa it occurs in the subtropical eastern parts of the country: in the Eastern Transvaal, Swaziland, Natal and Transkei (Verschuren 1957; Mutere 1973b; Kingdon 1974; Rautenbach 1982; Pienaar, Rautenbach & de Graaff 1980; Skinner & Smithers 1990; Happold, Happold & Hill 1987; Happold & Happold 1989) (Figure 1.1). The Angolan free-tailed bat and the little free-tailed bat, *Tadarida pumila*, have been found sympatrically throughout most of their geographical range, often sharing the same roost (Marshall & Corbet 1959; Mutere 1973b;

Figure 1.1. A map of the African continent indicating the distribution range of *T. condylura* (shaded area). The enlarged area (arrow) shows the locality of the two study areas in the Eastern Transvaal: Skukuza (S) in the Kruger National Park (KNP) and Komatipoort (K). Transvaal (T).

O'Shea & Vaughan 1980; Pienaar *et al* 1980; Van der Merwe, Rautenbach & Van der Colf 1986; Happold *et al* 1987; Happold & Happold 1989).

T. condylura is an average sized molossid with a mass of 16-34 g and a forearm length of 43.5-50 mm (Skinner & Smithers 1990). Information regarding its feeding and roosting biology are limited to casual observations. It is a crevice dweller, roosting in cracks in rocks and hollow trees. It also makes extensive use of man-made structures, roosting under the corrugated iron roofs of buildings, under bridges and in towers (Kingdon 1974; Skinner & Smithers 1990; Happold *et al* 1987; Happold & Happold 1989). *T. condylura* is a social species, roosting in groups ranging in size from a few to hundreds of individuals, and colonies of several hundred individuals have been recorded (Verschuren 1957; Marshall & Corbet 1959; Mutere 1973b; Kingdon 1974; Skinner & Smithers 1990; Happold *et al* 1987; Happold & Happold 1989).

Molossids are swift on the wing and they normally fly high (Sherman 1937; Krutzsch 1955a, b; Davis, Herreid & Short 1962; Mutere 1968). *T. condylura* forages above the trees, in clearings and gaps between the trees, usually high above the ground, and feeds predominantly on beetles, moths, bugs, winged termites and to a lesser extent on mosquitoes (Happold & Happold 1989; Kingdon 1974; Marshall & Corbet 1959; Mutere 1968; O'Shea & Vaughan 1980; Whitaker & Mumford 1978).

Although *T. condylura* is a common species throughout its range, information on its biology, especially reproductive biology, is limited. Although some work has been done elsewhere in Africa, virtually nothing is known about the species in Southern Africa. In the Eastern Transvaal, the species lends itself to a study on reproduction. Colonies are easy to locate and generally large enough for monthly sampling.

Seasonal changes in the environment may have far-reaching effects on the reproductive biology of bats and throughout the world it has been shown that reproductive patterns in bats are adjusted in such a way as to maximise reproductive success by allowing optimal use of favourable environmental conditions (Gustafson 1979; Krutzsch 1979; Oxberry 1979; Van der Merwe *et.al.* 1986; Wolda 1988). In the seasonal environment of the Eastern Transvaal, an attempt was made to determine to what extent *T. condylura*, a tropical species, has adjusted its reproductive pattern in order to respond favourably to seasonal changes in resource abundance. The aim of the present study is, therefore, to investigate the reproductive biology of *T. condylura* in the Eastern Transvaal through a histological examination of male and female reproductive tracts.

CHAPTER 2. STUDY AREA

Angolan free-tailed bats were collected at two different localities in the Eastern Transvaal (Figure 1.1). During 1988 and early 1989, bats were collected at Skukuza (24°59'S; 31°35'E) in the Kruger National Park as part of a comprehensive study on chiropteran reproductive patterns in the Kruger National Park. During 1989 and early 1990, collections were made at Komatipoort (25°26'S; 31°56'E), a town situated south of the Kruger National Park on the border between South Africa and Mozambique. Komatipoort lies approximately 62 km south-east of Skukuza (Figure 1.1).

Both sites are situated in the Southern Woodland Savanna biotic zone where the vegetation is characterised by an *Acacia nigrescens* and *Combretum apiculatum* association (Gertenbach 1983). The annual rainfall of Skukuza (not available for Komatipoort) varies between 500 and 550 mm with an average of 546 mm per year, most of which falls between November and March. Summers are hot and humid and although sporadic frost has been recorded, winters are moderate and dry.

Both sampling sites are situated on the banks of large rivers, viz. Skukuza on the bank of the Sabie River and Komatipoort at the confluence of the Komati- and Crocodile Rivers. The proximity of substantial bodies of water and the sub-tropical climate result in vast insect populations which can sustain large populations of free-tailed bats. These conditions, and the fact that free-tailed bats extensively utilise artificial roosts available in the form of man-made structures, ensure that both sites are ideal for studying free-tailed bats, particularly the two most common species, the little free-tailed bat, *Tadarida pumila*, and the Angolan free-tailed bat, *Tadarida condylura*. *T. condylura* was often

found in very large colonies (N > 500), especially in Komatipoort, where they made use of any man-made structure suitable to their needs. Most colonies were easily accessible, and at both sites, bats were collected from a number of colonies (see Materials and Methods).

CHAPTER 3. MATERIALS AND METHODS

3.1. COLLECTION OF MATERIAL

Monthly samples of male and female reproductive tracts were collected at Skukuza in the Kruger National Park from February 1988 to January 1989, and at Komatipoort in the Eastern Transvaal from April 1989 to March 1990.

During the present study, *T. condylura* were collected with macro mist nets (Rautenbach 1985) and bag traps. During 1988, both methods were used in collecting bats, whereas during 1989-1990, only bag traps were used. The macro mist net, an ultra-fine multifilament net, 30 x 6 m in size, was usually erected near open water where the bats were trapped in flight while foraging. Bag traps, made from large plastic bags, 1 m deep x 0.3 m radius (Plastilon Plastics) fitted with wire loops attached to the open ends, were suspended about 25 cm below the exit holes of roosts. This method proved to be very successful as the bats, upon emerging, habitually executed a shallow dive and landed in the bag. Most roosts had a number of exit holes and only those most frequently used were sampled. The remainder of the exit holes were temporarily sealed with paper plugs to ensure that no bats would escape. After all the bats had emerged, the traps were removed and the bats counted, sexed and aged. Adult females were palpated to determine their reproductive condition. A sample of five to ten adult bats of each sex was kept while the remainder of the colony was released at the site of capture. On occasions where bats were kept overnight, they were examined and released the following morning. The bats were processed in the field laboratory or kept in a special bat cage and transported to the University of Pretoria the following day.

During the 1989-1990 series of collections, in addition to

monthly sampling, more frequent collections were made from September 1989 to January 1990 during which females were collected every 5-15 days and males every 15 days. This was done to study more closely the histological changes during the reproductive cycle.

Colonies of *T. condylura* were located by searching for buildings from which large numbers of bats emerged at night or by examining buildings for signs of bat activity. When a colony was sampled for the first time, trapping success was usually high and most of the bats were captured. During subsequent trappings, however, *T. condylura* became progressively more trap shy, either by finding other exits or by staying in the roost. This problem was overcome mainly by sampling a number of roosts and by minimizing disturbance during trapping.

3.2. HISTOLOGY

The morphology and histology of the reproductive tract of many mammals have been described and throughout the years many different morphological and histological terms and guidelines have been used, resulting in an often confusing array of definitions. In order to prevent any confusion in using histological terminology, the histological definitions and terminology of Bloom and Fawcett (1975) were used as a guideline throughout the present study.

Bats were sacrificed by cervical dislocation and dissected in order to remove the reproductive tracts. The uteri and ovaries of females and the testicles of males were removed. Material was fixed in Bouin's fluid for 24 hours and then rinsed and stored in 70% ethanol. Large foetuses were removed from the uterus and fixed in AFA (a mixture of 95% ethyl alcohol, 40% formalin, glacial acetic acid and distilled water - 3:1:1:5 by volume). From April 1989 to July 1989, the right ovaries of females collected at Komatipoort were re-

moved from the reproductive tract and cold stored at -20°C for later endocrinological analysis (not for the purpose of the present study). This resulted in an unfortunate gap in the data on the ovarian development of females from Komatipoort during proestrus and oestrus.

Foetuses and testicles were weighed to 0.1mg using an analytical scale. The left testicle of each male was then dissected to separate the epididymis from the testis after which the epididymis and the testis were individually weighed. It was found that the testis was often punctured or damaged during dissection, resulting in an inaccurate estimate of testis mass. The epididymis, on the other hand, remained intact during dissection. It was decided, therefore, to rather calculate testis mass by deducting epididymis mass from testicle mass:

Testis mass = Testicle mass - epididymis mass

The right testicle of each animal was kept for histological purposes.

Following paraffin-wax embedding, tissues kept for histological purposes were serially sectioned at 5-8 μm, mounted on glass slides, and stained in Erlich's haematoxylin and counter-stained in eosin. The entire uterus and attached ovaries of females were sectioned and mounted, whereas only a few selected sections of the testicle were mounted. Sections were then examined using a light microscope.

Changes in seminiferous tubule area were investigated using a Quantimet 520 Image Analyser (Cambridge Instruments). Ten randomly selected cross-sectioned seminiferous tubules from each specimen were measured. Ovarian follicle diameter, oocyte and oocyte nucleus diameter as well as Leydig cell nucleus diameter were also measured, using an eyepiece micrometer. Ten follicles of each follicular stage and ten Leydig

cell nuclei were selected and measured to the nearest 0.5 μm.

Ovarian follicles were categorised and counted on the basis of follicle development and atretic changes. The following follicle counts were made: the total number of antral follicles, the number of secondary and Graafian follicles and the number of atretic antral follicles.

The variation in size between the right and left ovaries of adult females were determined by calculating the average diameter of each ovary using an eyepiece micrometer. Since most ovaries were oval in shape, the average diameter was determined by halving the sum of the widest diameter and the diameter at the widest point perpendicular to the first measurement.

Data were analysed using the following statistical procedures. As a result of small sample size, the possibility of pooling all data from each sample was tested using one way analysis of variance. This showed that individuals in one sample often differed significantly with regard to these parameters. Sample means were therefore calculated using the means of individual animals. One way analysis of variance and standard Student's T-test were then used to test for inter-sample variations. Polynomal regression analysis was applied to the seminiferous tubule area data to check for significant bimodal trends. Simple linear regression analysis was used to determine the correlation between oocyte and oocyte nucleus diameter. Differences were considered to be significant at the 5% level.

3.3. SHORT TERM AND CYCLIC CHANGES IN TESTES POSITION

The effect of capture and handling on the position and short term movement of the testes in adult *T. condylura* was investigated during the breeding season by comparing the

position of the testes of recently captured males to that of males examined after a number of hours in captivity. Ten adult males of which both testes were recorded in the scrotal position immediately after capture, were kept in a bat basket for 24 hours before re-examination. Seasonal variations in the position of the testes were also investigated by recording the position of both testes of adult males immediately after capture.

3.4. FETAL GROWTH CURVES

Foetal growth curves of 129 foetuses collected between early October 1989 and March 1990 at Komatipoort were calculated using the Huggett and Widdas (1951) equation for mammalian foetal growth:

$$W^{1/3} = a(t_g - t_o),$$

where $W^{1/3}$ = the cube root of foetal mass (g), a = the specific foetal growth velocity, t_g = the gestation period and t_o = the value where the linear plot when lengthened intercepts the time axis. The t_o value was calculated by multiplying the gestation period with an arbitrary t_o value supplied by Huggett and Widdas (1951) for various gestation lengths. Hugget and Widas suggested a t_o value of 0.3 for a gestation period of 50-100 days. The present study, however, showed that a t_o value of 0.25 was more appropriate and produced conception and parturition dates which more closely paralleled the conception and parturition periods derived from histological examination of the reproductive tracts. Gestation period was determined from the conception and parturition dates. With first conceptions occurring in early September and first parturitions at the beginning of December, the gestation period was estimated at 85 days, giving a calculated t_o value of 21.25. A maximum foetal mass of 8.36g was recorded, resulting in a value of a = 0.03184 for specific

growth velocity. With these parameters available, the age (t) of all foetuses was calculated using the following equation:

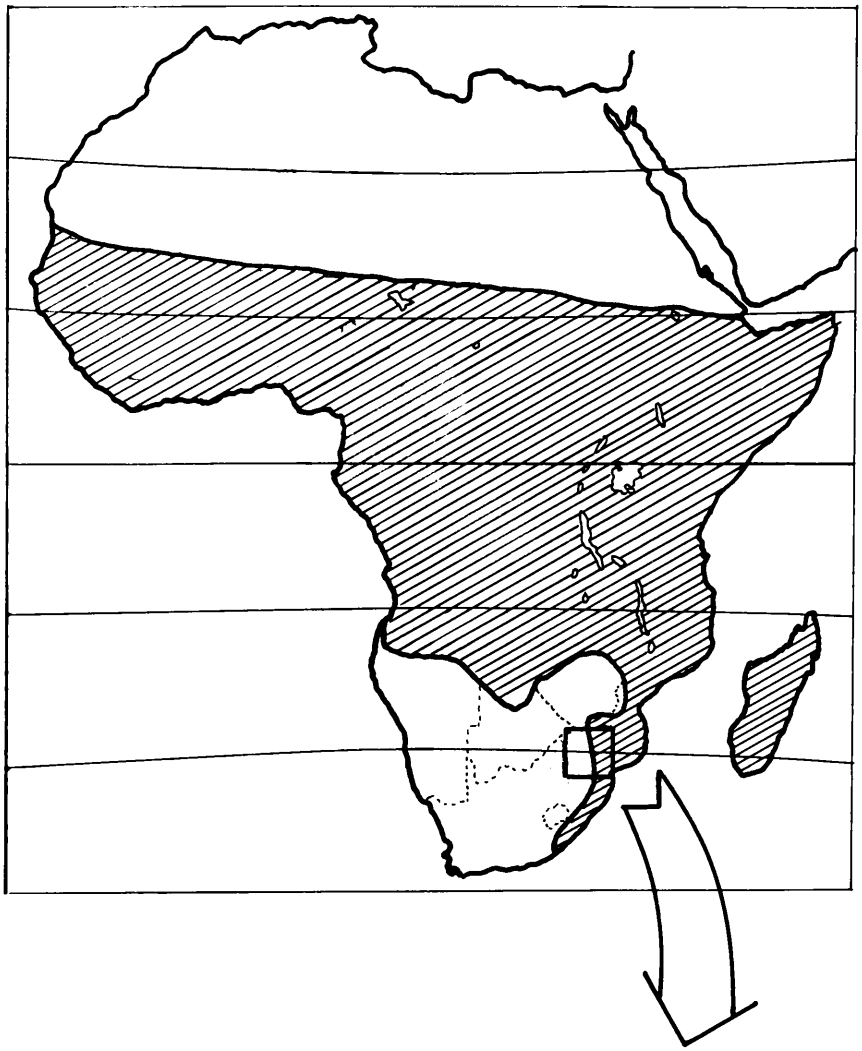
$$t = \frac{W^{1/3}}{0.03184} + 21.25$$

Since the foetuses were not all folded in the same manner in the uterus or in the sampling vial when fixed, crown-rump lengths varied too much to be used for age determinations. It was decided to rather use foetus body mass in calculating foetal age and plotting foetal growth curves. No foetuses were available from the collection made at Skukuza during 1988.

3.5. MARK-RECAPTURE EXPERIMENT

From November 1989 to February 1990, a mark-recapture programme using near-term and lactating female *T. condylura* from different colonies in Komatipoort was run in order to determine (a) to what extent births were synchronised, (b) the duration of gestation and lactation periods and (c) the interval between consecutive breeding cycles.

From colony 1 (Table 3.1), 86 near-term females (Group A) and 50 lactating females (Group C) were banded and released on 22 November and 11 December, respectively. From colony 2, 125 near-term females (Group B) and 100 lactating females (Group D) were banded and released on 27 November and 3 January, respectively. Bats from these two colonies were subsequently recaptured at intermittent intervals during December 1989 and January 1990 (Table 3.1). The number of bats recaptured, and the band number and reproductive condition of each banded bat were recorded.



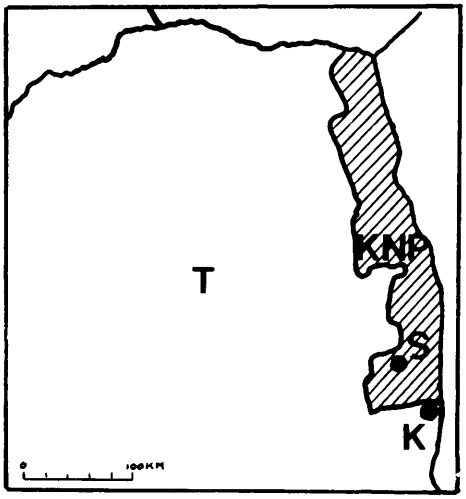


Table 3.1 Mark and recapture dates for female *T. condylura* during the mark-recapture programme from November 1989 to March 1990.

GROUP	MARK AND RECAPTURE DATES											
	22/11	27/11	30/11	7/12	11/12	13/12	17/12	28/12	1/1	3/1	1/2	3/3
A	B		R		R		R		R		R	R
B		B		R		R		R		R	R	R
C					B		R		R		R	R
D										B	R	R

B = BANDED

R = RECAPTURED

CHAPTER 4. REPRODUCTION IN THE FEMALE

4.1 INTRODUCTION

Reproductive patterns displayed by members of the order Chiroptera are largely related to latitude. Due to the seasonal nature of resources in temperate and subtropical regions, availability of food is one of the major determinants in the timing of reproductive events. Late pregnancy, lactation and weaning are the most demanding periods in the reproductive cycles of most mammals (Heideman 1988). Animals in which such events are timed to coincide with optimum periods of resource abundance, will be able to reproduce more successfully. In temperate regions, where adverse environmental conditions result in resource shortages for most of the year, mammalian species have become adapted to these conditions by undergoing hibernation during winter. The adaptive significance of hibernation is energy conservation and with reproduction representing such an energy demanding activity, hibernation can be expected to exert a profound effect on the reproductive biology of any species (Oxberry 1979). Reproductive patterns displayed by temperate chiropterans are, therefore, adjusted in such a way as to allow them to make optimal use of the short summer period of resource abundance (Jerret 1979).

Hibernating bats usually display seasonal monoestrus breeding patterns, with females conforming to two basic patterns (Krutzsch 1975; Jerret 1979; Oxberry 1979; Bernard 1980). Old and New World hibernating rhinolophids and vespertilionids, with the exception of the genus Miniopterus, display delayed ovulation. Proestrus and copulations are initiated during autumn prior to hibernation with spermatozoa being stored in the female reproductive tract until arousal in spring, when ovulation, conception and normal embryonic development ensues. Additional matings may occur during

intermittent arousal from hibernation. Members of the genus Miniopterus display a reproductive pattern characterised by delayed implantation with ovulation and conception concluded in late autumn, before the onset of hibernation. Embryonic development is arrested prior to hibernation and the unimplanted conceptus remains in the uterus until arousal in spring, when normal implantation and development is initiated (Krutzsch 1975; Bernard 1980; Oxberry 1979). Evidence has also been found for a third reproductive pattern, characterised by retarded embryonic development of the implanted conceptus (Wallace 1978; Oxberry 1979; Krutzsch & Crichton 1985).

In tropical and subtropical regions, animal species are subjected to less fluctuating environmental conditions and more prolonged periods of resource abundance. This reduces the need for extended periods of hibernation and for restriction of reproductive activity to one short reproductive cycle (Bernard 1980; Jerret 1979; Krishna 1985; Heideman 1988). It is, therefore, not unexpected that non-hibernating bats display a greater diversity with regard to the organisation of reproductive events (Jerret 1979; Oxberry 1979). Non-hibernating bats are represented by both mega- and microchiropteran species and although they usually inhabit warmer, lower latitude areas, a number of temperate species have also been documented (Oxberry 1979). The reproductive patterns displayed by non-hibernating species, characterised by synchronised spermatogenic and ovarian cycles with copulation, ovulation and conception occurring in early spring, can be divided into three categories (Jerret 1979).

1. Some non-hibernating species display seasonal monoestrus reproductive patterns. Most are found in temperate regions, although some tropical species also conform to this pattern e.g. *Otomops martiensseni* (Mutere 1973a). Species included in this group undergo a single annual reproductive cycle, with ovulation and conception occur-

ring in early spring and parturition of usually a single offspring following in early summer (Krutzsch & Crichton 1985; Table 4.1).

2. Seasonal polyoestrous breeders include bi- and trimodal breeders with reproductive cycles corresponding to periods of peak resource abundance. Most species displaying this pattern inhabit tropical or subtropical regions (Krutzsch & Crichton 1985; Table 4.1). Polyoestrous breeders normally yield one offspring at each parturition, resulting in two or more young over the breeding season. The interval between consecutive reproductive cycles are often characterised by a post-partum oestrus, allowing these species to fit more than one reproductive cycle into the breeding season (Harrison 1958; Krutzsch & Crichton 1985: Van der Merwe *et.al.* 1986; Happold & Happold 1989). Seasonal polyoestrous breeders often display unique specialisations such as prolonged embryonic diapause (*Artibeus jamaicensis*: Fleming 1971) and menstruation (*Glossophaga soricina*: Rasweiler 1972).

3. Aseasonal polyoestrous breeders are reproductively active throughout the year, with often little synchronisation between pregnancies. A number of tropical bats have been shown to exhibit this pattern (Oxberry 1979), including one African molossid, *Tadarida pumila* (Mutere 1973b; Table 4.1).

Information on reproduction in female molossids is fairly limited considering the size of the family. A recent literature survey revealed quantitative information on the reproductive patterns of only thirteen species, with casual observations reported on an additional five African tropical species. All information to date on female reproductive patterns in the family Molossidae are presented in Table 4.1. The majority of detailed reproductive studies dealt with New World temperate (n = 3) and tropical (n = 3) species and Old

Table 4.1 Female reproductive patterns displayed by the family Molossidae.

REGION	SPECIES	REFERENCE
1. SEASONAL MONOESTRUS BREEDERS		
NEW WORLD TEMPERATE	*Eumops perotis californicus*	Krutzsch 1955b
	Tadarida brasiliensis mexicana	Krutzsch 1955a, 1959; Davis Herreid & Short 1962; Jerret 1979
	T. brasiliensis cynocephala	Sherman 1937, Stephens 1962
NEW WORLD TROPICAL	*Molossus molossus*	Häusler, Möller & Schmidt 1981
	M. ater	Häusler, Möller & Schmidt 1981; Rasweiler 1988
OLD WORLD TEMPERATE	*Mormopterus planiceps*	Crichton & Krutzsch 1987
	Tadarida australis	Kitchener & Hudson 1982
OLD WORLD TROPICAL	*Otomops martiensseni*	Mutere 1973a
	~~*Tadarida aegyptiaca*~~	~~Kashyap 1980~~
2. SEASONAL POLYOESTRUS BREEDERS		
NEW WORLD TROPICAL	*Molossus fortis*	Krutzsch & Crichton 1985
OLD WORLD TROPICAL	*Tadarida pumila*	Marshall & Corbet 1959; Van ~~der Merwe~~ *et.al.* 1986
	T. condylura	Mutere 1973b; Happold & Happold 1989
	T. congica	Braestrup 1933
	T. limbatus	Braestrup 1933
	T. midas	Braestrup 1933
	T. nanula	Braestrup 1933
	T. thersites	Braestrup 1933
3. ASEASONAL POLYOESTRUS BREEDERS		
OLD WORLD TROPICAL	*Tadarida pumila*	Mutere 1973b

World temperate (n = 2) species from Australia. Old World tropical species are characterised by a dearth of detailed information.

Molossids have been shown to conform to all three reproductive patterns displayed by non-hibernating bats (Table 4.1). Seasonal monoestrous breeders are, with the exception of *Otomops martiensseni* (Mutere 1973a), restricted to temperate regions, while polyoestrous and aseasonal breeding appear to be the usual pattern for tropical regions (Jerret 1979). The majority of African molossids, including *T. condylura*, have mainly been studied in the tropical environment of central Africa and eight of the ten species studied to date conform to a seasonal polyoestrous pattern (Braestrup 1933; Marshall & Corbet 1959; Mutere 1973a, b; Van der Merwe *et.al.* 1986; Happold & Happold 1989. Unfortunately, very few detailed reproductive studies on African molossids are available and results are often based on macroscopical observations (Table 4.1). The little free-tailed bat, *Tadarida pumila*, is the only African molossid for which detailed information on female reproduction is available from both subtropical and tropical parts of southern Africa. It shows a geographical variation in its reproductive pattern from aseasonal polyoestrous in Uganda at 0°43'N (Mutere 1973b) to seasonal polyoestrous in the Eastern Transvaal, South Africa at 24°59'S (Van der Merwe *et.al.* 1986).

T. condylura has been studied at two localities in Africa, viz. in Uganda (0°43'N) (Mutere 1973b) and Malawi (14-16° S) (Happold & Happold 1989). Results from both studies suggested the species to be a bimodally seasonal polyoestrous breeder, producing two offspring a year. In Uganda, the reproductive cycles corresponded to peaks in rainfall during spring (August-November) and autumn (March-May), while in Malawi, the shorter summer rainfall period resulted in parturitions in early summer (November-December) and late summer (February-March) (Mutere 1973b; Happold & Happold 1989). Happold &

Happold (1989) suggested the possibility of a post-partum oestrus after parturition of the first offspring. Both of these studies were, however, based entirely on macroscopical observations. To date no detailed histological data are available on reproduction in this species. Data from other parts of the geographical range of this species are limited to casual observations at intermittent intervals during the year (Braestrup 1933; Happold & Happold 1989). In Southern Africa, Skinner & Smithers (1990) only mentioned pregnant females in January and Pienaar *et.al*. (1980) recorded neonates during the summer months.

The family Molossidae, throughout its almost global distribution, displays remarkable stability with regard to the morphological arrangement of the female reproductive tract. The right-left asymmetry characteristic of the female reproductive tract is widely recognised as the most pronounced form of dextral dominance among chiropterans studied to date, with the right ovary and right uterine horn being morphologically and functionally dominant over the left (Sherman 1937; Krutzsch 1955a, b; Davis *et.al* 1962, Mutere 1968, 1973a, b; Wimsatt 1975; Krutzsch & Crichton 1985; Van der Merwe *et.al*. 1986; Crichton & Krutzsch 1987).

4.2 RESULTS

4.2.1 MORPHOLOGY OF THE FEMALE REPRODUCTIVE TRACT

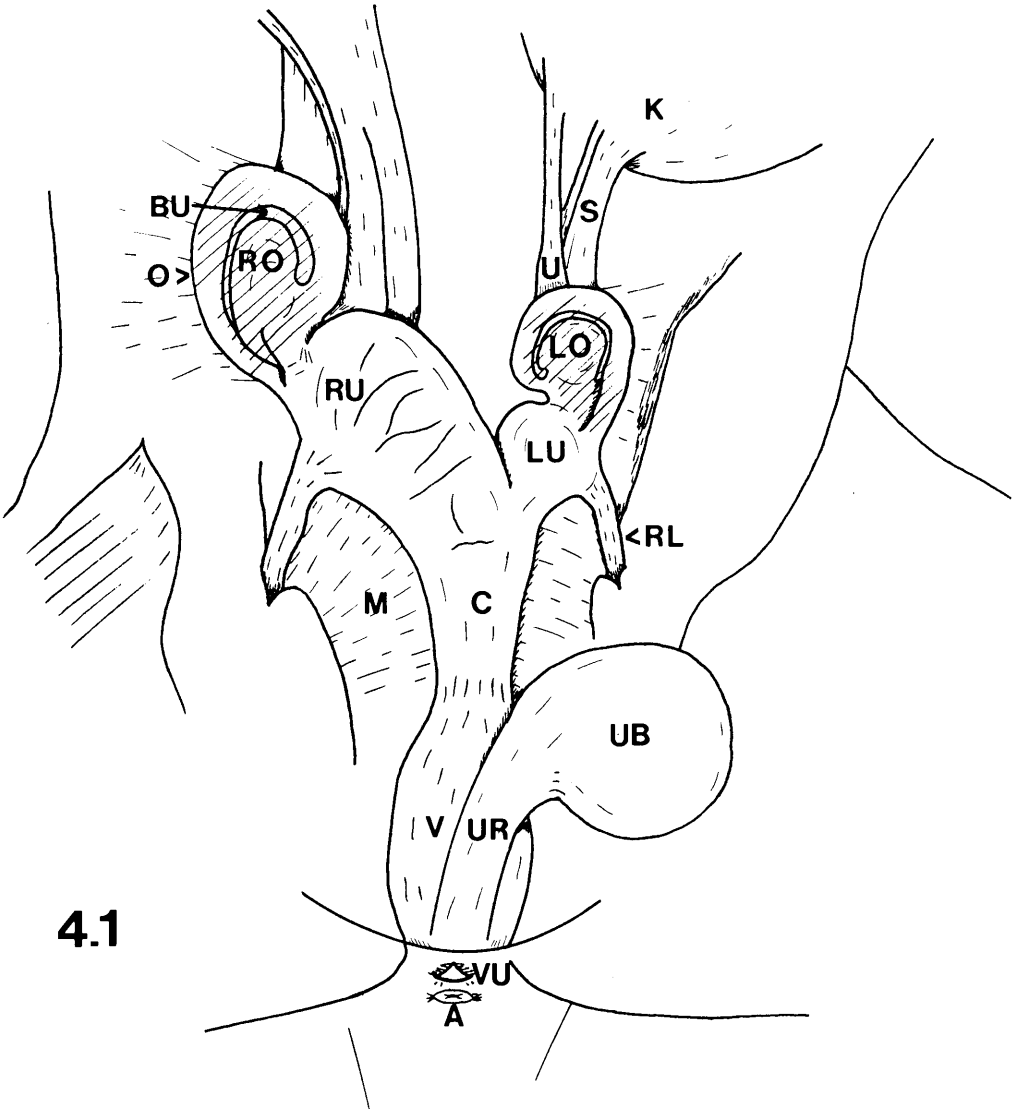
The uterus of the female *T. condylura* was bicornuate with the tubular uterine horns of adult females exhibiting dextral dominance (Figures 4.1 & 4.2). In juveniles and nulli-parous adult females, the uterine horns were identical in size, but after the initial pregnancy the horns became asymmetrical, the right horn being larger and more rounded and distended than the left horn. The uterus was dorso-ventrally flattened and Y-shaped with the uterine horns joining the uterine corpus at an approximate 110° angle. The uterine corpus extended caudally as a relative short, straight tube to the region of the cervix. The vagina extended caudally from the short cervical region, widening at its external opening into a prominent vulva which was situated ventral to the anus (Figure 4.1 & 4.2).

The uterus was attached to the ventro-lateral wall by a pair of symmetrical, non-pigmented round ligaments which extended ventrally from the lateral ends of the uterine horns. The round ligaments formed the cranial edge of the broad ligament or mesometrium from where it passed through the inguinal canal to attach to the medial ventral surface of the thigh. The mesometrium extended from the lateral aspect of the uterus to the ventro-lateral abdominal wall (Figure 4.1).

The curved oviducts almost completely encircled the ovaries, becoming slightly widened distally to terminate in the fimbriated ostia which were situated ventro-medially of the ovaries (Figure 4.1 & 4.2). The oviducts were initially narrow in the infundibulum, but widened in the ampulla region before narrowing again in the isthmus. The oviducts curved around the ovaries to join the uterine horns at their cra-

Figure 4.1. Line drawing of the reproductive system of the female *T. condylura* (ventral view). Illustrated are: Anus (A); Bursa (BU); Corpus (C); Kidney (K); Left ovary (LO); Left uterine cornu (LU); Mesometrium (M); Oviduct (O); Round ligament (RL); Right ovary (RO); Right uterine cornu (RU); Suspensory ligament (S); Ureter (U); Urinary bladder (UB); Urethra (UR); Vagina (V); Vulva (VU).

Figure 4.2. Photomicrograph showing the morphology of the reproductive system of the female illustrated in Figure 4.1. For labels, see Figure 4.1.



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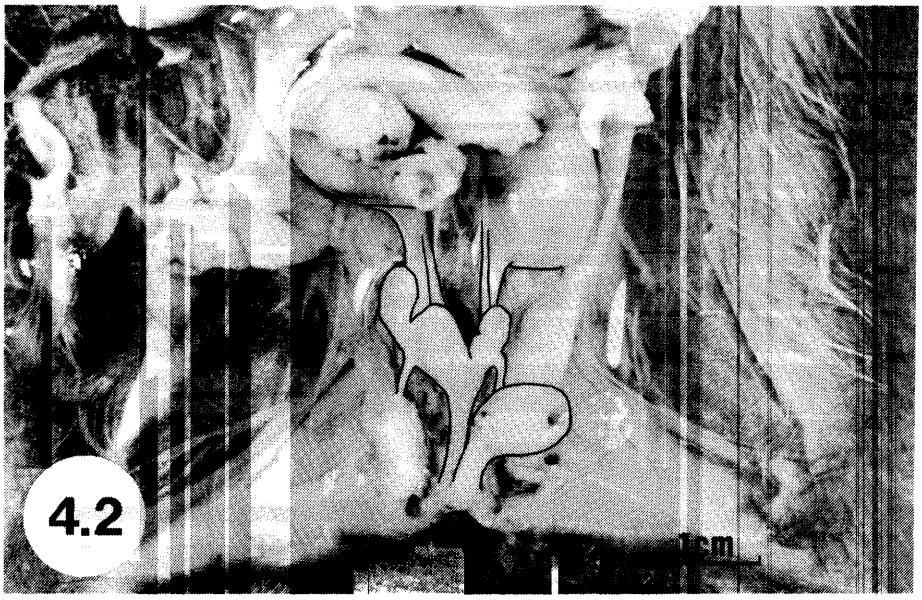

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nial ends. The ovaries were suspended to the cranial ends of the uterine horns by the ovarian ligament (Figure 4.3).

The ovaries were enclosed in an ovarian bursa (Figure 4.1 & 4.2) formed by the mesophalinx stretching from one side of the oviductal loop to the other, thus forming an enveloping bursa around the ovary (Figure 4.1). The ovaries were suspended to the dorso-lateral abdominal wall by the mesovarium (Figure 4.1).

The ovaries, like the uterus, displayed conspicuous dextral dominance with the mean diameter of the right ovary (3072.6 ± 201.1 μm, n = 8) of parous females differing significantly from that of the left ovary (1202.9 ± 122.1 μm, n = 8, $P<0.001$, d.f. = 14). The ovaries of nulli-parous females were equal in size.

4.2.2 HISTOLOGY OF THE OVARY, OVIDUCT AND UTERUS

4.2.2.1 HISTOLOGY OF THE OVARY

4.2.2.1.1 Dextral dominance

The ovaries of *T. condylura* displayed dextral dominance in size and structure which resulted in the absence of advanced follicular growth stages in the left ovary. The right ovary was functionally and morphologically dominant, displaying follicular activity with all stages of follicular development being present throughout the year (Figure 4.3). Ovulation and corpus luteum formation were invariably associated with the right ovary.

The left ovary contained only primordial and primary follicles, with the former dominating all left ovaries examined (Figure 4.4). Primary follicles were not abundant and hypertrophy of the granulosa cells never progressed beyond the bilaminar or trilaminar stage. The left ovary was also

characterised by an abundance of interstitial cells.

Because of the absence of general follicular events in the left ovary, the following discussion on the histology of the ovary will focus mainly on the right ovary.

4.2.2.1.2 General histology of the ovary

The ovary consisted of a well developed outer cortex, which contained the ovarian follicles, and an inner medulla (Figure 4.3). The demarcation between the two regions was mostly poorly defined. The cortex had a cellular appearance as a result of the abundant interstitial cells (Figure 4.5).

The medulla was characterised by abundant connective tissue which enveloped a mass of blood, nerve and lymphatic vessels. At the hilus region, the blood vessels were very prominent relative to the size of the ovary, forming a well defined rete system, the rete ovarii (Figure 4.3 & 4.6).

The ovary was covered by a layer of epithelial cells, the germinal epithelium, which varied from squamous to cuboidal (Figure 4.5). The tunica albuginea, lying adjacent to the germinal epithelium, consisted of a thin layer of connective tissue which was difficult to distinguish from the theca cells of large follicles.

4.2.2.1.3 Interstitial tissue

The inter-follicular spaces in the cortex was character-ised by abundant interstitial cells together with blood capillaries and fibroblasts (Figure 4.5). The interstitial cells appeared to undergo seasonal variations in size. During the breeding season, the interstitial cells resembled lutein cells, appearing hypertrophied and polyhedral with round to ovoid, prominent nuclei. During reproductive quiescence, the interstitial cells were involuted with little cytoplasm and

Figure 4.3. Right ovary showing the cortex (C) with all stages of follicular development, as well as the medula (M) with abundant connective tissue and blood vessels, the ovarian ligament (L), the ovarian bursa (B) surrounding the ovary and a section of the ampulla (arrow-head). x40.

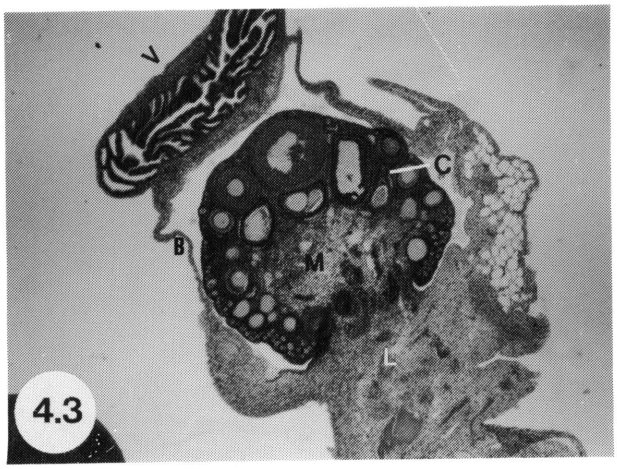
Figure 4.4. Left ovary showing abundant primordial follicles and two developing primary follicles. The ovary is enclosed by the ovarian bursa. x100.

Figure 4.5. Cortex of the right ovary showing the germinal epithelium (G), interstitial tissue (I), primordial (P) and early primary follicle (EP). One follicle (BP) is becoming bilaminar. The theca folliculi of the latter is differentiating into the theca interna (arrow-head) and the theca externa (double arrow-head). x400.

Figure 4.6. Right ovary showing the corpus (C), medulla (M) and the rete ovarii (R) with blood vessels (arrows). x100.

Figure 4.7. Right ovary showing a young secondary follicle (S) with antrum (arrow-head) and an early atretic Graafian follicle (G). x200.

Figure 4.8. Right ovary of a near-term female showing two healthy (G) and two atretic large Graafian follicles (A) and the corpus luteum of pregnancy (CL) which has been deformed by the large Graafian follicles. x40.



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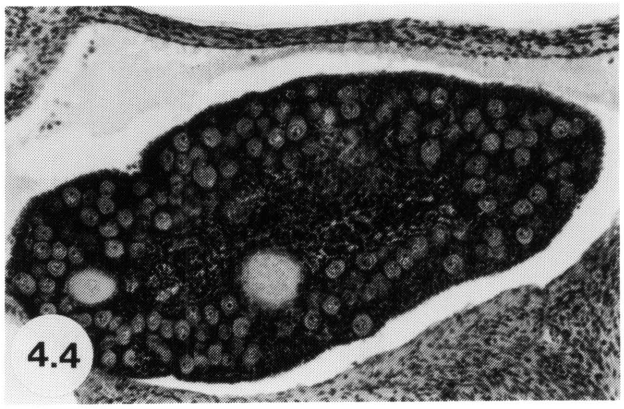
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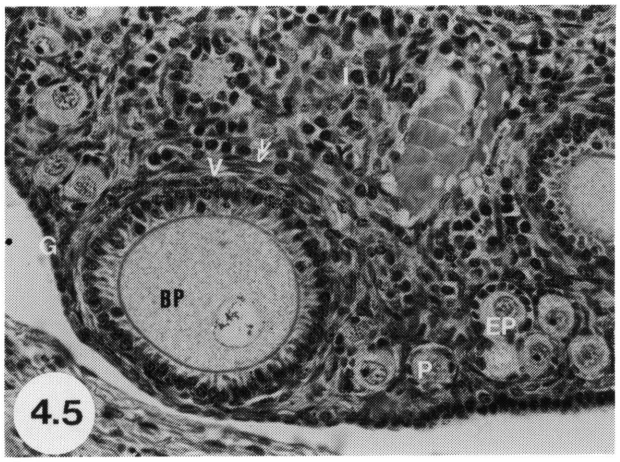

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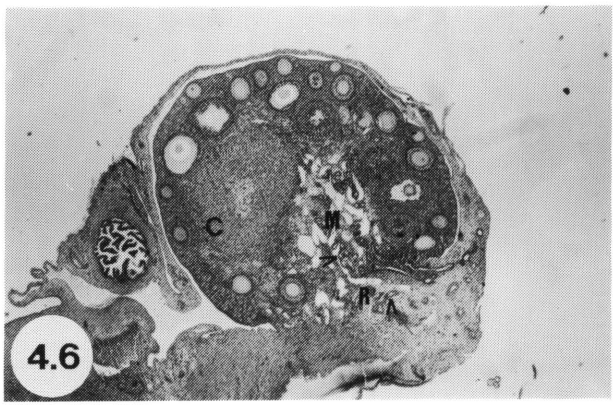

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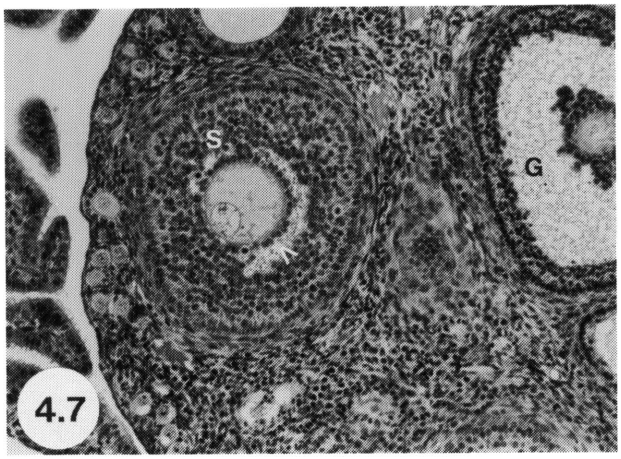

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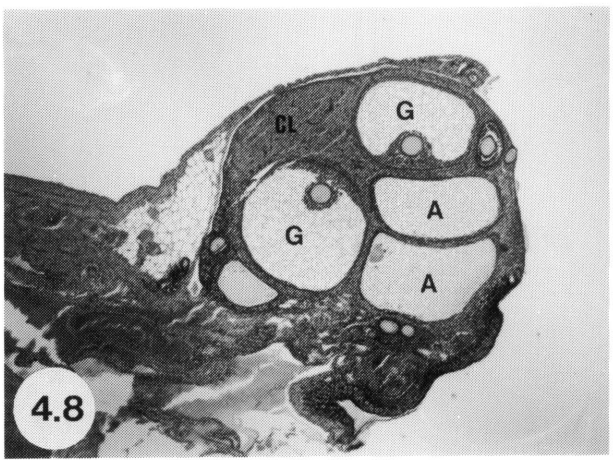

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the nuclei were small with an ovoid to irregular outline.

4.2.2.1.4 Ovarian follicles

Four types of follicles could be distinguished on the basis of the development of the follicular cells. Data on follicle diameter, and oocyte and oocyte nucleus diameter in relation to the four different stages in follicle development are presented in Table 4.2.

(i) Primordial follicles

Primordial follicles were the most abundant type of follicle throughout the year. They were located at the periphery of the ovary just interior to the tunica albuginea (Figure 4.5). Primordial follicles contained a small oocyte surrounded by a single layer of flattened to spindle-shaped follicular cells (Table 4.2). These cells had small, ovoid to flat nuclei and little cytoplasm. Oocytes were dominated by large, eccentric, round nuclei with prominent, dark-staining nucleoli. Distinct chromatin strands were visible in the nuclei.

(ii) Primary follicles

The development of primordial follicles into primary follicles was marked by the transition of the squamous follicular cells into cuboidal cells (Figure 4.5). The cuboidal cells first became low columnar and then through mitotic proliferation gave rise to a stratified epithelium of granulosa cells, thus transforming the unilaminar primordial follicle into a multilaminar follicle. Prior to the onset of follicular cell proliferation, the oocyte showed a three to four-fold increase in size and the nucleus almost doubled in size (Table 4.2). The oocyte nucleus was characterised by distinct chromatin strands, a single, large nucleolus and an uneven, almost jagged outline. The formation of the zona pellucida, a homogeneous deeply staining layer surrounding the oocyte, was

TABLE 4.2 Ovarian follicle diameter and oocyte and oocyte nucleus diameter during the four stages of follicle development (n = 10 for all stages)

STAGE	FOLLICLE (μm)	OOCYTE (μm)	OOCYTE NUCLEUS (μm)
PRIMORDIAL	22-37	18-24	12-13
PRIMARY	40-148	25-85	14-28
SECONDARY	151-296	86-98	29-33
GRAAFIAN	308-711	92-99	29-33

initiated early in the life of the primary follicle, usually before the initiation of follicular cell proliferation.

As the primary follicles increased in size, they gradually moved deeper into the cortex. Following the initial development of cuboidal follicular cells, stromal cells surrounding the follicle formed a sheath, the theca folliculi. The theca folliculi was composed of flattened cells with concentrically arranged spindle-shaped nuclei. At the time of first follicular cell proliferation, the theca folliculi differentiated into two layers, an outer theca externa composed of two to three layers of connective tissue cells, and an inner theca interna composed of large secretory cells with distinct spherical to ovoid nuclei (Figure 4.5). Primary follicles showed a rapid increase in size as a result of the proliferation of the granulosa cells and growth of the oocyte (Table 4.2).

(iii) Secondary follicles

The transition from primary to secondary follicles was characterised by the appearance of fluid-filled cavities in the stratum granulosum (Figure 4.7). These cavities, filled with liquor folliculi, eventually coalesced into one large fluid-filled antrum. As a result of the rapid accumulation of follicle fluid, the secondary follicle developed very rapidly and the antrum changed from several smaller spaces separated by irregular partitions of follicular cells into a large continuous cavity. The rapid increase in size of the follicle during this stage was mainly due to the accumulation of fluid in the antrum with the oocyte showing little growth (Table 4.2).

(iv) Graafian follicles

The transition into the Graafian follicle stage was not as

clearly defined as in previous stages, but a Graafian follicle was identified as having a large continuous fluid-filled antrum with the oocyte pressed to the side of the follicle (Figure 4.8). The oocyte, bulging distinctly into the antrum, was surrounded by a few layers of granulosa cells which formed the cumulus oophorus. The cumulus oophorus remained attached to the granulosa wall by a stalk of granulosa cells which varied from a few cells to a broad band of cells. Graafian follicles showed a rapid increase in size due to the accumulation of follicular fluid, ranging from ±300 μm in young Graafian follicles to >700 μm in pre-ovulatory follicles (Table 4.2). Proliferation of granulosa cells continued, although pre-ovulatory follicles showed a decrease in mitotic structures.

Ovaries of reproductively active females often contained a number of Graafian follicles. In one specimen four Graafian follicles developed side by side, completely dominating the ovary (Figure 4.8).

4.2.2.1.5 Follicular growth

Growth of ovarian follicles in relation to growth of their oocytes and oocyte nuclei are plotted in Figure 4.9. The data indicate a rapid increase in the diameter of both the oocyte and oocyte nucleus during the primordial and primary follicle stages with a concomitant small increase in the size of the follicle. The oocyte and oocyte nucleus attained their maximum size at the early antral follicle stage after which no further growth was observed. The subsequent rapid increase in follicle diameter was caused by proliferation of the granulosa cells and the accumulation of liquor folliculi. The data also show a significant linear correlation between growth of the oocyte and growth of the oocyte nucleus (r^2 = 0.933, $P<0.001$, Figure 4.10).

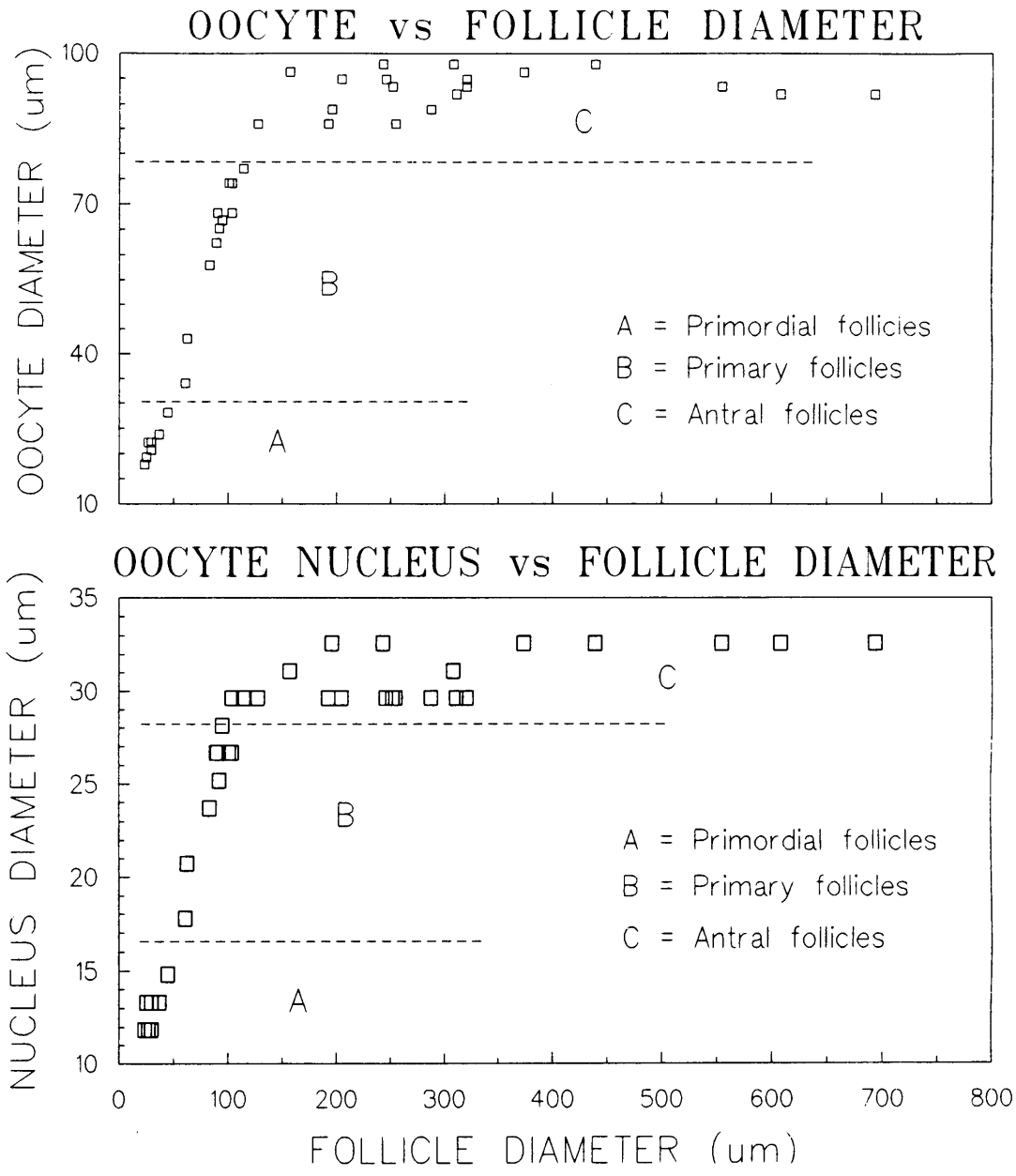


Figure 4.9. Diameter of primordial, primary and antral follicles plotted against follicle oocyte and oocyte nucleus diameter.

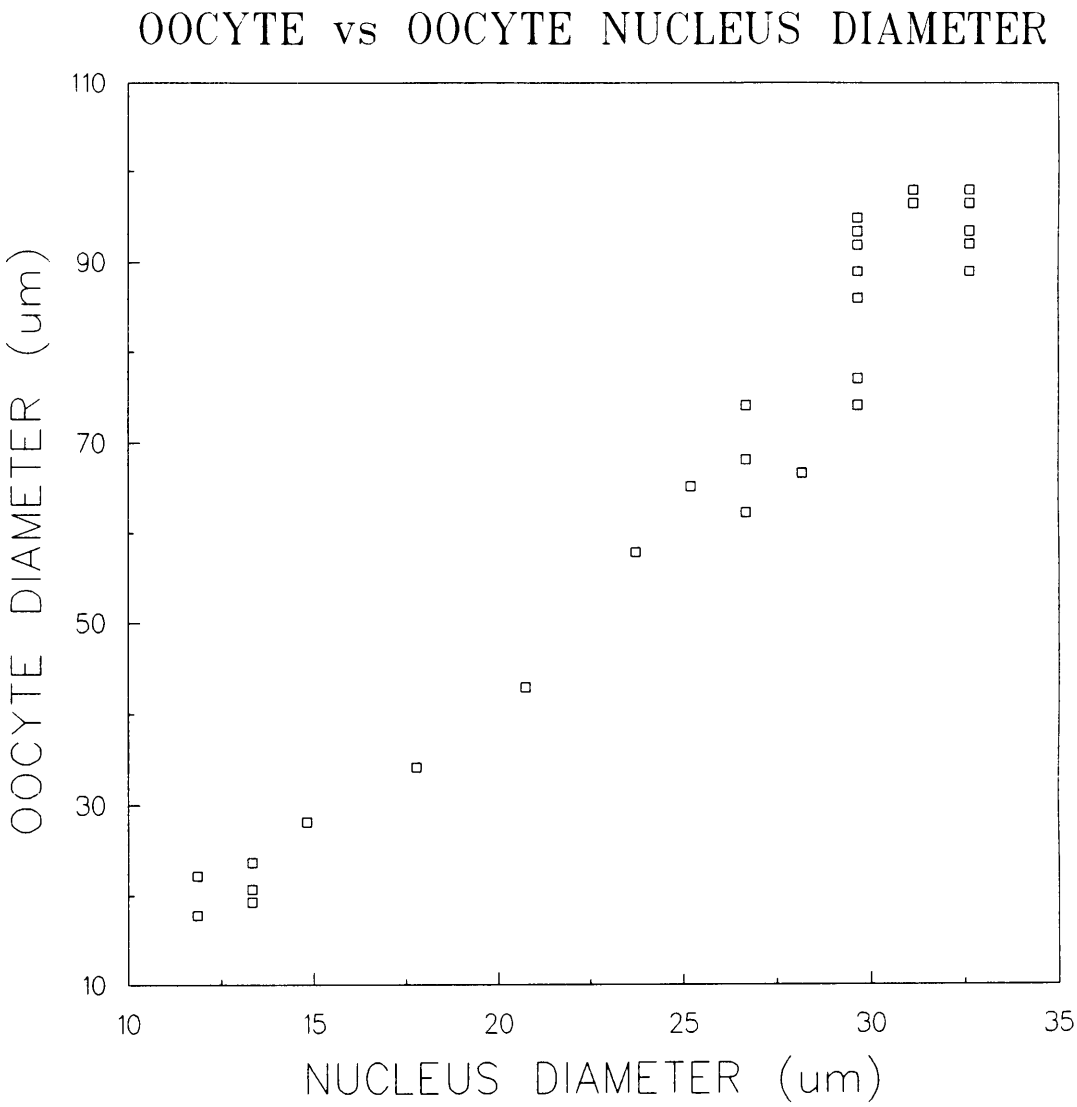


Figure 4.10. Follicle oocyte diameter plotted against oocyte nucleus diameter.

4.2.2.1.6 Follicular atresia

Atresia of primary, secondary and Graafian follicles were observed during all months of the year for which information was available. Atretic primordial follicles were not observed.

Two types of follicular atresia were observed. The first, known as Type I atresia, was characteristic of atretic primary follicles and showed early degeneration and fragmentation of the oocyte before atresia of the granulosa was initiated (Figure 4.11). The second, known as Type II atresia, was characterised by the degeneration of the granulosa cells before atretic changes in the oocyte were initiated (Figure 4.12). Although secondary and Graafian follicles displayed both types of atresia, the second type was more commonly observed. Secondary and Graafian follicles also showed a wide range of atretic changes with some showing complete degeneration of the granulosa cells with the oocyte still intact, while in others the oocyte became fragmented (Figure 4.13) with degeneration of the granulosa cells only following later. In these follicles, the theca interna displayed little hypertrophy. The glassy membrane, originating from the basal membrane, became distinct in later stages and persisted until the end, forming the corpus fibrosum atreticum. The latter was rarely observed, suggesting rapid removal of atretic follicular remains.

In follicles displaying Type II atresia, the first sign of follicular degeneration was the appearance in the antrum of loose granulosa cells with pycnotic nuclei (Figures 4.12 & 4.13). Mitosis of granulosa cells ceased, although infrequent mitotic figures were sometimes seen until late in atresia. Pycnosis of the granulosa cell nuclei gradually increased as the membrana granulosa became thinner and the antrum became filled with degenerating granulosa cells. At this stage, macrophages and polymorphonuclear leucocytes

Figure 4.11. Graafian follicle displaying Type I atresia. The oocyte had become fragmented (arrow-heads) and the cumulus oophorus had disappeared. The membrana granulosa was almost intact (M) with only a few pycnotic granulosa nuclei in the granulosa. x200.

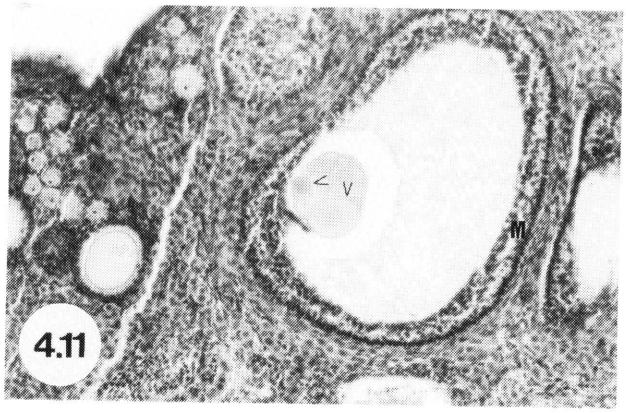
Figure 4.12. Two secondary follicles displaying Type II atresia. The first (A) show typical initial atretic changes with the first granulosa cells becoming pycnotic (arrow-head) while the oocyte was still unchanged although detached from the membrana granulosa. The membrana granulosa of the second secondary follicle (B) was almost depleted and the oocyte was still intact, although the nucleus had become condensed. x200.

Figure 4.13 Graafian follicle showing advanced Type II atresia with the membrana granulosa broken down completely and pycnotic nuclei in the antrum. The oocyte showed fragmentation (arrow-heads) and the zona pellucida was breaking up (double arrow-head). x400.

Figure 4.14. Right oviduct showing the pars interstitialis (P), isthmus (I), ampulla (A) and fimbrae (F) of the infundibulum. Uterine lumen (U); Right ovary (R). x40.

Figure 4.15. Section of the isthmus of the right oviduct, showing the mucosa with low, slightly branched folds, cuboidal to low columnar epithelium (arrow-head) and thin lamina propria (L). The muscularis (M) and serosa (S) can also be seen. x400.

Figure 4.16. Section of the ampulla of the right oviduct, showing highly folded and branched mucosa with tall columnar epithelium cells. Ciliated cells (arrow-head) are most abundant. x400.



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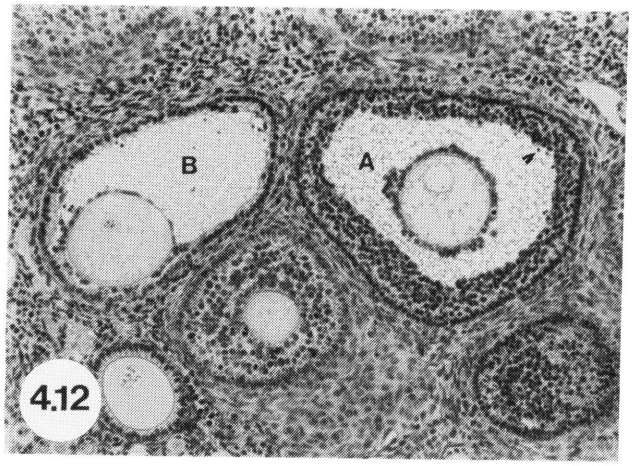

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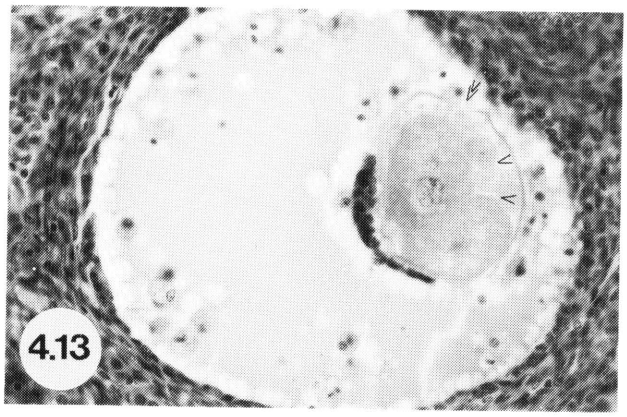
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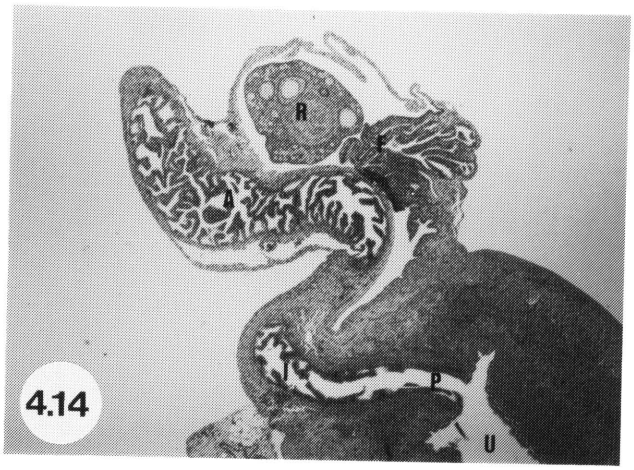

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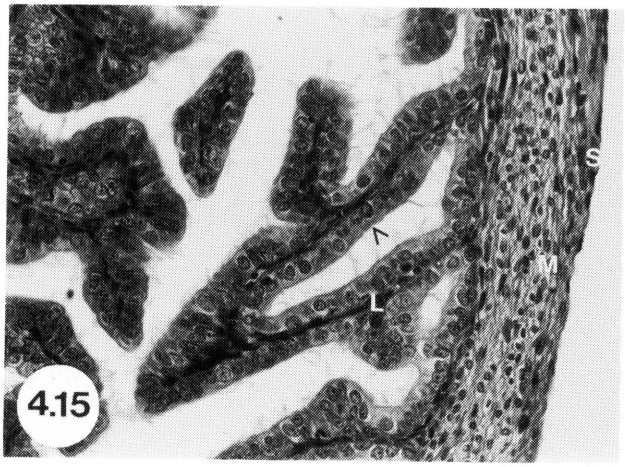

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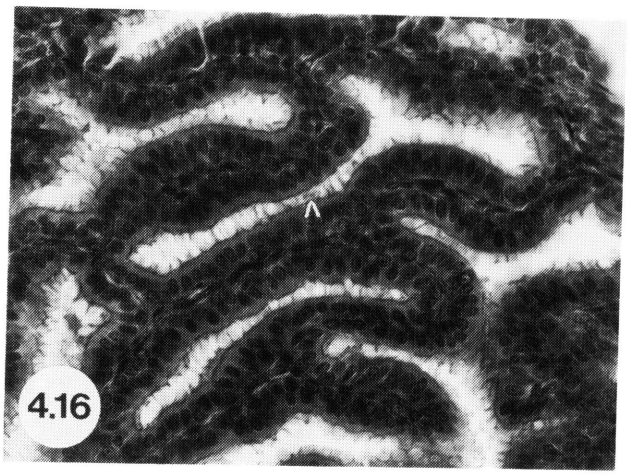
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appeared in the antrum. Concurrent with pycnosis of the granulosa cell nuclei, the cumulus oophorus cells broke loose and their nuclei also became pycnotic, until eventually the oocyte floated freely and naked in the antrum. The liquor folliculi became smooth and cloudy, loosing its distinct mesh-like appearance.

Considerable intra-sample variation was observed, e.g the atretic antral follicles observed in the ovary of one female collected on 6 December 1989 displayed Type I atresia, i.e. the membrana granulosa were almost intact, while the oocytes displayed meiotic spindles or complete cell fragmentation. Other females from the same sample showed the opposite with all atretic antral follicles displaying Type II atresia.

4.2.2.2 HISTOLOGY OF THE OVIDUCT

The oviduct consisted of four sections (Figure 4.14):

(i) The intramural part or the pars interstitialis situated in the uterine wall connecting the oviduct to the uterus,

(ii) the isthmus, which is the portion of the oviduct adjacent to the uterus,

(iii) the ampulla, which is the longest and most dilated portion of the oviduct and

(iv) the infundibulum, a funnel-shaped section closely applied to the ovary which ended in a fringe of finger-like processes called fimbriae (Figure 4.14).

The wall of the female reproductive tract was composed of three layers, viz. an inner mucosa, a central muscularis and an outer serosa (Figure 4.15). The mucosa showed numerous folds which varied in number and height in different parts of the oviduct (Figure 4.14). The mucosa of the infundibulum and ampulla was highly folded forming numerous elaborately bran-

ched laminar folds (Figure 4.16). The mucosal folds in the isthmus was less pronounced and branched, while in the pars interstitialis the folds were reduced to low ridges (Figure 4.15).

The oviduct wall was lined by epithelium which ranged from cuboidal to tall columnar epithelial cells, the nuclei of which contained mostly two distinct nucleoli. Two types of cells were distinguished, viz. ciliated and non-ciliated cells. The epithelium showed a gradual increase in height towards the ovary with the isthmus and pars interstitialis epithelium consisting of cuboidal to low columnar cells (Figure 4.15). The epithelium of the infundibulum and ampulla consisted of low to tall columnar cells with ciliated cells predominant (Figure 4.16). The composition of the epithelium also changed with non-ciliated cells becoming much more prominent in the isthmus and the intramural section.

The mucosa displayed a thin lamina propria containing connective tissue and small blood vessels. The muscularis constituted a few layers of outer longitudinally and inner circularly arranged smooth muscle cells. The serosa consisted of a thin layer of connective tissue covered by simple squamous epithelium (Figure 4.15).

4.2.2.3 HISTOLOGY OF THE UTERUS

Macroscopically the uterus consisted of bicornuate uterine horns which fused caudally to form the uterine corpus. Microscopical examination of the uterine corpus, however, showed that although the uterine horns fused externally, the lumina of the two horns remained separated almost throughout the corpus (Figure 4.17 & 4.18). The two lumina only fused near the caudal end of the corpus. The lumen of the corpus was divided by an inner wall consisting of a muscularis layer which was a continuation of the myometrium, and an outer,

double layer of highly folded mucosa (Figure 4.18 & 4.19). The mucosa consisted throughout the corpus of simple columnar epithelial cells which flattened out at the caudal end of the corpus, eventually becoming almost cuboidal. Two types of cells were recognised, viz. secreting and ciliated cells, with secreting cells dominating the epithelium. A thin lamina propria extended into the mucosal folds, consisting mainly of connective tissue and small blood vessels. The muscularis showed predominantly longitudinal smooth muscle fibres, although the centre region, which displayed a number of blood vessels, contained some oblique fibres (Figure 4.19). No active uterine glands were seen in the corpus, although shallow solitary glands sometimes appeared in the cranial end of the corpus.

The wall of the bicornuate uterine horns consisted of three layers, the endometrium (mucosa), the myometrium (muscularis) and the perimetrium (serosa).

(i) Endometrium

The surface epithelium of the endometrium ranged from cuboidal to simple columnar epithelium and consisted of two types of cells, viz. ciliated and non-ciliated cells. Interspersed along the epithelium were the openings of relatively long, slightly convoluted tubular uterine glands, which extended down to the basal region of the endometrium (Figure 4.20). The glands were lined with epithelium similar to that of the uterine lumen, although less ciliated cells were observed. The glands showed distinct variations in structure, size and activity in relation to the reproductive condition of the female. Glands were sometimes simple tubes, but often they were branched and dilated at their fundic ends deep inside the endometrium.

The lamina propria, or endometrial stroma, displayed large, irregular cells with ovoid nuclei embedded in a

Figure 4.17. Line drawing of the reproductive tract of the female *T. condylura* showing the morphology of the uterine corpus (C) with the mucosa inner wall (W) dividing the corpus lumen into two separate lumina. The dextrally dominant right uterine horn (RU) and right ovary (RO) are also illustrated. Left uterine cornu (LU); Endometrium (E).

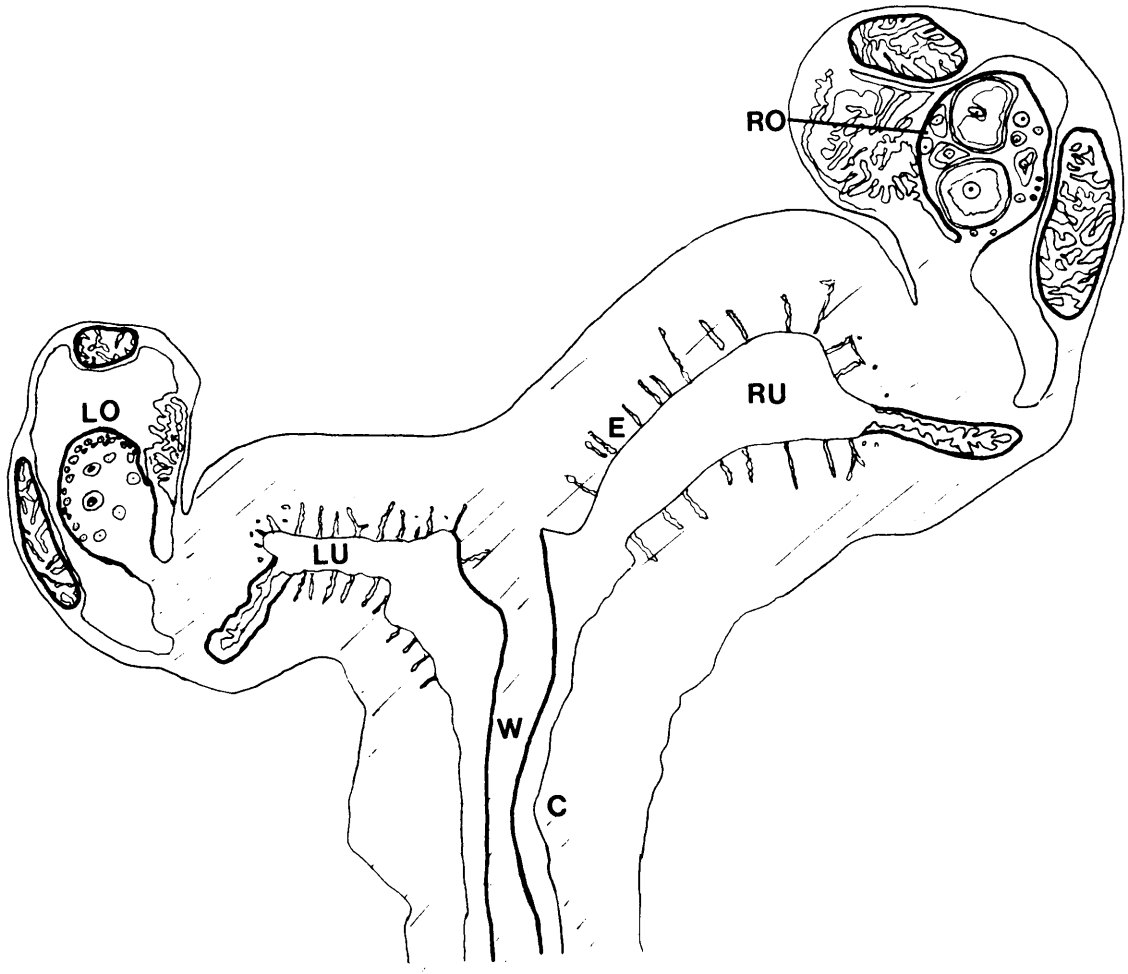


Figure 4.18. Section of the uterus showing the morphology of the corpus (C). The mucosal inner wall (W) dividing the corpus lumen into two separate lumina (L) is continuous with the endometrium (E) of the uterine cornu (U). x40.

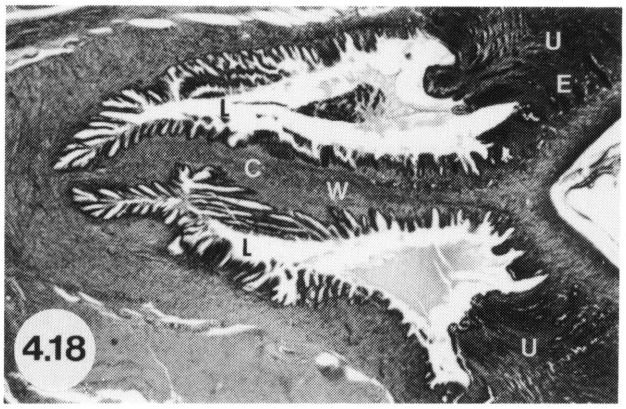
Figure 4.19. Mucosal inner wall of the corpus (W) showing the columnar epithelium (arrow-head) and mucosa (M). Muscularis of the corpus wall (MC). x 400.

Figure 4.20. Endometrium of the uterine cornu, showing the functionalis (F), basalis (B), part of the myometrium (M) and tall columnar epithelium (E) lining the endometrium. Low columnar epithelium (white arrow-head) lines the uterine glands (G). The stroma shows large ovoid stromal cells (dark double arrow-head), connective tissue and blood capillaries (dark arrow-head). Uterine lumen (U). x400.

Figure 4.21. Wall of the uterine cornu showing the morphology of the myometrium: stratum submucosum (A), stratum vasculare (B) with numerous blood vessels (white arrow-head), stratum supravasculare (C) and stratum subserosum (D). The perimetrium (P) is covered with squamous epithelium (dark arrow-head). Basalis (BA); Functionalis (F). x200.

Figure 4.22. Post-partum right uterine horn of pregnant female collected on 6 December showing blood and debris (arrow-head) in the uterine lumen (U) with the endometrium (E) still low but recovering rapidly. x40.

Figure 4.23. Eight cell stage embryo in the ampulla of a female collected on 6 December. x200.



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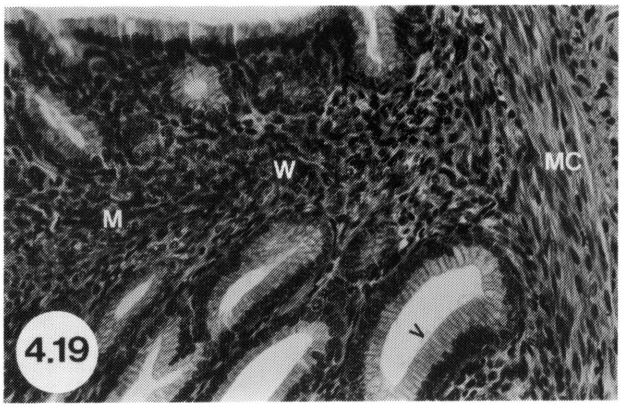

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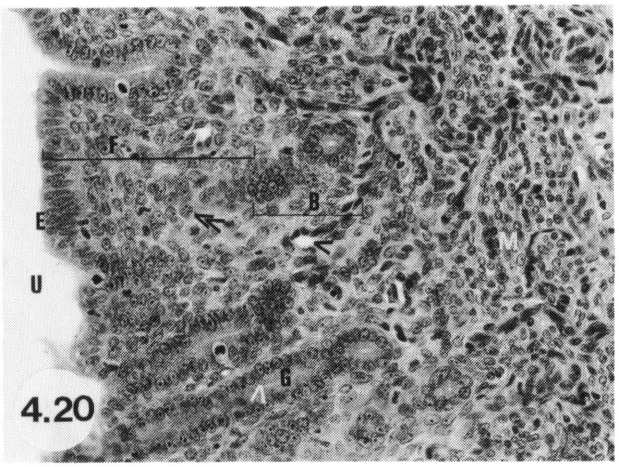

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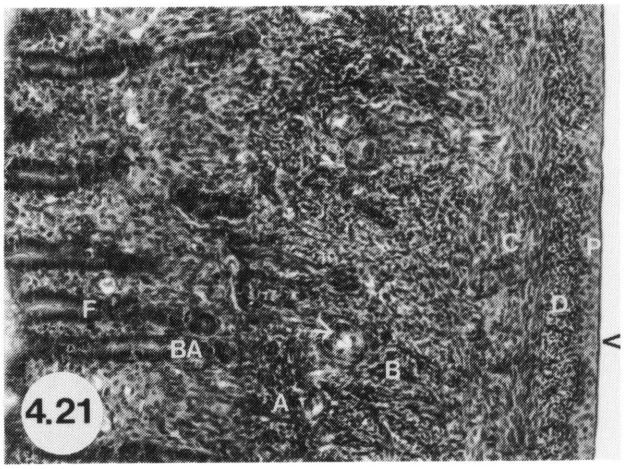

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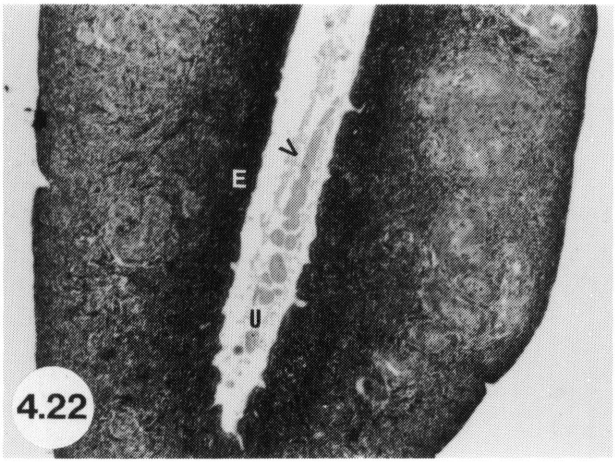

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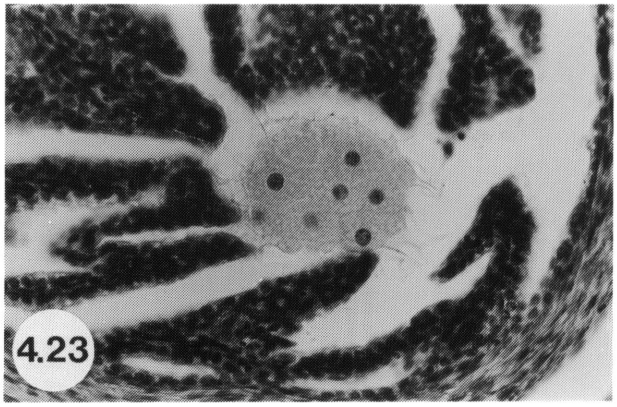

4.23

network of fine reticular fibres (Figure 4.20). During proestrus, the stroma became highly vascularised showing abundant coiled arteries. The endometrium was subdivided into two zones, the upper functionalis containing the straighter sections of the glands, and the deeper basalis, which contained the basal contorted portions of the glands (Figure 4.20).

(ii) Myometrium

The transition between the endometrium and the myometrium was not always clear, as glands often extended into the myometrium. Four layers were distinguished in the myometrium on the basis of the orientation of muscle fibres (Figure 4.21).

The first layer adjacent to the endometrium, the stratum submucosum, was thin and difficult to distinguish, consisting predominantly of longitudinal fibres, although oblique fibres were also observed. The second layer, the stratum vasculare, dominated the myometrium due to its thickness and the presence in the layer of many large blood vessels. Circular fibres arranged in bundles separated by connective tissue strands were predominant. The third layer, the stratum supravasculare, contained fibres arranged longitudinally. The outermost layer, the stratum subserosum, showed regularly arranged circular fibres.

(iii) Perimetrium

The perimetrium consisted of a thin layer of connective tissue covered by a single layer of squamous epithelium (Figure 4.21).

4.2.3 FEMALE REPRODUCTIVE PATTERN

4.2.3.1 GENERAL PATTERN

The female Angolan free-tailed bat displayed a bimodally seasonal reproductive pattern, with reproductive activity extending from early September to early May, followed by reproductive quiescence from May to August. The breeding season consisted of two consecutive reproductive cycles, i.e. the first from September to early December and the second from mid December to early April. The interval between the two reproductive cycles was marked by a post-partum oestrus with adult females becoming pregnant one to three weeks after parturition while still nursing their young. Females were found to be polyoestrus and monotocous, each producing one offspring at a time. All adult females examined were pregnant during each of the two reproductive cycles. Gestation period was approximately 85 days.

Reproductive activity in the female *T. condylura* was categorised into five stages on the basis of the reproductive status and follicular activity in the right ovary, viz. proestrus, oestrus, metestrus, pregnancy and lactation. Proestrus is generally characterised by the presence in the ovary of large secondary and Graafian follicles, whereas during anoestrus, ovaries are said to become quiescent displaying little follicular activity (Kitchener & Halse 1978). Since the ovaries of female *T. condylura* remained active and displayed all stages of follicular development throughout the year, females never became reproductively dormant and it was concluded that *T. condylura* never really exhibited true anoestrus.

4.2.3.2 FIRST REPRODUCTIVE CYCLE

Females (n = 17) collected at Komatipoort and Skukuza during the period May to July were reproductively quiescent

showing no spermatozoa or embryos in the reproductive tract. Copulation were initiated during August, with the first spermatozoa being observed in the reproductive tract of one female in early August.

In females from Komatipoort examined during 1989, ovulation and conception were initiated in early September (Tables 4.3 & 4.4). One of six females (17%) collected on 4 September was pregnant with a four cell stage embryo. On 11 September, six of the seven females (86%) collected were pregnant with conceptuses ranging from two to sixteen cell stages embryos (Table 4.3 & 4.4). Two of the five non-pregnant females collected on 4 September, had large pre-ovulatory Graafian follicles in their right ovaries. By the end of September, all females examined were pregnant with conceptuses ranging from implanting bilaminar blastocysts to early somite stage embryos. The first embryo at the primitive streak stage of development was recorded on 25 September. From early October to late November, all adult females examined were found to be pregnant.

Females from Skukuza examined during 1988 displayed a similar pattern, with ovulations and conceptions initiated during early September. Four females examined on 21 August had not yet copulated or conceived, whereas on 29 September, all five specimens examined were pregnant with conceptuses ranging from morulae in the uterus to implanting blastocysts (Table 4.4).

The onset of parturition during late November 1988 and 1989 indicated the end of the first reproductive cycle. At Komatipoort, all females (n = 112) examined through palpation on 27 November 1989 were pregnant with the majority carrying near-term foetuses (Table 4.5). The first lactating females were observed on 1 December, and on subsequent occasions, increasing numbers of lactating females were found (Table 4.5). The last near-term females were collected on 17 Decem-

TABLE 4.3 Conception in female *T. condylura* during 1988 and 1989 at the onset of the first reproductive cycle.

LOCALITY	DATE	NO EXAMINED	% PREGNANT
1988 (SKUKUZA):			
	29 SEP	5	100%
1989 (KOMATIPOORT):			
	4 SEP	6	17%
	11 SEP	7	86%
	15 SEP	5	40%
	20 SEP	5	100%
	25 SEP	5	100%
	29 SEP	5	100%

TABLE 4.4 Conceptus development in female *T. condylura* during the first reproductive cycle in 1988 and 1989.

DATE	BAT	CONCEPTUS	LOCALITY	Z.P.
1988 (SKUKUZA):				
29 SEP	1	UNILAMINAR BLASTOCYST	UTERUS LUMEN	-
	2	IMPLANTING BLASTOCYST		
	3	MORULA (>32 CELL)	UTERUS LUMEN	-
	4	MORULA (>32 CELL)	UTERUS LUMEN	-
	5	IMPLANTING BLASTOCYST		
1989 (KOMATIPOORT):				
4 SEP	1	4 CELL STAGE	AMPULLA	+
11 SEP	1	8 CELL STAGE	AMPULLA	+
	2	4 CELL STAGE	AMPULLA	+
	3	4 CELL STAGE	AMPULLA	+
	4	16 CELL STAGE	AMPULLA	+
	5	4 CELL STAGE	AMPULLA	+
	6	2 CELL STAGE	AMPULLA	+
20 SEP	1	16 CELL STAGE	ISTHMUS	-
	2	UNILAMINAR BLASTOCYST	UTERUS LUMEN	-
	3	16 CELL STAGE	ISTHMUS	-
	4	MORULA (>32 CELL)	UTERUS LUMEN	-
	5	16 CELL STAGE	ISTHMUS	-
25 SEP	1	EMBRYO (EARLY PRIMITIVE STREAK)		
	2	IMPLANTING BLASTOCYST		
	3	UNILAMINAR BLASTOCYST	UTERUS LUMEN	-
	4	IMPLANTING BLASTOCYST		
	5	EMBRYO (PRIMITIVE STREAK STAGE)		
29 SEP	1	EMBRYO (EARLY SOMITE STAGE)		
	2	EMBRYO (EARLY PRIMITIVE STREAK STAGE)		
	3	EMBRYO (PRIMITIVE STREAK STAGE)		
	4	EMBRYO (EARLY PRIMITIVE STREAK STAGE)		
	5	IMPLANTING BLASTOCYST		

Z.P. = ZONA PELLUCIDA (+ = PRESENT, - = SHED)

TABLE 4.5 The transition between pregnancy and lactation at the end of the first and the second reproductive cycles at Komatipoort during 1989 and early 1990.

DATE	NO EXAMINED	%PREGNANT	%LACTATING
FIRST REPRODUCTIVE CYCLE:			
22 NOV	68	100	0
27 NOV	112	100	0
1 DEC	29	90	10
6 DEC	50	52	48
11 DEC	208	7	93
13 DEC	109	1	99
17 DEC	42	2	98
27 DEC	107	0	100
1 JAN	49	0	100
SECOND REPRODUCTIVE CYCLE:			
1 FEB	78	100	0
3 MAR	63	70	30
1 APR	41	0	100

ber, indicating that all females had given birth within the three week period 27 November to 17 December.

Little information is available regarding the end of the first reproductive cycle for females from Skukuza, except that on 28 December all twelve females examined were lactating.

4.2.3.3 POST-PARTUM OESTRUS

Histological examination of the genital tract of female *T. condylura* from Komatipoort and Skukuza showed that females display a post-partum oestrus following the birth of their first offspring. The reproductive status of females examined during December 1988 and 1989 are presented in Table 4.6.

One of the nine females (No. 8) from Komatipoort examined on 6 December displayed a distended, post-partum uterus containing blood and debris in the lumen, suggesting recent parturition (Table 4.6, Figure 4.22). However, she was already pregnant again with a young corpus luteum in the right ovary and an eight cell stage conceptus in the ampulla of the right oviduct (Figure 4.23). Since all females examined through palpation on 27 November were still pregnant (Table 4.5), these data suggest that this specimen had given birth and conceived again within ten days.

Of the six and seven females examined on 11 and 17 December, 33% and 56% had conceived, respectively, even though most of them had distended, post partum uteri containing debris and blood (Table 4.6). By the end of December, all adult females examined were pregnant while still lactating.

Examination of females from Skukuza provided similar evidence for a post-partum oestrus. As seen from Table 4.6, eleven of the twelve females (92%) examined on 28 December

were pregnant with conceptuses ranging from a 4 cell stage embryo to an embryo at the early somite stage. From the data presented on conceptions during the first reproductive cycle in Table 4.4, it can be seen that first parturitions in 1988 also started in early December. Females from Skukuza, therefore, also conceived again within three weeks of giving birth to their first offspring.

Information obtained from the mark-recapture programme during 1989 provided further evidence for a post-partum oestrus in *T. condylura* (Table 4.7). Seven pregnant females banded on 22 November were recaptured on 30 November and again on 17 December, after which their reproductive tracts were histologically examined. On 30 November, all seven females were carrying near-term foetuses (established by means of palpation), whereas on 17 December, six of them were found to be lactating. Of these, four were pregnant again with conceptuses ranging from a sixteen cell stage embryo in the ampulla to morulae in the uterus. Within eighteen days of giving birth to their first offspring, these four females had conceived again and were pregnant with their second offspring. The seventh female was still pregnant with her first offspring.

The ovaries of females carrying near-term foetuses examined during November and early December contained abundant large secondary and Graafian follicles alongside the corpus luteum of pregnancy (Figure 4.8). Pregnant females examined in early December all showed ovaries with degenerating corpus lutea and large Graafian follicles.

4.2.3.4 SECOND REPRODUCTIVE CYCLE

Information on the second reproductive cycle is not as detailed as is the case with the first cycle. From Tables 4.6 and 4.7, it can be seen that as a result of a post-partum

TABLE 4.6 Reproductive status and conceptus development in female *T. condylura* examined during December 1988 and 1989 at the onset of the second reproductive cycle.

DATE	BAT	STATUS	CONCEPTUS	SITE	Z.P.
1988 (SKUKUZA):					
28 DEC	1	P + L	MORULA (>32 CELLS)	UTERUS LUMEN	-
	2	P + L	MORULA (>32 CELLS)	UTERUS LUMEN	-
	3	P + L	4 CELL STAGE	AMPULLA	+
	4	L	---		
	5	P + L	IMPLANTING BLASTOCYST		
	6	P + L	EMBRYO (PRIMITIVE STREAK STAGE)		
	7	P + L	IMPLANTING BLASTOCYST		
	8	P + L	BILAMINAR BLASTOCYST	UTERUS LUMEN	-
	9	P + L	BILAMINAR BLASTOCYST	UTERUS LUMEN	-
	10	P + L	EMBRYO (PRIMITIVE STREAK STAGE)		
	11	P + L	EMBRYO (EARLY SOMITE STAGE)		
	12	P + L	EMBRYO (PRIMITIVE STREAK STAGE)		
1989 (KOMATIPOORT):					
6 DEC	1	NT	FOETUS (4.9 g)		
	2	P	FOETUS (0.68 g)		
	3	NT	FOETUS (7.45 g)		
	4	L	---		
	5	L	---		
	6	L	---		
	7	L	---		
	8	P + L	8 CELL STAGE	AMPULLA	+
	9	L	---		
11 DEC	1	P + L	2 CELL STAGE	AMPULLA	+
	1	L	---		
	2	L	---		
	3	L	---		
	4	L	---		
	5	P + L	2 CELL STAGE	AMPULLA	+
17 DEC	1	P + L	MORULA (>32 CELLS)	UTERUS LUMEN	-
	2	L	---		
	3	NT	FOETUS (5.88 g)		
	4	L	---		
	5	P + L	8-16 CELL STAGE	AMPULLA	+
	6	P + L	UNILAMINAR BLASTOCYST	UTERUS LUMEN	-
	7	P + L	MORULA (16-32 CELL)	ISTHMUS	-
26 DEC	1	P + L	EMBRYO (PRIM. STREAK STAGE)		
	2	P + L	IMPLANTING BLASTOCYST		
	3	P + L	16-32 CELL STAGE	ISTHMUS	-
31 DEC	1	P + L	IMPLANTING BLASTOCYST		
	2	P + L	EMBRYO (PRIM. STREAK STAGE)		

P = PREGNANT
L = LACTATING
NT = NEAR-TERM
Z.P.= ZONA PELLUCIDA (+ = PRESENT; - = SHED)
--- = NO CONCEPTUS

TABLE 4.7 Data on post-partum oestrus in female *T. condylura* obtained through the mark-recapture programme during 1989.

BAT NO.	22 NOV.	30 NOV.	17 DEC.	CONCEPTUS ON 17 DEC
1	P^1	NT	P^2 + L	16 CELL STAGE IN AMPULLA
2	P^1	NT	P^2 + L	MORULA IN ISTHMUS
3	P^1	NT	P^2 + L	MORULA IN UTERUS
4	P^1	NT	P^2 + L	MORULA IN UTERUS
5	P^1	NT	L	-
6	P^1	NT	L	-
7	P^1	NT	NT	(5.88g FOETUS)

P^1 = PREGNANT DURING FIRST CYCLE

P^2 = PREGNANT DURING SECOND CYCLE

NT = NEAR-TERM

\- = NO CONCEPTUS

L = LACTATING

oestrus, ovulations and conceptions were initiated in the first three weeks of December. By the end of December, almost all adult females from Skukuza and Komatipoort were pregnant (95%, n = 17) with conceptuses ranging from morulae still in the oviduct to somite stage embryos.

During 1990, first parturitions marking the end of the second reproductive cycle must have occurred during late February, since 30% of the females examined on 3 March had already given birth. All females examined through palpation in early April (n = 41) had given birth and were lactating (Table 4.5). Some of those examined histologically during April 1990 had only recently given birth as their uteri were still distended and filled with debris and blood.

4.2.3.5 LACTATION

The duration of lactation in female *T. condylura* was determined through the mark-recapture experiment conducted during late 1989 and early 1990. The mark-recapture data with regard to lactation are presented in Table 4.8.

The lactation period was estimated to be between 50-60 days. Three females from group A (no 2, 3 & 6) and two females from group C (no 2 & 3), found to be lactating on 11 December, were still lactating when recaptured on 1 February. The duration of lactation in these females, therefore, was more than 52 days. Two females from group B (no 1 & 2), were lactating when recaptured on 13 December and again on 1 February, indicating that the duration of lactation in these females exceeded 50 days. The remaining females from group A (no 1, 4 & 5), still pregnant when recaptured on 30 November, were lactating when recaptured on 11 December and 1 January. On 1 February, however, when they were again recaptured, they were not lactating. The lactation period in these females had, therefore, not exceeded 62 days.

TABLE 4.8 Lactation period in female *T. condylura* as determined through the mark-recapture programme during 1989 and 1990.

GROUP	DATE	BAT	DATES									
No.	BANDED	No.	22 NOV	27 NOV	30 NOV	11 DEC	13 DEC	17 DEC	1 JAN	3 JAN	1 FEB	3 MAR
A	22 NOV	1	P	-	P	L	-	L	L	-	P	
		2	P	-	P	L	-	L	-	-	L	
		3	P	-	P	L	-	-	-	-	L	
		4	P	-	P	L	-	L	L	-	P	
		5	P	-	P	L	-	L	L	-	P	L
		6	P	-	P	L	-	L	-	-	L	
B	27 NOV	1		P	-	-	L	-	-	L	L	
		2		P	-	-	L	-	-	L	L	
		3		P	-	-	L	-	-	L	P	
C	11 DEC	1				L	-	L	L	-	P	
		2				L	-	L	-	-	L	
		3				L	-	L	L	-	L	
D	3 JAN	1								L	-	L
		2								L	L	L
		3								L	L	L

L = LACTATING (NONPREGNANT OR PREGNANT)

P = PREGNANT BUT NOT LACTATING

- = NOT RECAPTURED

Two of the three females from group D (no 2 & 3) were still nursing their first offspring on 1 February (Table 4.8). When recaptured on 3 March, they were again lactating following the birth of their second offspring. In these females, therefore, less than a month had elapsed from the time when the first lactation period was terminated to the start of the second lactation period.

Although very little data is available on lactation following the second parturition, it is expected to be similar to that observed during the first period. If this is the case, then the second lactation period could extend well into June, particularly since some females are expected to give birth only during late March. This assumption is based on the fact that 70% of the females were still pregnant on 3 March (Table 4.5).

Of special interest is the mark-recapture record of specimen no. 5 (group A, Table 4.8). Pregnant when banded and released on 22 November, this female was recaptured on six occasions during the period December to March. These data clearly emphasise the fact that females undergo two pregnancies in rapid succession. It is also additional evidence for a post-partum oestrus, because only 92 days (30 November to 3 March) had elapsed from the time that this female was carrying her first offspring to the time that she was nursing her second offspring. With the gestation period estimated at 85 days, it shows that she had conceived again within a maximum of seven days following the birth of her first offspring.

4.2.4 HISTOLOGICAL CHANGES ASSOCIATED WITH REPRODUCTION

The reproductive tract of female *T. condylura* displayed a distinct bimodal seasonal pattern in development and activity.

4.2.4.1 GENERAL OVARIAN AND UTERINE DEVELOPMENT

Ovarian follicle counts were made during 1988 from February to December (Figure 4.24) and during 1989 and 1990 from August to March (Figure 4.25). Examination of the right ovaries of adult females during 1988 showed that the mean antral follicle population varied significantly concurrent with the breeding season ($F = 4.7$, $d.f. = 26$, $P<0.01$). The lowest antral follicle counts were made during reproductive quiescence, i.e. April to June (9.3 - 12.0), whereas the highest values were recorded from December to March (23.4 - 26.0). More frequent collections during 1989 showed a significant bimodal pattern in antral follicle numbers during the breeding season, with the first peak in late September and the second in late December ($F = 2.311$, $d.f. = 58$, $P<0.05$). A mid-season low was recorded during October.

A closer look at the different follicle stages, showed that the secondary follicle population closely followed the bimodal pattern observed in the antral follicle population. The Graafian follicle population also varied significantly during 1988 ($F = 3.0$, $d.f. = 26$, $P<0.05$, Figure 4.24), and 1989 ($F = 2.4$, $d.f. = 75$, $P<0.02$, Figure 4.25), showing a rapid increase during August. This was followed by a more gradual increase during September and October resulting in a peak at the end of December. The rapid increase in Graafian follicle numbers during August resulted in a concomitant decrease in the number of secondary follicles over the same period (Figure 4.24 & 4.25). In late July or early August, the antral follicle population was composed almost entirely of secondary follicles (1988: 95.8%; 1989: 86.2%), but by late August or early September, secondary follicles constituted only about a half of the antral follicles (1988: 56.2%; 1989: 59.6%).

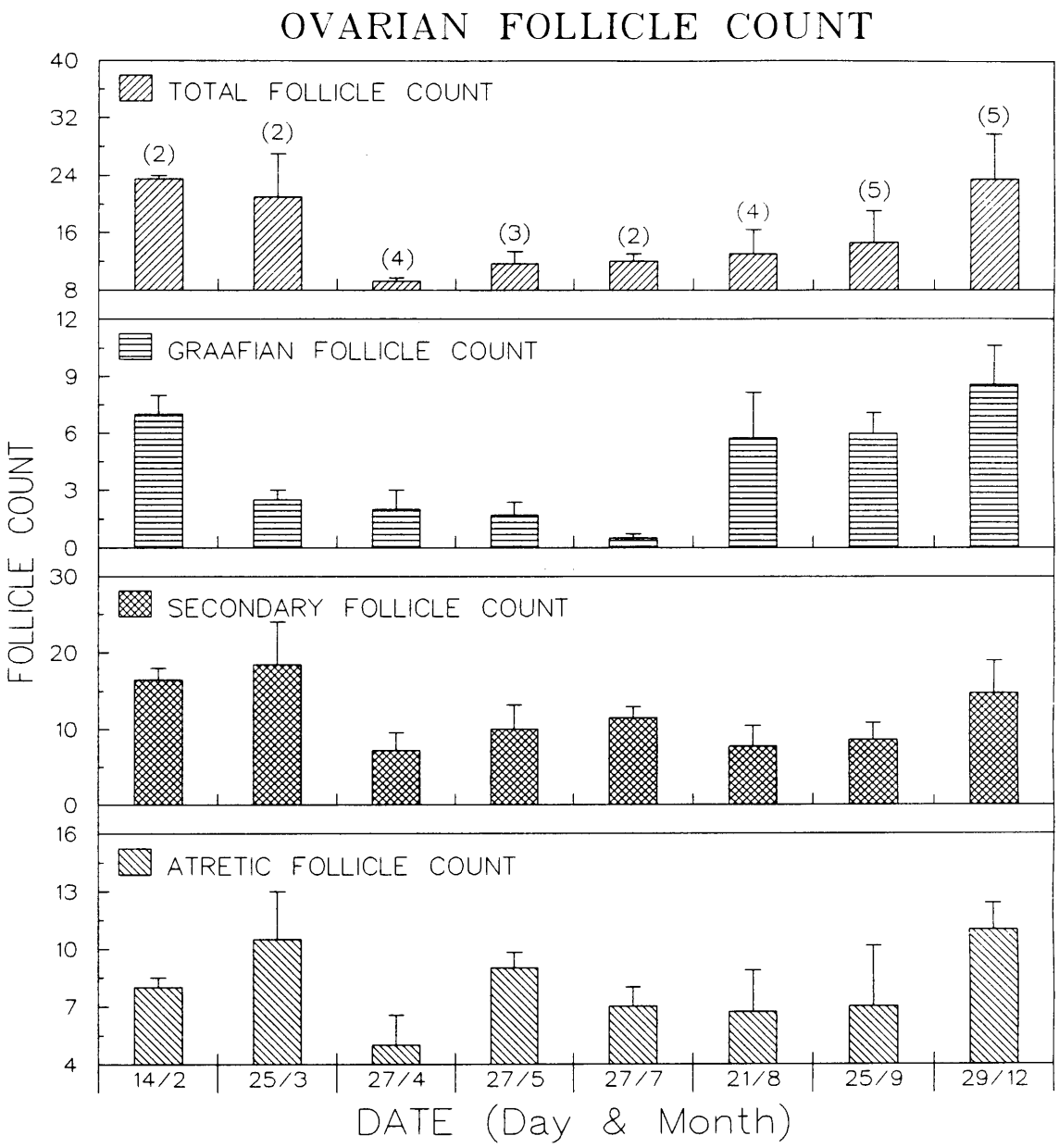


Figure 4.24. Mean number of antral follicles (± 1 SD) in right ovaries of female *T. condylura* collected at Skukuza during 1988 (Sample sizes are indicated in brackets).

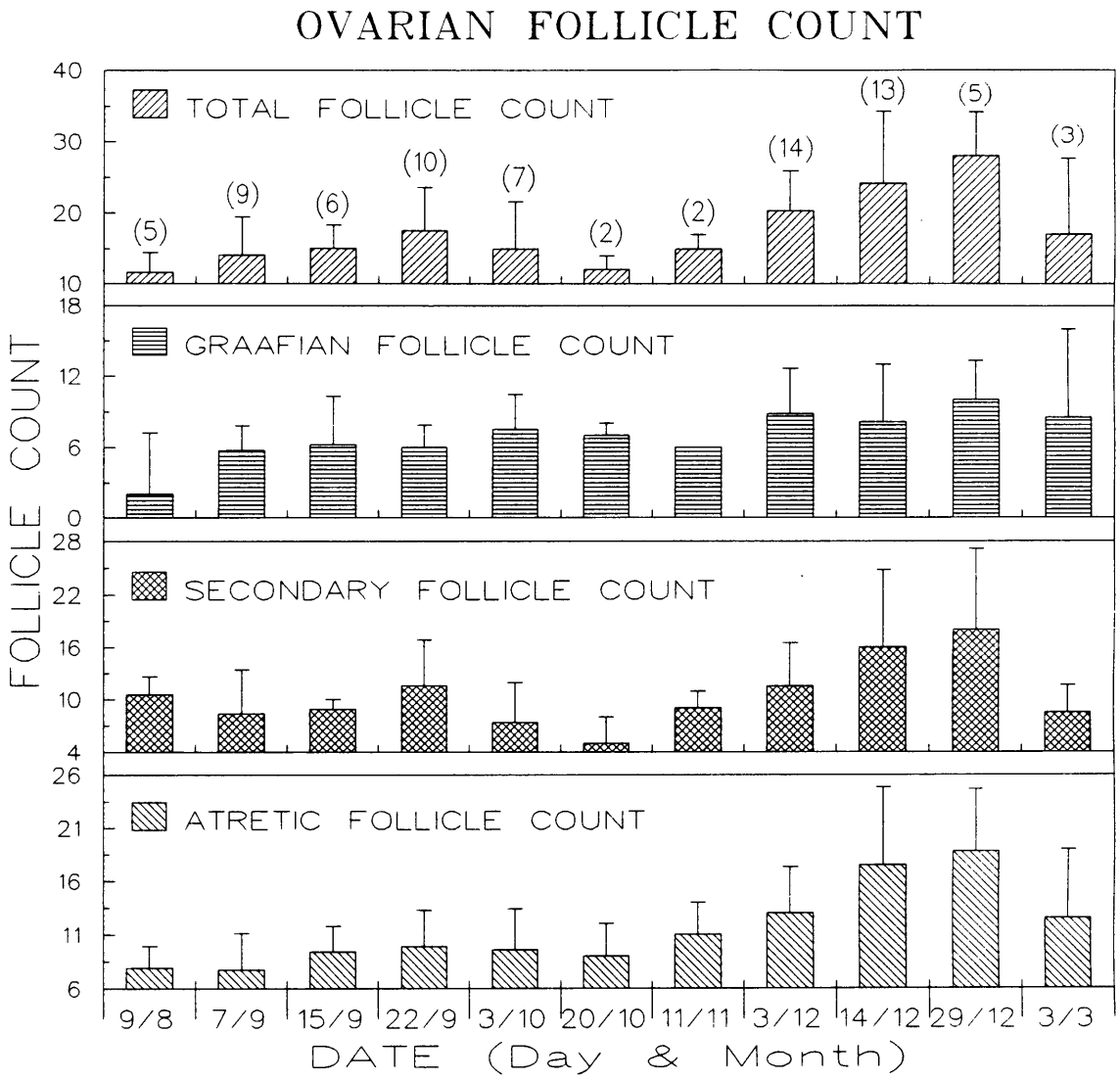


Figure 4.25 Mean number of antral follicles (± 1 SD) in right ovaries of female *T. condylura* collected at Komatipoort during 1989 (Sample sizes are indicated in brackets).

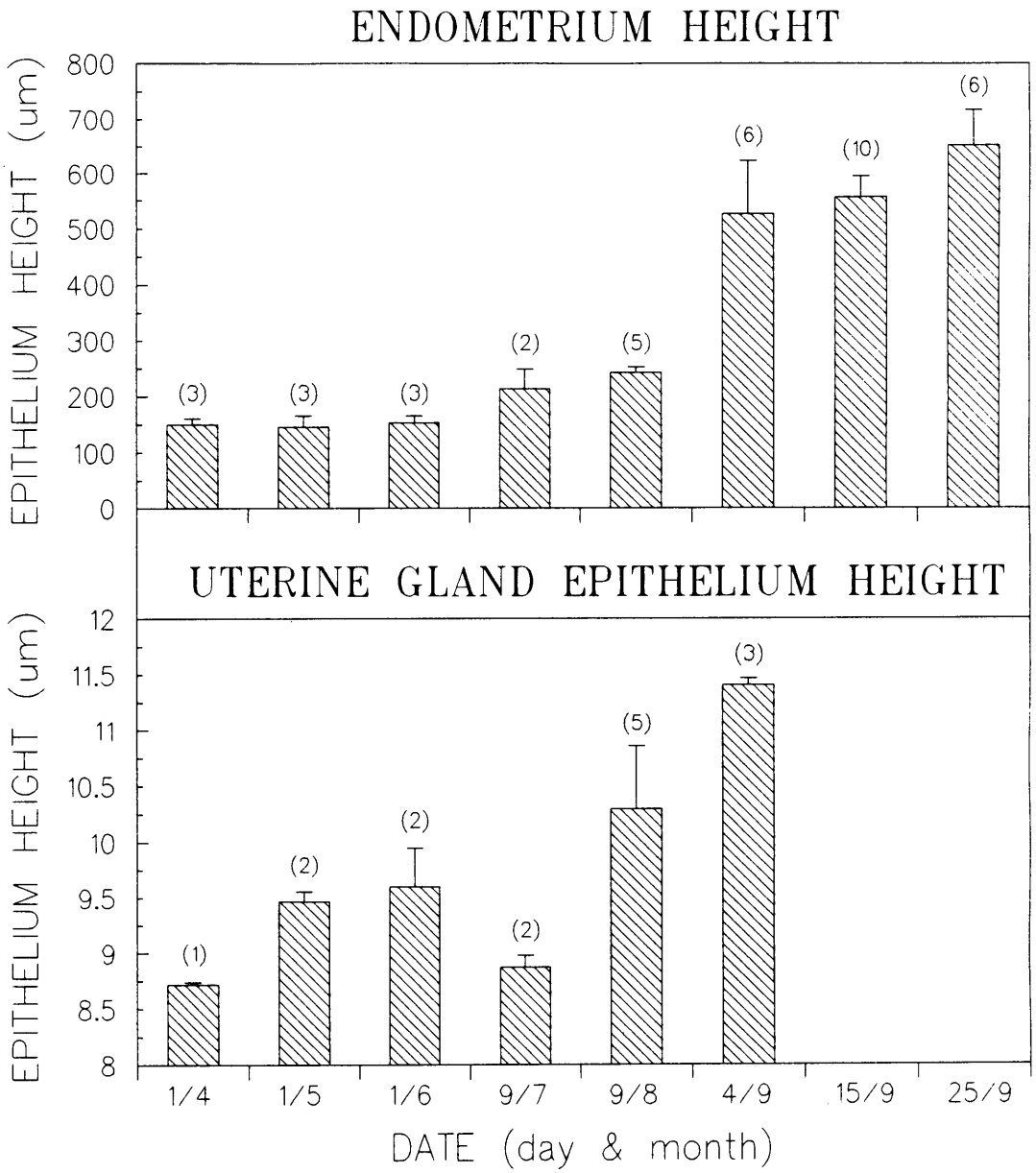


Figure 4.26. Mean height (+ 1 SD) of endometrium and uterine gland epithelium in uteri of female *T. condylura* collected during proestrus, oestrus and pre-implantation development (Sample sizes indicated in brackets).

The number of atretic antral follicles varied significantly during 1988 ($F = 2.8$, $d.f. = 26$, $P<0.05$, Figure 4.24), although no definite seasonal pattern was observed. The lowest value was recorded during late April (5.0 ± 1.8) and highest values during December (11.5 ± 1.6) and March (10.5 ± 2.5). During 1989, the atretic follicle population also varied significantly during the breeding season ($F = 3.06$, $d.f. = 75$, $P<0.005$, Figure 4.25), showing an increase in numbers from September (7.7 ± 1.4) through November with a peak in late December (18.8 ± 2.7). This was followed by a decrease through late summer to March (12.5 ± 5.5).

The endometrium showed a fourfold increase in height during 1989 from baseline levels in early April (150 ± 7.6 μm) to implantation in September (655 ± 29.1 μm, $F = 95.5$, $d.f. = 24$, $P<0.001$, Figure 4.26). The endometrium gradually thickened from April through June and July, followed by rapid proliferation of the endometrium during August and September prior to implantation in late September. Uterine gland epithelium varied significantly in height from April (8.7 ± 0 μm) through May to August to late September (11.5 ± 0.3 μm), showing a rapid increase during July, August and September ($F = 5.4$, $d.f. = 12$, $P<0.05$).

4.2.4.2 PROESTRUS

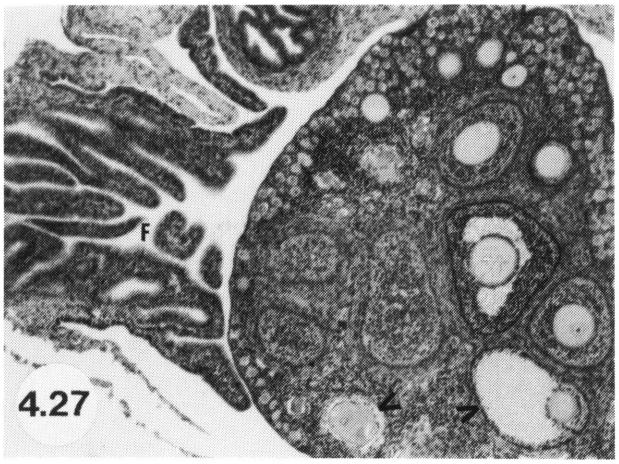
All right ovaries of adult females from Skukuza examined during proestrus, i.e. April to July, were found to be active, displaying all stages of follicular development. Ovaries were least active during late March and early April, being dominated by primordial and primary follicles, although a few antral follicles were also present (Figure 4.27). From late April to July, ovarian activity gradually increased with secondary follicles becoming increasingly more dominant. Although Graafian follicles were only found in small numbers at this stage, one specimen collected in April showed four large Graafian follicles which dominated the right ovary. By

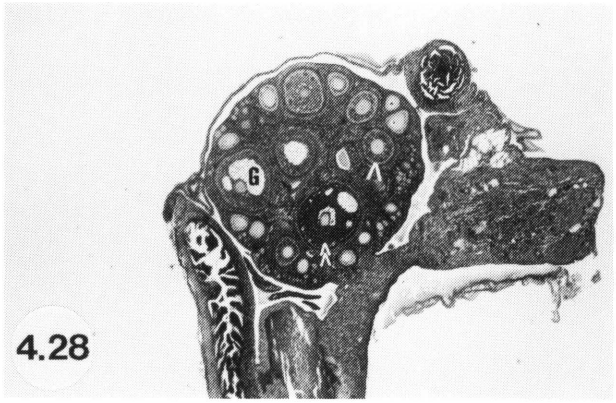
the end of July, ovaries were characterised by large primary and secondary follicles (Figure 4.28)

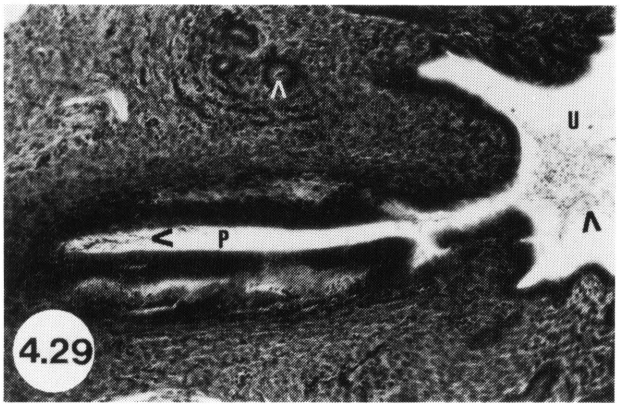
The uteri of adult females examined from early April to early July showed little activity and the uterine lumina were wide and mostly empty. The uteri of three females collected in early April, however, displayed signs of recent parturition with the uterine lumina containing debris composed of blood, leucocytes and loose endometrial cells. These uteri were still distended and regeneration of the endometrial epithelium was still in progress. The endometrium was poorly developed and showed little activity during this stage, although a gradual increase in height of the endometrium was observed during June and July (Figure 4.26). The endometrium was composed primarily of the deeper basalis bordered by a thin outer functionalis. Uterine glands were poorly developed and straight with the gland lumina empty and narrow.

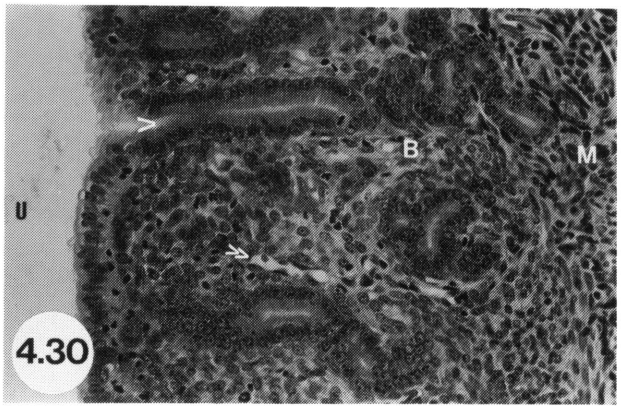
4.2.4.3 OESTRUS

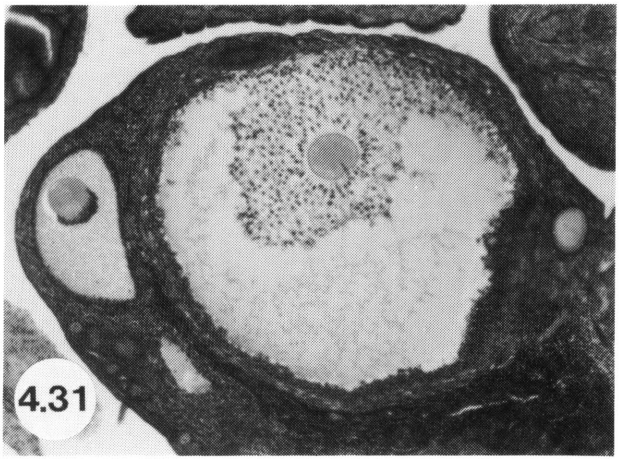
The onset of oestrus in August was marked by the presence of spermatozoa in the uterus. First spermatozoa in the female reproductive tract were recorded in early August, indicating the initiation of copulations. In most females, however, spermatozoa were only observed in uteri during late August or early September just prior to first ovulations and conceptions. The majority of spermatozoa were observed in the uterine lumen and in the uterine glands, while in the oviduct, abundant spermatozoa were only observed in the caudal end of the isthmus, the pars interstitialis and in the oviductal entrance (Figure 4.29). Spermatozoa were equally abundant in the right and the left uterine horns. Concurrent with the appearance of spermatozoa in the uterus, macrophages and polymorphonuclear leucocytes became evident in the uterine lumen, resulting in the rapid removal of spermatozoa, especially in the oviduct and the cranial end of the uterine horns.

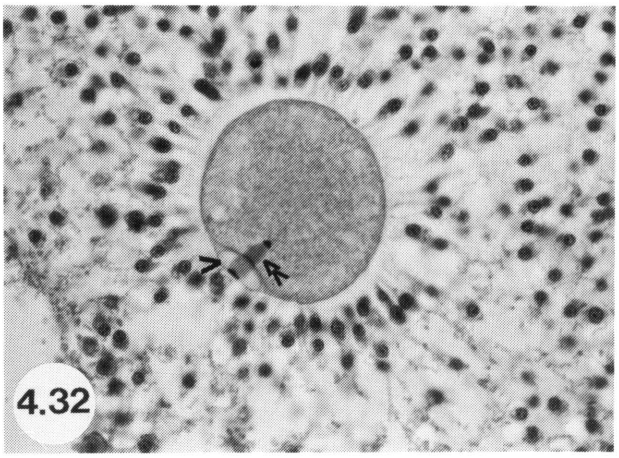












The right ovary was characterised by a rapid increase in the number of Graafian follicles from early August to early September concomitant with a slight decrease in the number of secondary follicles. (Figures 4.24 & 4.25).

As a result of rapid hypertrophy of the endometrial stroma, the endometrium showed a twofold increase in height during the period early August to early September (Figure 4.26), caused mainly by proliferation of the functionalis. The uterine glands showed an increase in activity. Concurrent with the thickening of the endometrium, the glands became longer with their basal sections becoming more convoluted. Gland lumina remained narrow and empty (Figure 4.30).

4.2.4.4 OVULATION

First ovulations occurred during the first week of September, during which time the female reproductive tract showed a rapid increase in development and activity. Two females examined in early September each contained a large pre-ovulatory Graafian follicle (Figure 4.31). The follicle completely dominated the ovary, pushing other follicles aside and causing the ovary to bulge outwards. The cumulus oophorus cells had expanded and had become slightly dispersed. Formation of the first polar body was nearly completed, with the first maturation spindle still intact (Figure 4.32). Pre-ovulatory Graafian follicles were observed in 60% (n = 6) of the non-pregnant females examined during September.

The endometrium showed a further increase in activity and height (Figure 4.26), mainly due to rapid proliferation of the stroma in the functionalis. This resulted in further lengthening of the uterine glands. The glandular epithelium was high columnar and the gland epithelium more dilated than during August. Numerous finely coiled arteries became prominent in the functionalis.

4.2.4.5 METOESTRUS

Following ovulation, the right ovaries were dominated by the recently ruptured Graafian follicles which began to luteinise. The follicle cavities were still large containing abundant blood, loose degenerating granulosa cells and polymorphonuclear leucocytes (Figure 4.33). Luteinisation of the membrana granulosa proceeded rapidly, although a blood filled cavity was still present in corpora lutea observed during October. Rapid proliferation of the luteal cells caused strands of newly formed cells to invade the blood-filled cavity (Figure 4.34). Luteal cells took on a characteristic appearance shortly after the corpus luteum was formed (Figure 4.35). Initially, the nuclei of the luteal cells showed evenly distributed nucleoplasm, but the nucleoplasm soon became concentrated as a dark band around the periphery of the nucleus, giving the nuclei a very distinct vacuolated appearance.

Luteal activity was maintained throughout pregnancy and healthy corpora lutea were observed until late in pregnancy. Towards the end of pregnancy, corpora lutea were usually still well developed, although often deformed by large Graafian follicles (Figure 4.8). Although the corpora lutea began to wane prior to parturition, remnants of the corpora lutea were sometimes found even after parturition.

During the period following ovulation and conception up to the time of implantation, the uterus attained a typical pre-implantation appearance. The endometrium attained its maximum height and the uterine glands became very prominent and highly contorted (Figure 3.36) and the gland lumina was filled with secretion. Glandular epithelium was high and the epithelial cells were distinctly columnar with the nuclei situated basally. Coiled capillaries became a distinctive feature of the endometrium and the blood vessels in the myometrium showed a rapid increase in volume.

Figure 4.33. Right ovary with a young corpus luteum (CL) displaying a large central cavity. x100.

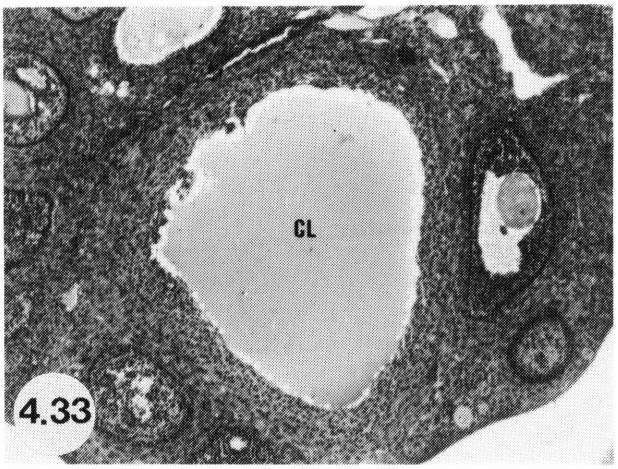
Figure 4.34. Right ovary showing a well developed corpus luteum with blood (arrow-head) still present in the centre. x100.

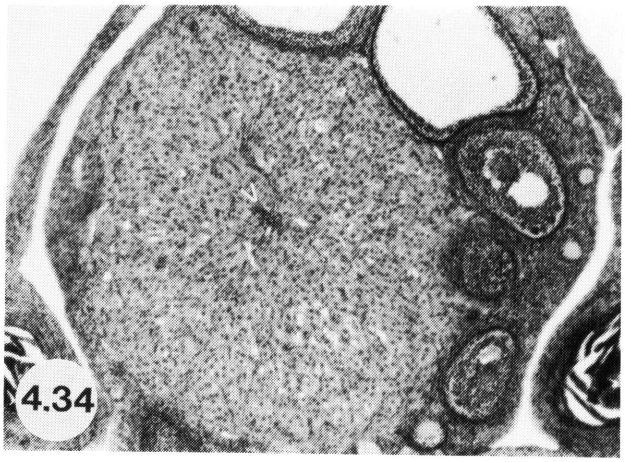
Figure 4.35. Section of a corpus luteum showing the typical structure of the luteal cells. The nuclei showed large nucleoli and the nucleoplasm had become concentrated around the periphery of the nucleus, giving the nucleus a vacuolated appearance (white arrow-head). Strands of connective tissue (double arrow-head) and blood capillaries (dark arrow-head) are common. x400.

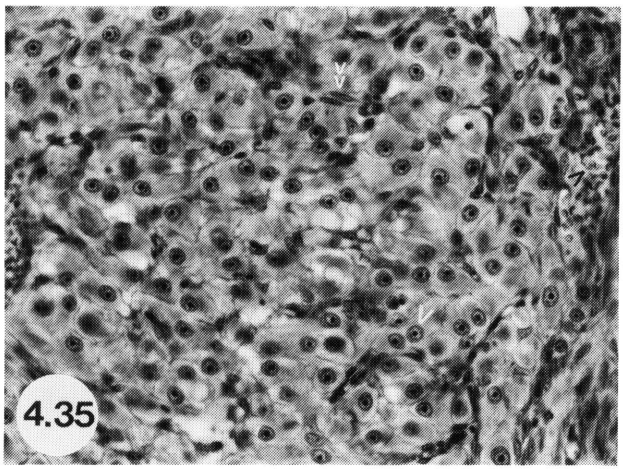
Figure 4.36. Right uterine horn with a morula in its lumen, showing a thick, highly proliferated endometrium with long, convoluted uterine glands. x40.

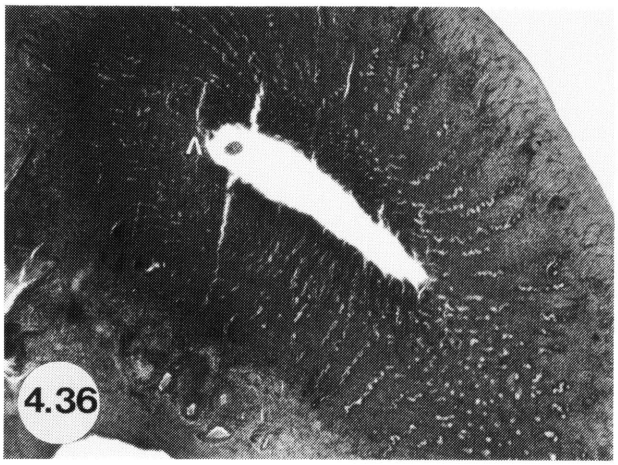
Figure 4.37. Zygote with first polar body still attached (arrow-head) in the ampulla of the right oviduct. x400

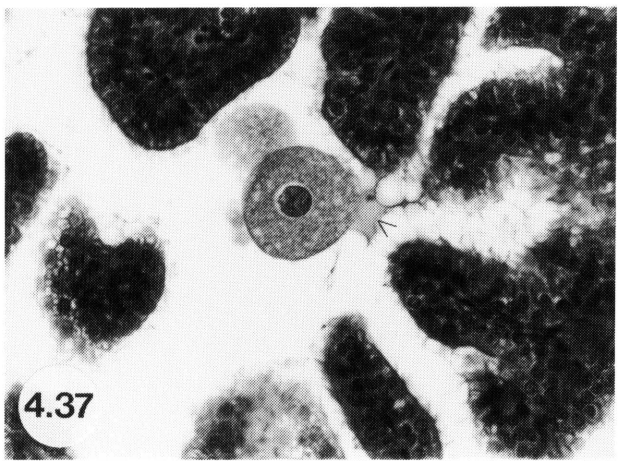
Figure 4.38. 16 Cell stage conceptus in the ampulla/isthmus junction region showing the break-up of the zona pellucida (arrow-heads). The first polar body can still be seen (double arrow-head). x400.

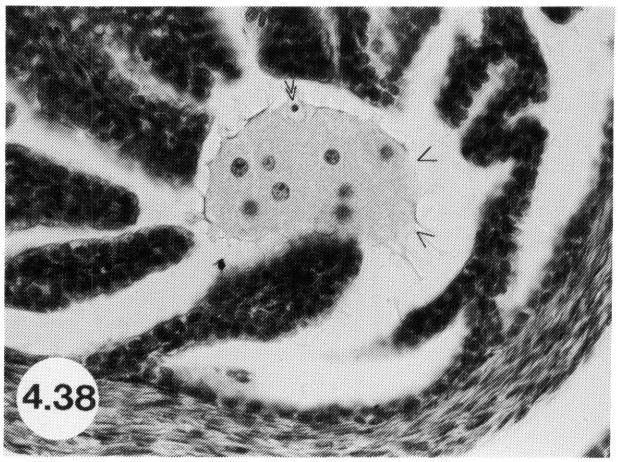












(i) OVIDUCTAL PASSAGE

Data on the stages of embryonic development in the oviduct of female *T. condylura* during the first and second reproductive cycle are presented in Tables 4.4 and 4.6, respectively. Conception always occurred in the right oviduct following ovulation from the right ovary. Embryos observed during oviductal passage ranged from a zygote with the polar body still attached (Figure 4.37), to well developed morulae (>32 cells). No blastocyst was observed in the oviduct.

The data available made it difficult to determine the duration of oviductal passage. On 11 September, all females examined contained two to sixteen cell stage conceptuses in the ampulla (Table 4.4). The first conceptuses observed in the uterus were recorded between 20 and 25 September. Four of the five embryos recorded on 25 September were either already implanted or in the process of implantation (Table 4.4). If the five smaller embryos observed on 11 September were 1-3 days old, it implied that implantation occurred about 14-16 days post conception. The duration of oviductal passage is then estimated at approximately 7-10 days.

Shedding of the zona pellucida in female *T. condylura* occurred in the oviduct in the region of the ampulla-isthmus junction (Figure 4.38). No embryos with intact zona pellucidae were observed in the uterus.

(ii) PREIMPLANTATION DEVELOPMENT

The embryo entered the uterus as a morula where it was transformed into a unilaminar, free-lying blastocyst. Proliferation of the embryonic endoderm was initiated prior to implantation and the embryo implanted as a bilaminar blastocyst (Figure 4.39).

Degeneration of the endometrial epithelium began before implantation. One outstanding feature of the pre-implantation uterus of female *T. condylura* was the formation of a distinct decidua at the implantation site on the mesometrial side (Figure 4.40). This area was characterised by marked oedema of the stroma. It was first observed in the endometrium during the early blastocyst stages. Stromal cells became irregularly interspersed in a network of reticular fibres, giving the area a spongy appearance (Figure 4.41). The left uterine horn also revealed deciduation in its cranial end, even though implantation never occurred there. In the right horn, the decidua zone was formed in the region of the future discoidal placenta.

(iii) IMPLANTATION

Implantation in *T. condylura* was centric and superficial. First attachment of the trophoblast to the endometrium was mesometrial. The formation of a pre-placental pad was also initiated on the mesometrial side (Figure 4.42), while orientation of the embryonic disc was antimesometrial (Figure 4.43). Implantation always occurred in the cranial end of the right uterine horn.

At the time of implantation, initial breakdown of the endometrial epithelium by the trophoblast occurred lateral to the abembryonic pole of the bilaminar blastocyst (Figure 4.43). Degeneration of glandular epithelial cells was initiated as evidenced by cellular debris and pycnotic nuclei in many glands as well as in the endometrial stroma. Immediately after initial attachment, proliferation of the trophoblast in the abembryonic hemisphere occurred, which eventually gave rise to a double layered structure consisting of an outer layer of syncytiotrophoblast, and an inner layer of cytotrophoblast. The cytotrophoblast maintained its cellular arrangement, whereas the syncytiotrophoblast became syncytial when the stroma was invaded. The trophoblast adjacent to the

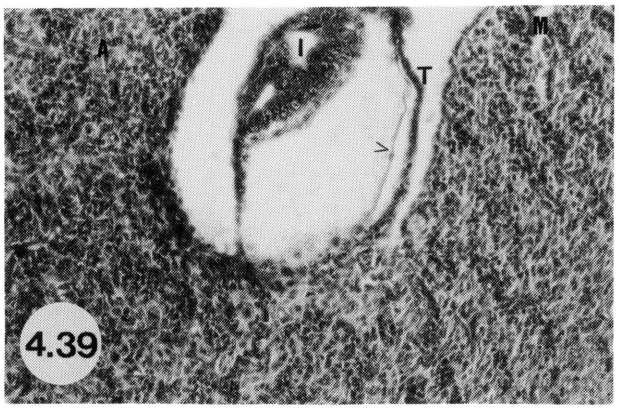
Figure 4.39. Implanting bilaminar blastocyst in the cranial end of the right uterine horn showing the endoderm (arrow-head), trophoblast (T) and inner cell mass (I). Initial attachment of the trophoblast is mesometrial (M) and the inner cell mass is oriented antimesometrially (A). x200.

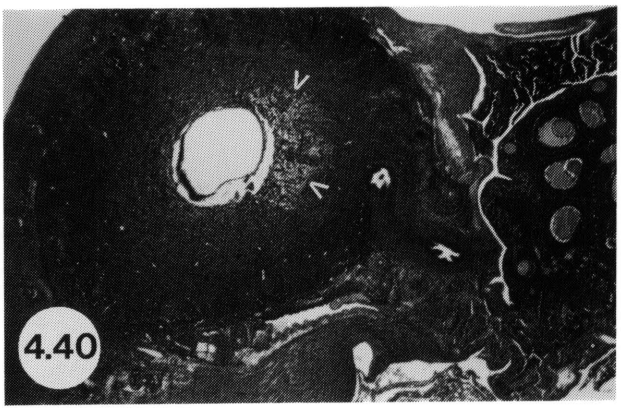
Figure 4.40. Right uterine horn with implanting blastocyst showing the decidua situated (arrow-heads) adjacent to the oviductal entrance. x40.

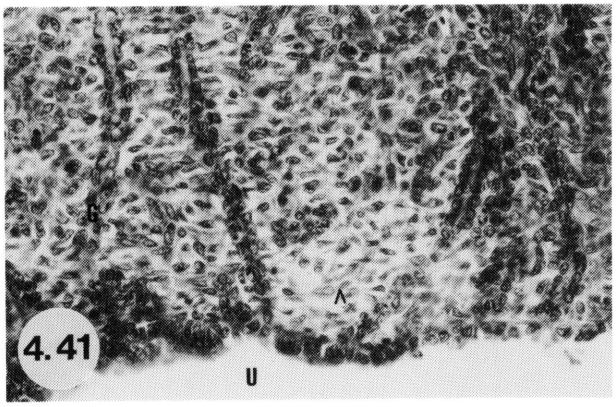
Figure 4.41. High magnification of the decidua showing connective tissue (arrow-head) and uterine glands (G). Uterine lumen (U). x400.

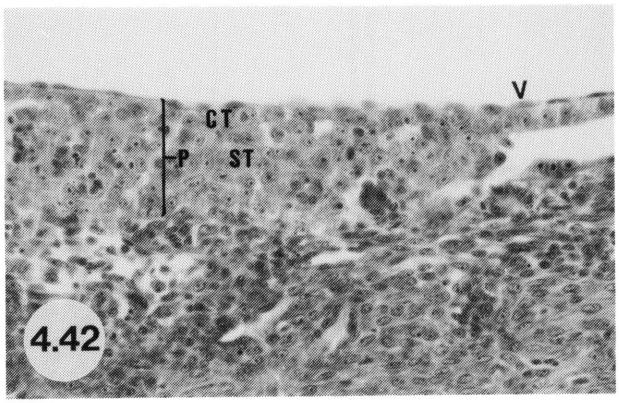
Figure 4.42. Pre-placental pad (P) at the site of first trophoblast attachment in the right uterine horn. The syncytiotrophoblast (ST) is starting to invade the endometrium. Cytotrophoblast (CT); Endodermal cells (arrow-head). x400.

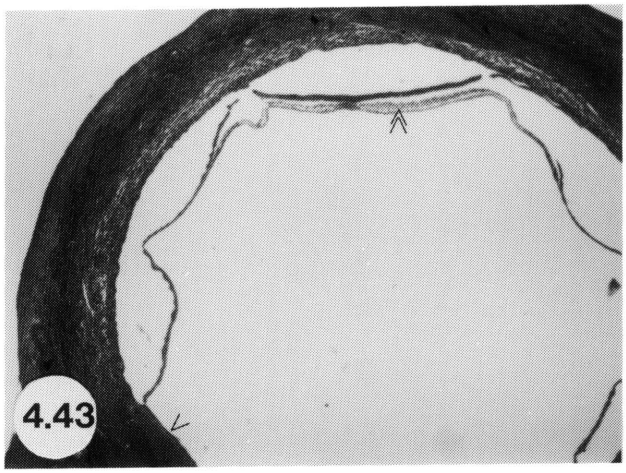
Figure 4.43. Primitive streak stage embryo showing the pre-placental pad slightly lateral from the abembryonic pole (arrow-head) and early mesodermal development (double arrow-head). The embryonic disk is oriented antisometrially. x200.











decidua, at the site of first attachment, proliferated rapidly to form a distinct, multi-layered pre-placental pad (Figure 4.42). This pad of trophoblast cells acted as a precursor to the future definite discoidal placenta, which in *T. condylura*, was not formed diametrically opposite the embryonic disc, but slightly lateral to the abembryonic pole in the vicinity of the oviduct entrance (Figure 4.43). Trophoblastic invasion of the endometrial stroma during implantation was restricted initially to columns of trophoblast cells which began to invade the stroma.Proliferation of the trophoblast over the embryonic hemisphere was retarded and a bilaminar arrangement of the trophoblast was not observed in this region until after the primitive streak stage.

At the primitive streak stage, the pre-placental pad had further increased in size with the syncytiotrophoblast cells actively invading the stroma. Pycnotic nuclei and abundant leucocytes were seen in this area. The primitive streak stage was characterised by the appearance of mesodermal cells between the embryonic disc and the endoderm (Figure 4.43).

4.2.5 FETAL GROWTH CURVES

During 1989 and early 1990, 129 foetuses ranging in size from 0.005g to 8.36g were collected at Komatipoort, examined and weighed. Foetal body mass against age and cube root of foetal body mass against age are plotted in Figure 4.44.

Since the collection dates were known, the exact age of each foetus could be calculated from the foetal body mass. By extrapolating the age of the foetus from the date of collection, the actual conception and parturition dates for each foetus were calculated. In this manner additional data to that presented in section 4.2.3. on the distribution of conceptions and parturitions were obtained. These data for the first and second reproductive cycles are illustrated in Figures 4.45 and 4.46, respectively.

From Figure 4.45 it can be seen that 90% (n = 98) of the females conceived during the period 2-20 September at the start of the first reproductive cycle. First conception occurred on 2 September, and with conception estimated to follow not more than two days after ovulation, first ovulations must have occurred at the end of August. Conception in adult females were synchronised with 83% (n = 90) of the females conceiving during the two week period 6-20 September. Only 7% (n = 7) of females conceived later than 26 September, the latest being recorded on 23 October. All females (n = 6) which conceived later than 1 October were nulliparous young females, which are believed to have reached sexual maturity only prior to the breeding season.

First parturition occurred on 28 November. The majority of births occurred during the first two weeks of December with 83% (n = 90) of births occurring during the period 1-15 December (Figure 4.45). Only 7% (n = 7) of births occurred after 16 December, with the latest being recorded on 22 January.

The second reproductive cycle was initiated in late November with first conceptions estimated to be as early as 28 November (Figure 4.46). Conceptions peaked during the first two weeks of December (70%, n = 14), with 45% (n = 9) of the females conceiving during the period 8-14 December. Only 20% (n = 4) of conceptions occurred later than 20 December and the last conception was recorded on 11 January. Parturitions started on 23 February and 75% (n = 15) of the births occurred between 23 February and 14 March. The last females gave birth on 6 April.

The above results provided further evidence for a postpartum oestrus following the birth of the first offspring. Figure 4.45 and Figure 4.46 illustrate the extent of overlap between parturition at the end of the first reproductive

cycle and conception at the start of the second cycle. During the first cycle, 83% (n = 90) of births occurred between 1-15 December, while during the same period, 70% (n = 14) of the females conceived for the second time. It can be seen, therefore, that females ovulated and conceived within a few days after parturition.

When the two reproductive cycles are compared, conceptions and births appeared to be more synchronised during the first cycle, with 83% of the females conceiving during a 14 day period compared to 70% for the same period during the second cycle. On the other hand, conceptions and births extended over almost two months during the first cycle, compared to just over five weeks during the second cycle. It must, however, be stressed that the number of foetuses collected during the first cycle (n = 109) by far exceeded the number collected during the second cycle (n = 20). This made a comparison between the two cycles more difficult.

Mean body mass of near term foetuses in relation to neonatal body mass are presented in Table 4.9. These data show that neonates displayed a substantial post-partum drop in mean body mass, viz. neonates which were weighed within a few hours after birth showed a 38% post-partum decrease in mean body mass. The exact age of these neonates were known and their umbilical cords were still moist and soft when they were weighed. Neonates only seemed to regain their immediate pre-parturition mass about two to three days after birth (Table 4.9). Near-term foetal mass was 25.8% of non-pregnant maternal body mass (s.e.m. = 1.2%, n = 7, 22.3-31.3%), compared to 21.0% in the case of neonates weighed immediately after birth. (s.e.m. = 0.6%, n = 3, 20.0-21.6g).

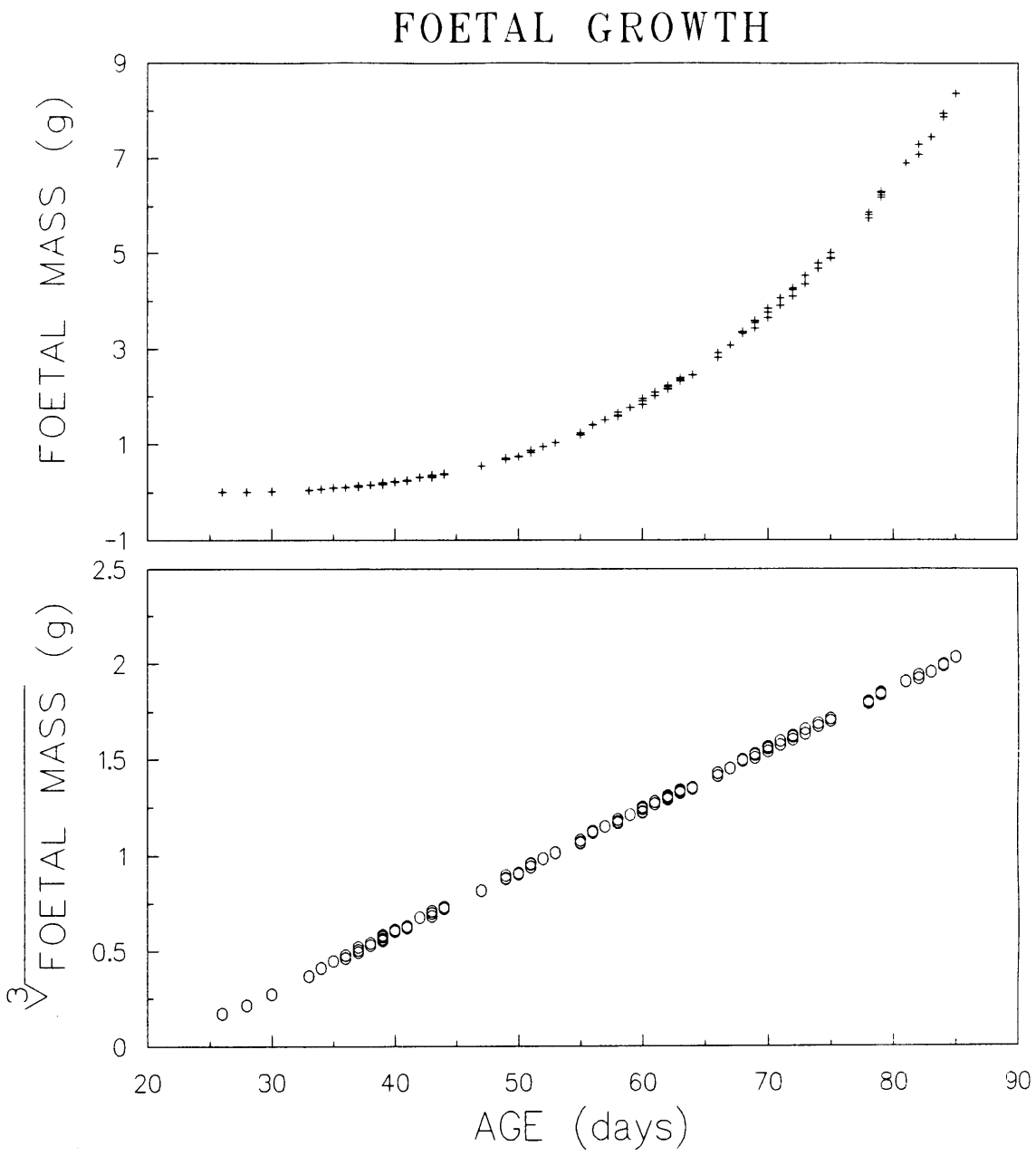


Figure 4.44. Foetal body mass and cube root of foetal body mass plotted against foetal age in days.

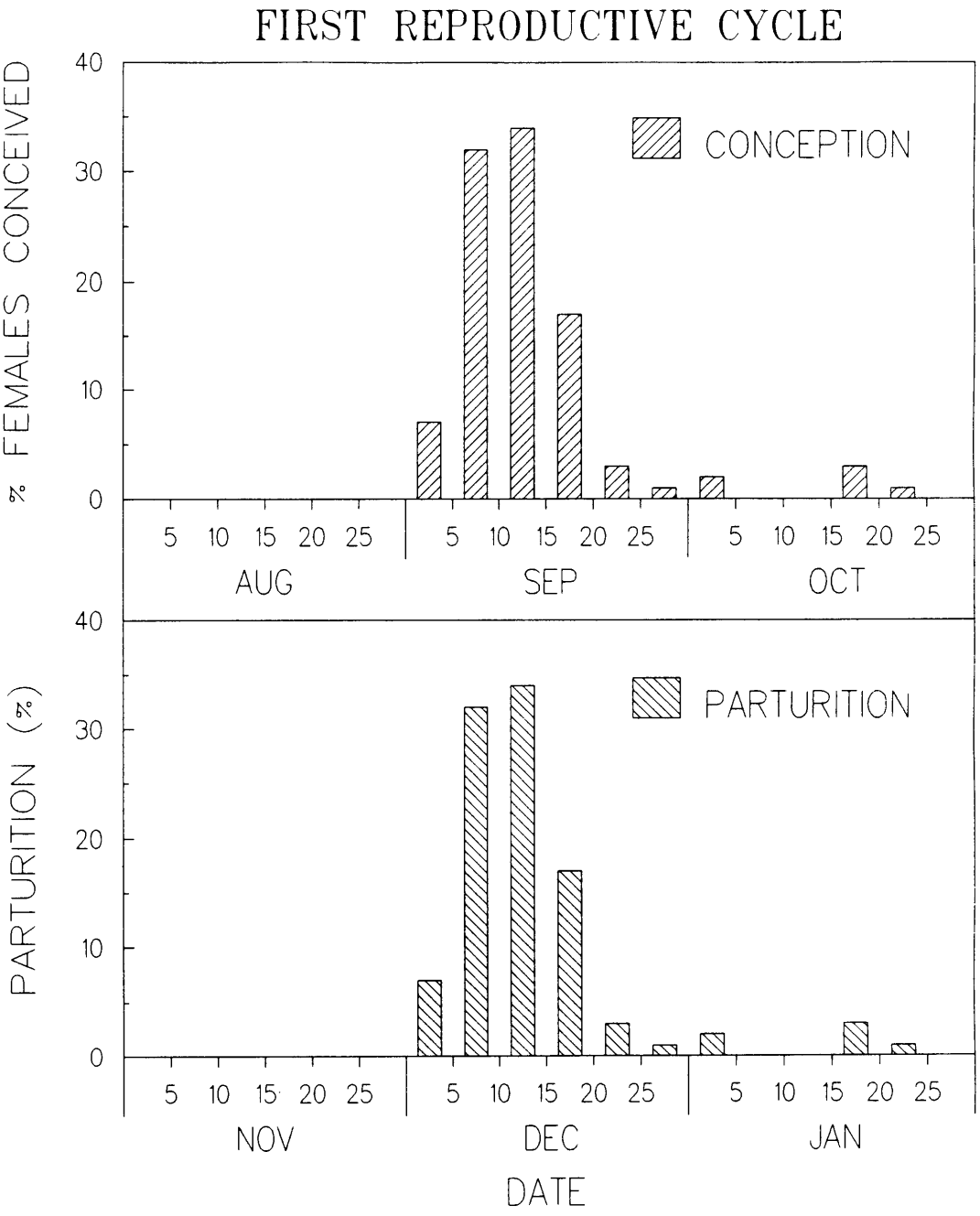


Figure 4.45. Conceptions and parturitions in female *T. condylura* during the first reproductive cycle.

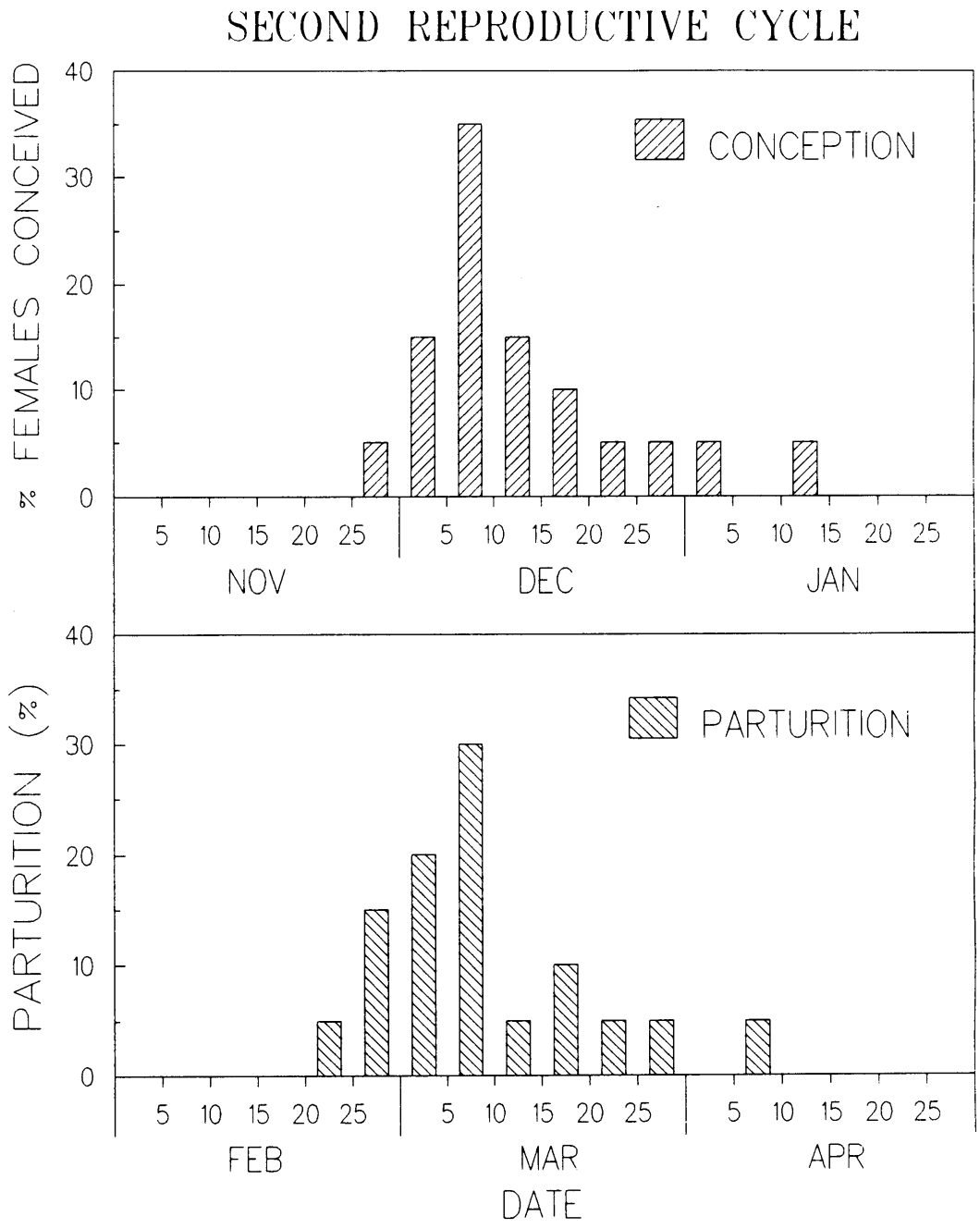


Figure 4.46. Conceptions and parturitions in female *T. condylura* during the second reproductive cycle.

TABLE 4.9 Mean body mass of *T. condylura* near-term foetuses and neonates. Only foetuses weighing more than 7g were used.

STATUS	AGE	MASS (g)	RANGE (g)	N	REMARKS
Foetus	>80 days	7.54±0.17	7.0-8.36	8	---
Neonate	<10 hours	4.64±0.08	4.58-4.8	3	Umbilical cord soft
Neonate	1-2 days	6.82±0.46	5.87-7.85	4	---
Neonate	>2days	9.25±0.28	8.79-9.66	3	first body hair

4.3 DISCUSSION

The reproductive pattern observed in female *T. condylura* in the Eastern Transvaal correspond to previous reports from elsewhere in Africa, suggesting a bimodal reproductive pattern for the species throughout its range (Braestrup 1933; Freeman 1981; Mutere 1973b; Pienaar *et.al.* 1980; Skinner & Smithers 1990; Happold & Happold 1989). Earlier reports do, however, show a geographical variation in the timing and succession of reproductive events. In the aseasonal environment of central Africa (Uganda, 0°43'N), the breeding season was extended over ten months with a four month interval between pregnancies (Mutere 1973b). Mutere (1973b), however, found no evidence of a post-partum oestrus. Births were recorded during February-March and July-September with lactation and weaning occurring during the two periods of peak precipitation, i.e. spring (September-November) and autumn (March-May; Mutere 1973b). In the more seasonal environment of southern Malawi (16°'13S, Happold & Happold 1989) and the Eastern Transvaal (present study), the situation was different with females showing two pregnancies in quick succession with a post-partum oestrus following parturition of the first offspring. In both the latter areas, rainfall was concentrated during a single summer rainy season with the remainder of the year showing much reduced precipitation (Gertenbach 1983; Van der Merwe *et.al.* 1986; Happold & Happold 1989). It is therefore not unexpected that *T. condylura* in these areas displayed a shorter breeding season and a post-partum oestrus in order to fit both pregnancies into the relatively short period of resource abundance (Van der Merwe *et.al.* 1986; Happold & Happold 1989). Happold and Happold (1989) augmented on the relation between latitude and the timing of reproductive events and showed a six to seven month interval between consecutive births in Uganda (0°43'N), four to five months in Kenya (2°18'S) and three to four months in southern Malawi (16°13'S). The interval between births in the Eastern Transvaal was also about three to four months.

In a seasonal environment such as the Eastern Transvaal, insect abundance is highest during the annual rainfall season and temperature peaks and reproductive events in bats are, therefore, geared to gain optimum benefit from such periods of high resource abundance (Jansen & Schoener 1968; Van der Merwe *et.al.* 1986; Van der Merwe, Rautenbach & Giddings 1987; Rautenbach, Kemp & Scholtz 1988; Wolda 1988). Late pregnancy, lactation and weaning are energetically strenuous events which are particularly geared to coincide with optimum resource conditions. Parturition of the second offspring in *T. condylura* occurred during the period March to April and lactation and weaning extended well into autumn and even early winter. Rainfall data for Skukuza show that peak precipitation occurred during the months November to February (70-97mm), with rainfall decreasing rapidly after February. Mean monthly precipitation during March, April and May were 64%, 44% and 20%, respectively, of the mean value for the three months of peak precipitation (December to February). Lactation and weaning following the second reproductive cycle, therefore, occurred at a time when insect abundance is expected to decline, causing female *T. condylura* to experience possible food shortage. This could lead to an increase in mortality rate among neonates.

Female *T. condylura* displayed similar reproductive patterns at the two study sites in the Eastern Transvaal. Although no temperature and rainfall data are available for Komatipoort, the two areas are only about 40 km apart, and environmental conditions are believed to differ very little. At both study areas, adult females showed two reproductive cycles, with similar conception and parturition periods. Unfortunately, foetuses were not collected from Skukuza and more precise data on the initiation of conception and parturition were, therefore, not available.

Only one other molossid species studied in detail has been

reported to be a bimodally seasonal breeder, i.e. *Molossus fortis*, a New World tropical species (Krutzsch & Crichton 1985). A comparison between this species and *T. condylura* in the Eastern Transvaal showed a very close resemblance with regard to the reproductive pattern and the morphology and development of the reproductive tract. *Molossus fortis*, studied at a subtropical latitude (18°N), also displayed two reproductive cycles in rapid succession with a post-partum oestrus during the interlude (Krutzsch & Crichton 1985). A study at lower latitudes would reveal whether this species in a more tropical environment would display a similar geographical variation in the timing of reproductive events as observed in *T. condylura* or whether it would perhaps conform to an aseasonal pattern as found in *T. pumila* (Mutere 1973b).

During the present study, no data were collected to confirm at what age females reached sexual maturity. In *Molossus fortis* (Krutzsch & Crichton 1985), *Tadarida brasiliensis cynocephala*, *T. b. mexicana* (Krutzsch 1955a, 1959; Short 1961) and *M. sinaloae* (Heideman, Erickson & Bowles 1990) females have been shown to reach sexual maturity during their first year. In Uganda, females of *T. pumila*, *Otomops martiensseni* and *T. condylura* have also been suggested to become sexually mature during their first year (Marshall & Corbet 1959, Mutere 1973a, b).

Although fairly uncommon, the occurrence of post-partum oestrus cycles have been reported in a number of chiropteran species, especially megachiropterans (Mathews 1939; Ramakrishna 1947; Harrison 1958; Gopalakrishna 1964; Lim 1970; Thomas & Marshall 1984; Krishna 1985). Post-partum oestrus cycles have also been recorded in some molossids, viz. *Molossus fortis* (Krutzsch & Crichton 1985), *Tadarida pumila* (Harrison 1958; Van der Merwe *et.al.* 1986, 1987) and *T. aegyptiaca* (Kashyap 1980).

Sufficient evidence has been found during the present study to indicate a post-partum oestrus in *T. condylura* in the Eastern Transvaal. The right ovaries of females with near-term foetuses showed large developing Graafian follicles alongside the corpora lutea of pregnancy. Similar observations were reported in a pipistrelle, *Pipistrellus mimus* (Krishna 1985) and in two molossids displaying a post partum oestrus, *Molossis fortis* (Krutzsch & Crichton 1985) and *Tadarida pumila* (Van der Merwe *et.al.* 1987).

During 1989, all females examined through palpation on 27 November were still pregnant and first lactating females were recorded on 1 December (Table 5.4). Histological examination, however, showed that females had conceived again as early as 6 December and by 17 December, 56% of the females were again pregnant (Table 4.6). These data show that the majority of female *T. condylura* entered their second pregnancy not more than two weeks after parturition of their first offspring.

This was confirmed by evidence derived from mark-recapture data (Table 4.7). Four of the seven near-term females recaptured on 30 November had conceived prior to examination on 17 December. Of these, two contained uterine morulae. With oviductal passage of the conceptus was estimated to be approximately 7-10 days, conception in these two females must have occurred between 7-10 December, indicating that parturition and conception had occurred within a maximum of eleven days.

Female *T. condylura* with post-partum uteri were found to be pregnant while nursing their young. Van der Merwe *et.al.* (1987) reported that *T. pumila* females were capable of becoming pregnant with uteri still in the regenerative (proliferative) phase of development, although the endometrium in most cases regenerated to the secretory phase before implantation occurred. As in *T. condylura*, the uterine walls of female *T. pumila* lacked sufficient time to recover to the pre-pregnant proliferative state of uterine development, but

only regenerated to the extent where implantation was possible (Van der Merwe *et.al*. 1987).

The significance of a post-partum oestrus is that it shortens the breeding season by reducing the interlude between consecutive pregnancies. This is of particular importance in the subtropical climate of the Eastern Transvaal in that it enables tropical species inhabiting a seasonal environment to remain polyoestrus breeders. Without a post-partum oestrus, *T. condylura* would probably only have sufficient time for one reproductive cycle. As suggested by Krutzsch & Crichton (1985), post-partum oestrus cycles will become a more widely recognised phenomenon among molossids once more detailed information on reproduction in tropical molossid species become available.

Microchiropterans display long periods of intrauterine development relative to other small mammals, with gestation periods ranging from about 50 days to almost eight months (Orr 1970). *T. condylura* in the Eastern Transvaal displayed a gestation period of about 85 days, which corresponds to previous reports for this species (Happold & Happold 1989) and falls within the range of 84-104 days reported for most other molossids (Sherman 1937; Kruzsch 1955a; Davis *et al* 1962; Kitchener & Hudson 1982; Krutzsch & Crichton 1985).

Data on the duration of lactation among molossids suggest post natal care of four to eight weeks. Van der Merwe *et.al*. (1986) and Happold & Happold (1989), and Krutzsch & Crichton (1985) reported lactation periods in *T. pumila* and *M. fortis* of more than 30 days and six weeks, respectively. Both species (6-15g) are slightly smaller than *T. condylura* (17-30g). Kitchener and Hudson (1982) reported an exceptional long lactation period in *T. australis* of three to four months. In *T. condylura*, Happold & Happold (1989) suggested a lactation period of approximately three months in Malawi, which coincides with *T. condylura* from the Eastern Transvaal.

In all molossids studied to date, the corpus luteum was associated with the right ovary. As in most molossids, excepting *Molossus fortis* (Krutzsch & Crichton 1985), the corpus luteum in *T. condylura* persisted to the end of term (Sherman 1937; Stephens 1962; Jerret 1979; Crichton & Krutzsch 1987; Van der Merwe *et.al.* 1986; Rasweiler 1988). Krutzsch & Crichton (1985) proposed that Graafian follicles found in the ovaries of near-term female *M. fortis* suggested that the corpus luteum began to wane prior to parturition. Near-term *T. condylura* were, however, found with large Graafian follicles alongside the corpus luteum of pregnancy, showing that folliculogenesis continued unaffected even in the presence of a corpus luteum. Most molossids contained a single corpus luteum, but *M. fortis* (Krutzsch & Crichton 1985) and *T. brasiliensis* (Sherman 1937; Krutzsch 1955a, 1959) sometimes had more than one.

In *T. australis*, an Old World temperate monoestrus breeder, antral follicle numbers showed a typical unimodal pattern with a steady increase in numbers from anoestrus through proestrus to reach a peak around ovulation, followed by a decrease after ovulation (Kitchener & Hudson 1982). The variation in follicle numbers observed in *T. condylura* followed a bimodal pattern concurrent with the breeding season. During August, prior to ovulation, ovaries were marked by a rapid increase in the number of Graafian follicles with a concurrent decline in secondary follicles. This phenomenon has also been recorded in *T. australis* (Kitchener & Hudson 1982).

Atretic changes in ovarian follicles followed the general pattern proposed by Ingram (1962) and Guthrie and Jeffers (1938). According to Guthrie and Jeffers (1938), mammalian ovarian follicles display two types of atresia. Type I, in which the granulosa cells degenerate before the oocyte, commonly occurs in secondary and Graafian follicles, while

Type II, in which the oocyte degenerates before the granulosa cells, is prevalent in primary follicles. Atretic secondary and Graafian follicles observed during the present study displayed both types of atresia. In *Mormopterus planiceps*, atretic follicles with cleaving oocytes and maturation spindles were distinct (Crichton & Krutzsch 1987).

In *T. condylura*, as in many tropical species (*Molossus fortis*: Krutzsch & Crichton 1985; *Macrotus californicus*: Crichton & Krutzsch 1990) and some temperate species (*Mormopterus planiceps*: Crichton & Krutzsch 1987), ovaries contain atretic follicles throughout the year. During 1989, *T. condylura* exhibited a significant increase in atretic follicles during the breeding season which parallelled seasonal changes in the total antral follicle population. Two peaks were observed concurrent with ovulation during the two reproductive cycles. Variation in atretic follicle abundance during the second reproductive cycle in 1988 appeared somewhat erratic and difficult to explain, with small sample size a possible cause. *T. australis* displayed no obvious seasonal trends in the abundance of atretic follicles, although highest values were also observed during oestrus and ovulation (Kitchener & Hudson 1982).

The order Chiroptera displays a higher incidence of asymmetry in the female reproductive tract than any other mammalian order (Wimsatt 1975, 1979). Earlier reports suggest that the family Molossidae are characterised by a general homogeneity in the morphology and function of the female reproductive tract (Kitchener & Hudson 1982; Krutzsch & Crichton 1985). The most profound expression of dextral dominance in the female chiropteran reproductive tract is observed in the family Molossidae (Wimsatt 1979). In *T. condylura*, as in all molossids studied to date, this involves a complete dominance of the right ovary and uterus over the left (Sherman 1937; Krutzsch 1955a,b, 1959; Davis *et al* 1962; Stephens 1962; Smithers 1971; Mutere 1973a, b; Wimsatt 1975,

1979; Jerret 1977, 1979; Kitchener and Hudson 1982; Rautenbach 1982; Krutzsch & Crichton 1985; Van der Merwe *et al* 1986).

The left ovaries of most molossids are significantly smaller than the right and are characterised by the absence of advanced follicular stages. As in the female Angolan free-tailed bat, early multi-laminar primary follicles represent the most advanced stage of follicular development (Kitchener & Hudson 1982; Krutzsch & Crichton 1985; Van der Merwe *et.al*. 1986; Crichton & Krutzsch 1987; Rasweiler 1988). The only exceptions in this regard seem to be *Tadarida cynocephala*, with Sherman (1937) reporting vesicular follicles in 12% of the left ovaries examined, and *Tadarida australis*, with one female showing a small vesicular follicle (Kitchener & Hudson 1982).

The morphology of ovarian follicles in *T. condylura* differed in some respects from that described for some other species. The oocyte of large antral follicles was situated peripherally and no distinct corona radiata could be distinguished. These results are similar to that reported for another molossid, *Tadarida australis* (Kitchener & Hudson 1982). In some vespertilionids, the ova are centrally located and are surrounded by a distinct corona radiata (Kitchener & Halse 1978).

In most molossids, young of the year have bilaterally symmetrical reproductive tracts, with the ovaries and uterine horns becoming asymmetrical only when the females reach sexual maturity (Jerret 1979; Mutere 1973b). Female *T. condylura* conformed to this pattern, whereas another molossid, *Tadarida australis*, displayed asymmetry in the juvenile ovaries from after birth (Kitchener & Hudson 1982).

Non-pregnant adult *T. condylura* from Uganda were reported to have bilaterally symmetrical uteri (Mutere 1973b), similar

to that observed in *Tadarida brasiliensis cynocephala* (Stephens 1962) and *Tadarida pumila* (Van der Merwe *et.al* 1986). The present study, however, showed that uterine horns of nulli-parous sexually mature females from the Eastern Transvaal were asymmetrical with the right horn larger and more distended. It is possible that juvenile females in Uganda were mistaken for nulli-parous sexually mature females as in most other molossids, the uterine horns of adult females during reproductive quiescence were also reported to be asymmetrical (Sherman 1937; Krutzsch 1955a, b, 1959; Mutere 1973b; Kashyap 1980; Kitchener & Hudson 1982; Krutzsch & Crichton 1985; Crichton & Krutzsch 1987; Rasweiler 1990).

The morphology of the uterine corpus of *T. condylura* differed from that found in most other molossids (Sherman 1937; Krutzsch 1955a, b; Davis *et al* 1962; Mutere 1973a, b; Jerret 1979; Kitchener and Hudson 1982; Krutzsch & Crichton 1985). In *T. condylura*, the lumina of the two uterine horns remained separated in the corpus, only fusing at the caudal end of the corpus. In *Molossus ater*, the lumina of the uterine horns also remained separated throughout the corpus, only fusing in the cervix (Rasweiler 1990). In most other molossids the two uterine horns opened into the corpus to form a single corpus lumen (Sherman 1937; Stephens 1962; Kitchener & Hudson 1982; Krutzsch & Crichton 1985; Crichton & Krutzsch 1987).

Oviductal passage in mammals vary considerably and range from three to four days in most mammals, four to seven days in some carnivores, and as long as 14-16 days in some bats (Wimsatt 1975). In bats, the oviduct plays a supportive role in the earlier development of the embryo, whereas in some other mammals this is suggested to happen in the uterus (Wimsatt 1975). In *M. ater*, oviductal passage was believed to be less than 5 days (Rasweiler 1990). In *Myotis lucifugus*, passage of the embryo into the uterus occurred within 48 hours (Rasweiler 1990).

The duration of pre-implantation development in molossids correspond to that observed in *T. condylura*, in which implantation was estimated to occur 14-16 days after conception. Implantation in *T australis* and *T. aegyptiaca* occurring 14 days and 10-15 days after conception, respectively (Kashyap 1980; Kitchener & Hudson 1982).

In most chiropterans, as in *T. condylura* and most other molossid species, endometrial hypertrophy occurs in both uterine horns (Stephens 1962). In this respect, *Tadarida brasiliensis cynocephala* appears to be an exception, as only the right cornu is affected during proestrus and oestrus (Stephens 1962).

The embryos of *T. condylura* entered the uterus at the morula stage. Similar observations were reported for *T. brasiliensis* (Sherman 1937; Stephens 1962) and *Molossus ater* (Rasweiler 1990). Data collected during the present study suggested that the zona pellucida in *T. condylura* was shed in the oviduct, resulting in a naked morula entering the uterus. This contradicts earlier reports which indicate that the zona pellucida in most Chiroptera (Rasweiler 1979) and particularly in molossids (Rasweiler 1990; Sherman 1937; Stephens 1962) are lost only in the uterus. Rasweiler (1979) has, however, suggested considerable diversity within the order and also variation within certain species. In *Vespertilio murinus, Peropteryx kappleri, Rousettus amplexicaudatus* and *Desmodus rotundus*, Rasweiler (1979) found evidence to suggest that the shedding of the zona pellucida is inconsistent and could occur either in the oviduct or in the uterus.

Implantation in molossids invariably occurs in the right uterine horn. The only records of implantation in the left uterine horn was reported to be in *Tadarida midas* (Smithers 1971) and *Tadarida cynocephala* (Sherman 1937).

Previous reports on implantation in molossids differ in some respects from observations made during the present study. Initial attachment of the trophoblast is antimesometrial or lateral in *Molossus rufus* (Rasweiler 1979) and *M. ater* (Rasweiler 1990), and circumferential in *Tadarida brasiliensis cynocephala* (Stephens 1962). *T. condylura* is the first molossid in which initial attachment has been found to occur mesometrially. As far as the orientation of the inner cell mass is concerned, *T. condylura* conforms to the general molossid pattern which depicts an antimesometrial orientation of the inner cell mass during implantation (Wimsatt 1975; Rasweiler 1979; Crichton & Krutzsch 1987; Rasweiler 1990). In all molossids the depth of implantation is superficial (Sherman 1937; Krutzsch 1955a; Davis *et al* 1962; Stephens 1962; Wimsatt 1975; Jerret 1979; Rasweiler 1979; Kitchener & Hudson 1982; Krutzsch & Crichton 1985; Rasweiler 1990). In *Molossus ater*, initial orientation of the inner cell mass was lateral, but this changed to antimesometrial shortly after implantation was initiated (Rasweiler 1990).

One outstanding feature of embryonic development in molossids, as in *T. condylura*, is that the embryonic disc is located opposite the discoidal placenta (Stephens 1962). In all other chiropterans studied to date, the definitive chorio-allantoic placenta develops dorsal to the embryonic shield (Stephens 1962; Gopalakrishna & Karim 1979; Rasweiler 1990). At present, it appears that molossids are the only chiropterans in which the definitive placenta and embryonic disc are located at opposite poles of the blastodermic vesicle.

With regard to the invasion of the endometrial stroma by the trophoblast, different groups of mammals display different patterns (Wimsatt 1975). In some mammals, including the bats, localised areas of trophoblastic proliferation and invasion are found which correspond to the distribution of subepithelial capillaries in such a manner that the columns of invasive cells are aimed at these vessels (Wimsatt 1975).

Localised invasion of the stroma by the trophoblast has also been found in *T. condylura*, resulting in broad columns of trophoblast cells invading the stroma.

The foetal growth curve obtained during this study conformed to the general pattern characteristic of non-hibernating mammals (Hugget & Widas 1951; Frazer & Hugget 1974; Van der Merwe 1979; Van der Merwe *et al* 1986). It should be noted that a t_o value of 0.25 was used in calculating the foetal growth curve and in extrapolating the conception and parturition dates in favour of the t_o value of 0.3 suggested by Hugget and Widdas (1951) for a gestation period of 50-100 days.

The drop in neonatal body mass immediately after birth found during the present study appears to be the norm among bats (Kurta & Kunz 1987). This has been attributed to the shock the neonate experiences during birth and the loss of body water through evaporation immediately after birth (Kurta & Kunz 1987). Like most other bats, *T. condylura* gives birth to relatively large young. Neonatal mass in *T. condylura*, *T. brasiliensis* (Davis *et al* 1962), *Molossus molossus* (Häusler, Möller & Schmidt 1981) and *M. ater* (Rasweiler 1990) averaged 21%, 23.6%, 23.2% and 20.6% of maternal body mass, respectively. These values are close to the average for chiropterans: 22.3% (range 12-43%; Kurta & Kunz 1987). In non-volant mammals, neonates averaged only 7.8% of maternal body mass (Kurta & Kunz 1987). Kurta and Kunz (1987) proposed that bats are unusual in that despite small litter sizes, large neonatal sizes and long gestation periods, they are altricial at birth.

5. REPRODUCTION IN THE MALE

5.1 INTRODUCTION

Reproductive patterns in bats are largely dependent on whether or not a species hibernates. True hibernators are restricted to temperate regions where their reproductive patterns have become adapted to the unfavourable conditions enabling them to make optimal use of the short summer season. Male reproductive patterns of hibernating species are characterised by an asynchronous, seasonal reactivation of the primary and accessory sexual organs, resulting in a temporal separation of primary and secondary reproductive functions (Gustafson 1979). Males of these species display two different reproductive patterns based on the timing and duration of the different reproductive stages in relation to hibernation. These patterns correspond to the general patterns employed by the respective females (Gustafson 1979).

The male reproductive pattern of Old and New World hibernating rhinolophid and vespertilionid bats, with the exception of the vespertilionid genus *Miniopterus*, is relatively well understood and is characterised by an asynchronous renewal of testicular and accessory gland cycles. In these species, spermatogenesis is initiated in early summer and reaches a peak in late summer and early autumn, after which the testes regress rapidly to the prepubertal state. Spermatozoa are stored in the epididymides throughout winter and the epididymides and accessory glands remain fully developed until spring. First copulations occur in autumn prior to hibernation and spermatozoa are stored in the uterus during winter, although additional copulations occur during intermittent arousals. The female pattern is characterised by delayed ovulation, with ovulations occurring shortly after emergence from hibernation in spring, followed by normal embryonic development. Sperm storage by both sexes and

delayed ovulation displayed by these species are closely associated with reduced metabolic levels during hibernation. Leydig cell activity closely follows that of the testes. Leydig cells become involuted prior to hibernation, followed by an increase in size and activity shortly after spring arousal (Krutzsch 1975; Gustafson 1979, 1987; Krutzsch & Crichton 1986).

In Old and New World temperate vespertilionids of the genus *Miniopterus*, the female pattern is characterised by delayed implantation. Spermatogenesis is initiated in late spring, reaches a peak in late summer, and the testes regress before winter. Mating and conception are concluded in late autumn, before initiation of hibernation. Leydig cells and accessory glands, in synchrony with spermatogenic activity, are active during summer and autumn, becoming atrophied in late autumn (Gustafson 1979; Krutzsch & Crichton 1990b).

Non-hibernating species, found in tropical and sub- tropical regions, display reproductive patterns which are characterised by synchronised male and female reproductive processes. Spermatogenic and accessory gland activity are most often synchronised with the onset of the oestrous cycle in the female. Spermatogenesis and oogenesis on the one hand, and copulation, ovulation and conception on the other hand are contemporary events (Krutzsch 1979; Van der Merwe *et al* 1986).

Male reproductive patterns in non-hibernating species correspond with the three different patterns observed in females:

(i) Males of species in which females display aseasonal or seasonal polyoestry, often show continuous spermatogenesis and accessory gland hypertrophy, resulting in reproductive activity and viable matings throughout the year. Such males are regarded as aseasonal breeders (Krutzsch 1979).

(ii) Bimodally seasonal breeders demonstrate two definite breeding peaks annually, although they sometimes display spermatogenic activity throughout the year (Krutzsch 1979).

(iii) The reproductive pattern of unimodal seasonal breeders conform to the female pattern of seasonal monoestry. Spermatogenic activity is restricted to a certain time of the year, after which complete involution of the primary reproductive organs occur (Krutzsch 1979).

Very little information regarding reproduction in male molossid bats is available. A literature survey on male reproduction revealed that quantitative information is available on only nine species of molossids, of which five are temperate species, one is a New World tropical species and the remaining three are Old World tropical species (Table 5.1).

Throughout their almost worldwide distribution, male molossid bats display remarkable morphological stability in the arrangement of the male reproductive tract. The limited data available suggest that there are very few variations other than size in the morphological details of their primary and secondary reproductive organs. Usually only minor differences exist in the size and composition of the accessory glands and in the presence of secondary male sexual characteristics (Sherman 1937; Krutzsch 1955a, b, 1979; Mutere 1973b; Krutzsch & Crichton 1987, 1990a).

Molossid bats do however display a large variation in the duration and composition of the reproductive pattern which seems to be correlated in part with geographic location (Sherman 1937; Krutzsch 1955a, b, 1979; Marshal & Corbet 1959; Mutere 1973a, b; Van der Merwe *et.al.* 1986; Krutzsch & Crichton 1987, 1990a; Happold & Happold 1989). Due to the

TABLE 5.1: Male reproductive patterns of the family Molossidae found throughout the world.

PATTERN	SPECIES	REFERENCE
	NEW WORLD - TEMPERATE SPECIES	
U.S.	*Tadarida brasiliensis mexicana*	Davis, Herreid & Short 1962
"	*Tadarida b. cynocephala*	Sherman 1937
"	*Eumops perotis californicus*	Krutzsch 1955b
"	*Molossus sinaloae*	Heideman, Erickson & Bowles 1990
	OLD WORLD - TEMPERATE SPECIES	
U.S.	*Mormopterus planiceps*	Krutzsch & Crichton 1987
	NEW WORLD - TROPICAL SPECIES	
A.C.	*Molossus fortis*	Krutzsch & Crichton 1990a
	OLD WORLD - TROPICAL SPECIES	
U.S.	*Otomops martienseni*	Mutere 1973a
B.S.	*Tadarida condylura*	Happold & Happold 1989; Mutere 1973b
A.C.	*Tadarida pumila*	Marshall & Corbet 1959; Mutere 1973b

U.S. = Unimodal Seasonal breeder

B.S. = Bimodal Seasonal breeder

A.C. = Aseasonal Continuous breeder

restrictive environmental conditions that prevail in temperate regions, molossid bats from these regions are mostly seasonal monoestrus breeders which sometimes display typical vespertilionid patterns with spermatozoa being stored during winter (Table 5.1) (Krutzsch & Crichton 1987). However, some molossids display patterns characterised by testicular recrudescence in autumn with maximal sperm production, copulation and ovulation occurring in late winter/early spring, followed by parturition in summer. New and Old World tropical molossid species display a variety of reproductive patterns ranging from seasonal monoestry to aseasonal polyoestry (Table 5.1).

Very little information regarding reproduction in male *T. condylura* is available. The only two studies dealing with reproduction in this species were merely based on general and macroscopical observations (Happold & Happold 1989; Mutere 1973b). In Uganda, Mutere (1973b) showed that female *T. condylura* are bimodally seasonal breeders, with males showing reproductive activity throughout the year. In Malawi, male *T. condylura* were found to be seasonal breeders (Happold & Happold 1989). There is, however, no information available on the histology of reproduction in this species.

5.2 RESULTS

5.2.1. MORPHOLOGY OF THE TESTIS AND EPIDIDYMIS

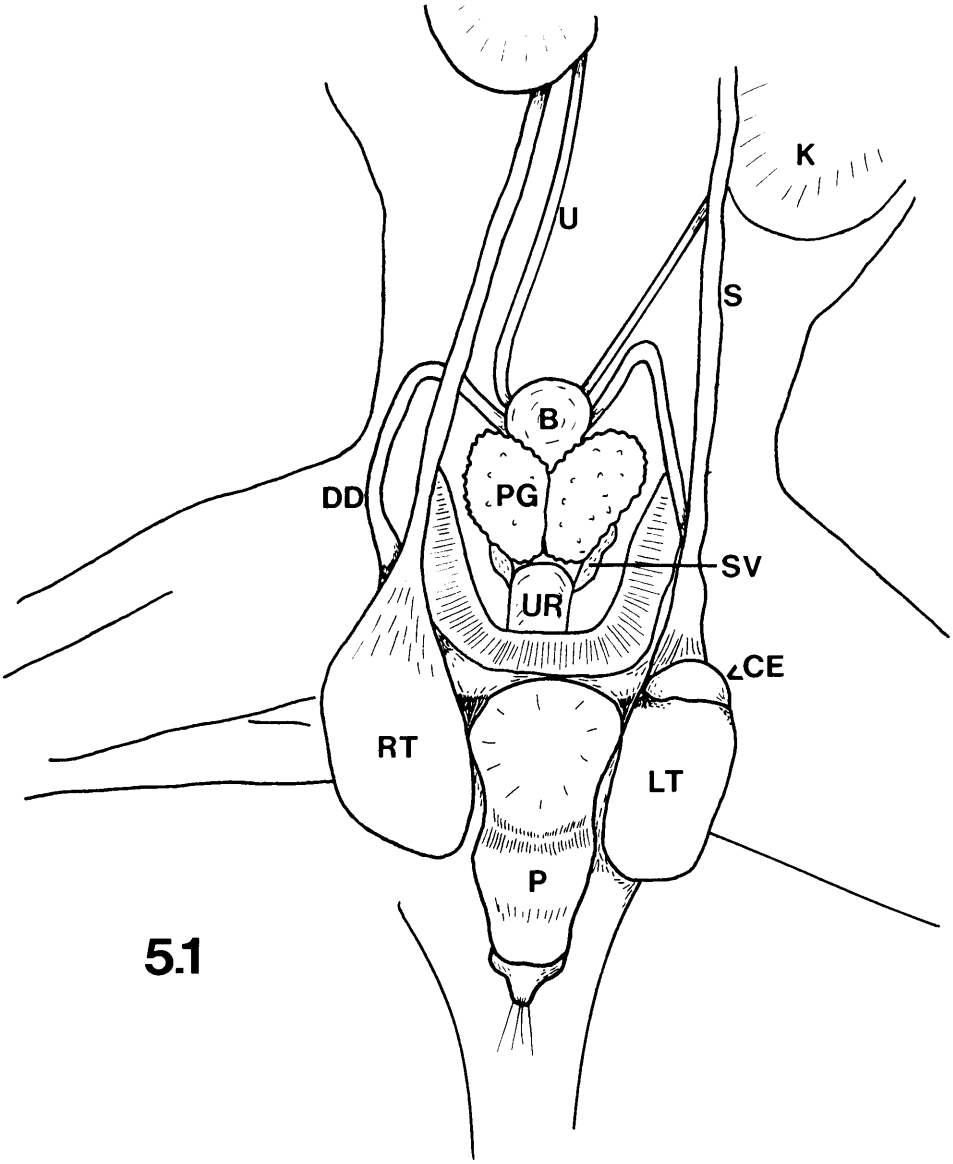
The gross anatomy of the testis and epididymis resembles that of most other microchiropteran species (Krutzsch 1955a, b, 1979; Krutzsch & Crichton 1987, 1990a). The testes are paired elongated, ovoid organs which are situated just beneath the skin on either side of the anus and are enclosed by a non-pigmented sacculation on the medial ventral surface of the thigh (Figures. 5.1 & 5.2). This pouch, which is formed by the fascia obliquus abdominus externus, also surrounds the epididymis and the part of the ductus deferens not enclosed in the inguinal canal. The pouch enlarges during the reproductive season to accommodate the enlarged testes and epididymides.

The epididymis consists of three distinct sections, the caput, corpus and cauda epididymis (Figure 5.3). The caput epididymis is attached to the cephalic curvature of the testis. The caput epididymis is connected to the cauda epididymis through the corpus epididymis, which lies on the medio-ventral surface of the testis. The cauda epididymis is appended to the caudal curvature of the testis. The ductus deferens, which arises from the cauda epididymis, extends across the medial-ventral side of the testis and enters the abdominal cavity through the inquinal canal as part of the spermatic cord (Figure 5.1 & 5.2).

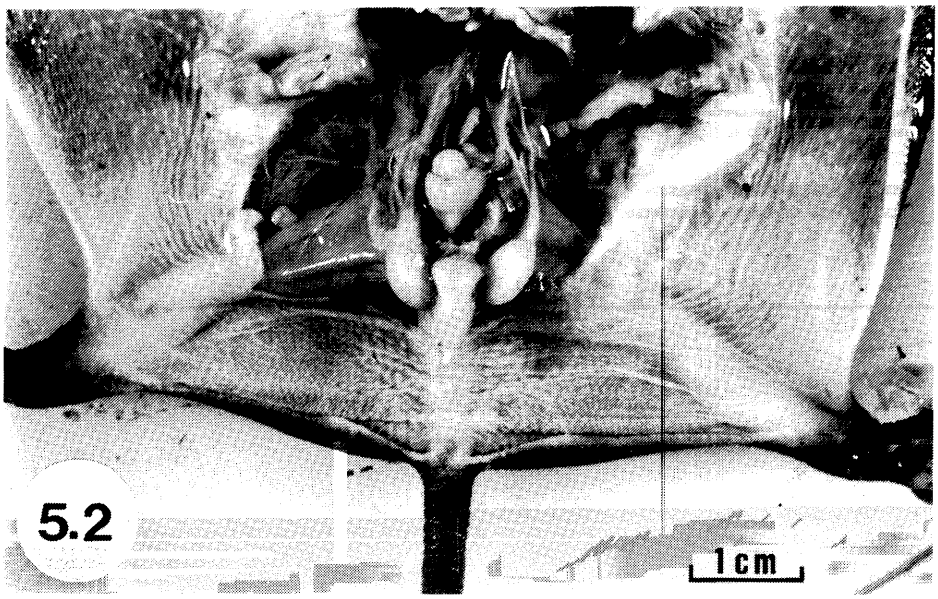
The testis is enclosed in a thick fibrous capsule, the tunica albuginea (Figure 5.4). On the medial-cephalic aspect, the mediastinum testis projects into the testis forming thin fibrous septa, the septula testis, which divide the testis into pyramidal segments, the lobuli testis (Figure 5.3). Each lobule comprises a number of highly tortuous seminiferous tubules. At the apex of each lobule, the seminiferous tubules pass into a short straight section, the tubuli recti, which

Figure 5.1. Line drawing of the reproductive tract of the male *T. condylura* (ventral view). Illustrated are: Urinary bladder (B); Caput epididymis (CE); Ductus deferens (DD); Kidney (K); Left testis (LT); Penis (P); Prostate gland (PG); Right testis (RT); Suspensory ligament (S); Seminal vesicle (SV); Ureter (U); Urethra (UR).

Figure 5.2. Photomicrograph showing the male reproductive system of the specimen illustrated in Figure 5.1. For labels, see Figure 5.1.

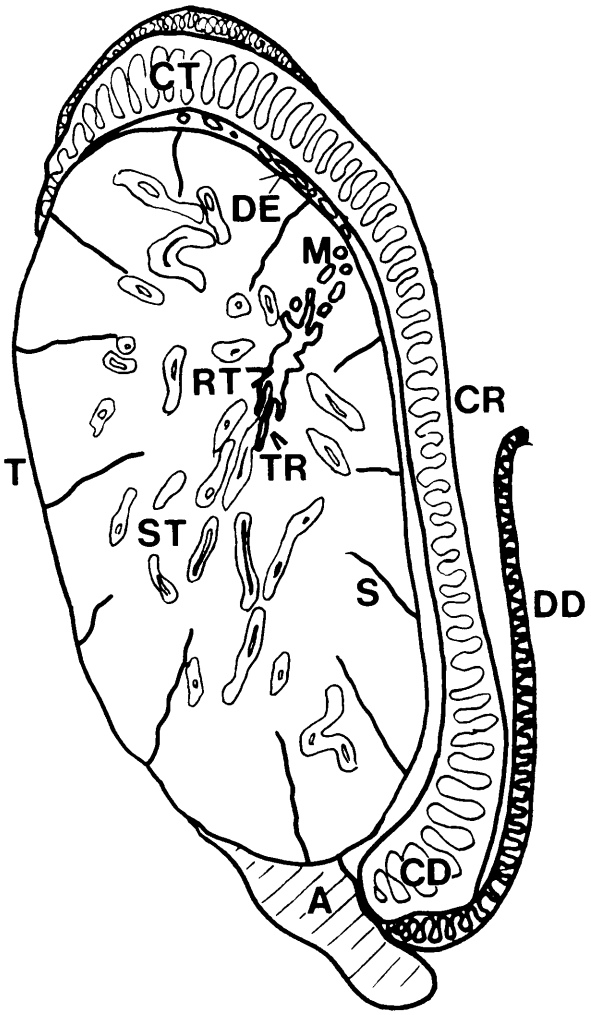


5.1



5.2

Figure 5.3. Line drawing of the testis showing: Adipose tissue (A); Cauda epididymis (CD); Caput epididymis (CT); Corpus epididymis (CR); Ductus deferens (DD); Ductus efferentes (DE); Mediastinum (M); Rete testis (RT); Septula testis (S); Seminiferous tubule (ST); Tunica albuginea (T); Tubuli rectis (TR).



then opens into the rete testis, a system of epithelium lined spaces in the mediastinum. From the rete testis, 3-4 convoluted ductuli efferentes extend cephalad towards the caput epididymis where they fuse to form the highly tortuous ductus epididymis. On the inside of the tunica albuginea, a layer of loose connective tissue, the tunica vasculosa testis, extends radially inwards forming strands of tissue which fill all the interstitial spaces among the seminiferous tubules (Figure 5.4). These areas between the seminiferous tubules also contain clusters of interstitial cells, called the Leydig cells (Figure. 5.4).

The testis is surrounded by a serous cavity, the tunica vaginalis propria testis, which consists of an outer parietal and inner visceral layer (Figure 5.4). The visceral layer is closely applied to the tunica albuginea on the cephalic and lateral side of the testis. On the cephalic side, the cavity covers the greater part of the caput epididymis. On the caudal side, it only extends for a short distance between the cauda epididymis and the caudal curvature of the testis (Figure 5.4). The tunica vaginalis propria testis is an outpocketing of the peritoneum that extends through the inguinal canal, facilitating movement of the testis from a scrotal to an abdominal position.

The right and left testes are almost similar in size with the right testis (40.7mg, n=8) slightly but not significantly larger than the left (39.5mg, n=8). Histological examination showed both testes to be functionally equivalent, becoming spermatogenically active and regressing simultaneously.

5.2.2 HISTOLOGY OF THE TESTIS AND EPIDIDYMIS

The histological structure of the testis and epididymis of *T. condylura* is, to a large extent, similar to that of other mammalian species. There are, however, some small differences

in the cytological organisation and structure in some parts of the testis and epididymis.

5.2.2.1 SEMINIFEROUS TUBULES

The seminiferous tubules were lined with spermatogenic epithelium which contained two cell types, the supporting cells or Sertoli cells, and the spermatogenic cells (Figures 5.5 to 5.7). Sertoli cells were large columnar or triangular shaped cells attached to the basal lamina. During spermiogenesis, developing spermatozoa were attached in bundles to the luminal ends of the Sertoli cells. Sertoli cells had ovoid to angular nuclei, each of which contained a single ovoid nucleolus.

Spermatogenic cells constituted several layers of cells throughout the year and included a number of morphologically distinguishable types (Figures 5.5 to 5.7):

(i) Spermatogonia: Three types of spermatogonia were recognised on the basis of nucleus shape: type A (pale), type A (dark) and type B spermatogonia. Type A (pale) spermatogonia, the most common during all reproductive stages, had ellipsoid nuclei with very fine chromatin granules giving the cell a characteristic pale appearance (Figure 5.5). Type A (dark) spermatogonia had spherical to ovoid nuclei with dark-staining smooth chromatin (Figure 5.6). Nuclear vacuoles, sometimes characteristic of these cells, were not observed. Type B spermatogonia had spherical nuclei containing dark-staining, sometimes patchy, coarse chromatin granules with a centrally located nucleolus (Figure 5.5).

(ii) Primary spermatocytes: Four morphologically different types of primary spermatocytes were distinguished on the basis of their nuclei characteristics: preleptotene, leptotene, zygotene and pachytene primary spermatocytes. Preleptotene primary spermatocytes had small spherical

Figure 5.4. Section of the testis showing the tunica vaginalis propria testis (dark arrow-head), the tunica vaginalis (dark double arrow-head) surrounding the testis and the tunica vasculare testis (white arrow-head) projecting strands of interstitial tissue between the seminiferous tubules (ST). Leydig cells can be seen in the interstitial spaces (double white arrow-head). The cauda epididymis (C) is indicated on the left. x100.

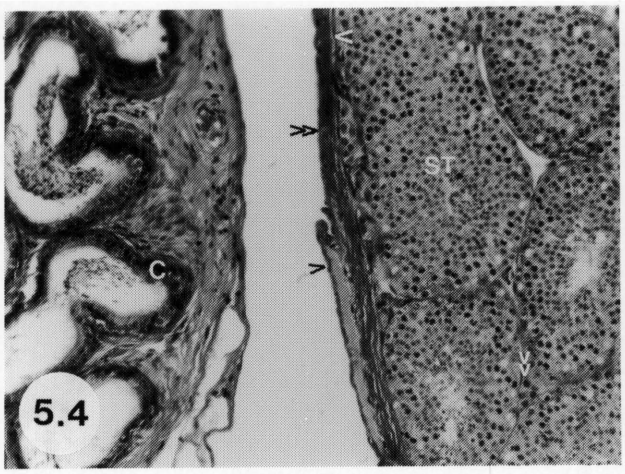
Figures 5.5. Seminiferous tubules showing germinal epithelium and interstitial tissue between tubules. Sertoli cells (S); Type B spermatogonia (B); Leptotene primary spermatocytes (L); Pachytene primary spermatocytes (P); Round spermatids (RS); Elongating spermatids (ES); Spermatozoa (SP); Fibroblasts (F); x400.

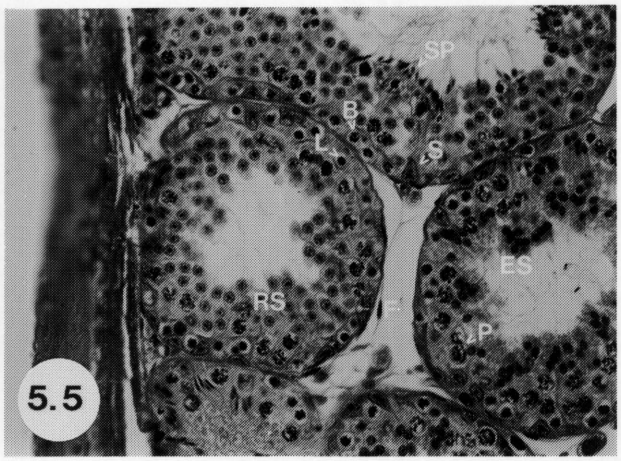
Figure 5.6. Seminiferous tubules showing germinal epithelium and interstitial tissue. Pale Type A spermatogonia (AP); Dark Type A spermatogonia (AD); Zygotene primary spermatocytes (Z); Leydig cells (LC); Blood vessels (BV). x400.

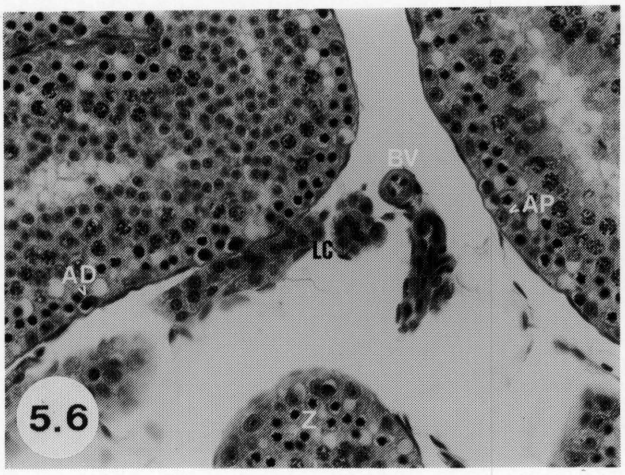
Figure 5.7. Seminiferous tubule showing meiosis. Secondary spermatocytes (SS); Meiotic bodies (M); Meiotic spindle (arrow-head); Round spermatids (RS). x400.

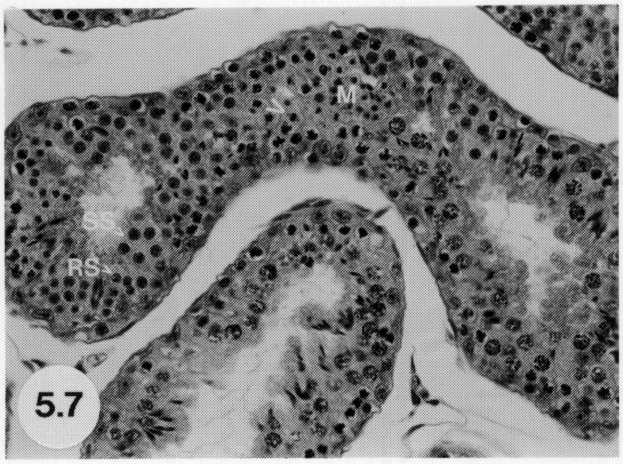
Figure 5.8. Terminal end of seminiferous tubules (ST) showing the disappearance of the germinal epithelium (G) with the tubuli recti (T) lined with Sertoli cells (arrow-head) only. The tubuli recti pass over into the rete testis (R) which is lined with cuboidal epithelium (double arrow-head). x400.

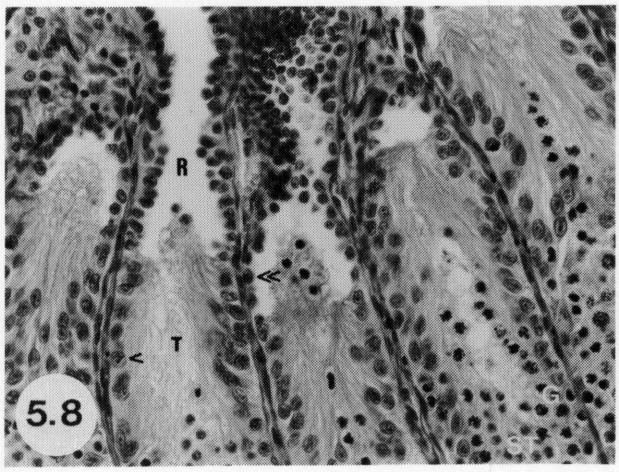
Figure 5.9. Ductus efferentes (D) with low columnar epithelium (arrow-head). Seminiferous tubule (ST); Tunica vaginalis (T); Adipose tissue (A). x400.

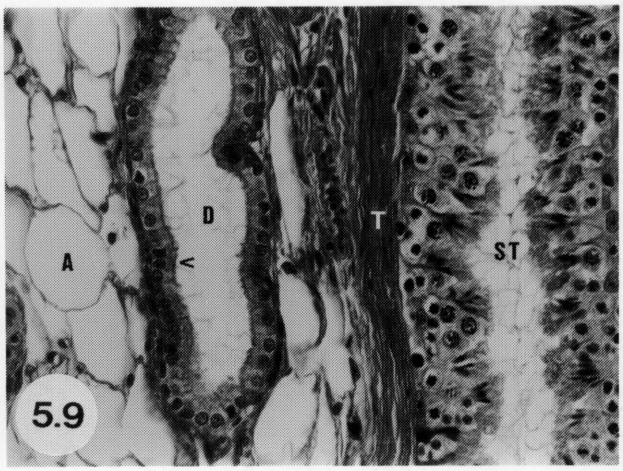












nuclei with dark patchy chromatin and no nucleoli. Leptotene primary spermatocytes had small, spherical, dark-staining nuclei with smooth, dense chromatin (Figure 5.5). Zygotene primary spermatocytes had spherical, very dark-staining nuclei with dense and flaky chromatin, giving the nuclei a characteristic spiky appearance (Figure 5.6). Pachytene spermatocytes had large spherical nuclei clearly showing the threadlike chromosomal strands typical of this stage (Figure 5.5). These last two types were very common during most stages of the spermatogenic cycle.

(iii) Secondary spermatocytes: These cells were observed very infrequently as they were short-lived, appearing only briefly during spermatogenesis. They had spherical nuclei with pale-staining cytoplasm, although chromatin granules were visible. They also appeared slightly larger than spermatids and were primarily associated with meiotic figures (Figure 5.7).

(iv) Spermatids: Round and elongated spermatids occurred in large concentrations during the entire breeding season. Round spermatids had small, round, pale- staining nuclei, often associated in groups (Figures 5.5 & 5.7).

(v) Spermatozoa: As a result of the staining technique used, very little detail of spermiogenesis could be distinguished. Developing spermatozoa were present at all times during the breeding season (Figure 5.5).

5.2.2.2 INTERSTITIAL TISSUE

Leydig cells, located in the intertubular spaces between seminiferous tubules, showed a considerable variation in size and morphology concurrent with fluctuating spermatogenic activity. They became enlarged during the breeding season with the nuclei changing from small, irregularly shaped bodies with dense chromatin during the period of spermato-

genic inactivity to large, spherical nuclei with light-staining, irregularly spaced chromatin during the breeding season. Their nuclei contained one or two prominent nucleoli. As in most microchiropterans, Leydig cells in *T. condylura* were abundant and occurred in clusters of varying size (Figure 5.6). In addition to the Leydig cells, the interstitial spaces contained fibroblasts, small bundles of collagen fibres, occasional macrophages and blood and lymph vessels (Figure 5.6).

5.2.2.3. EXCRETORY DUCTS

Germ cells disappeared from the seminiferous tubules at the apex of the lobuli testis, with the short terminal section of each seminiferous tubule lined by Sertoli cells only (Figure 5.8). The seminiferous tubules passed into the tubuli recti where the Sertoli cells were replaced by simple cuboidal epithelium, which lined both the tubuli recti and the rete testis (Figure 5.8). The epithelium of the ductuli efferentes, a system of tubules connecting the seminiferous tubules to the epididymis, consisted of low columnar cells. Two types of cells were recognised, viz ciliated and non-ciliated cells. This epithelium showed a smooth outline with most cells of equal height (Figure 5.9).

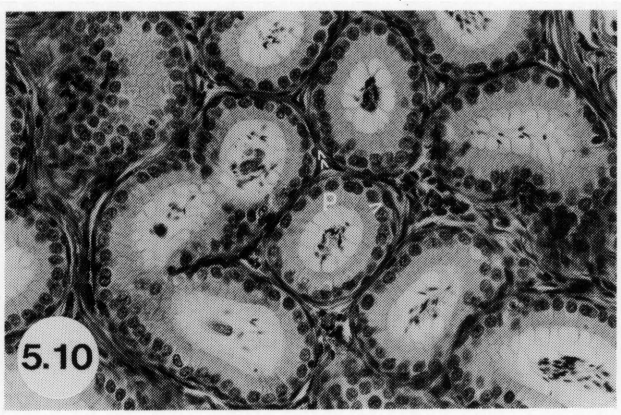
5.2.2.4. EPIDIDYMIS

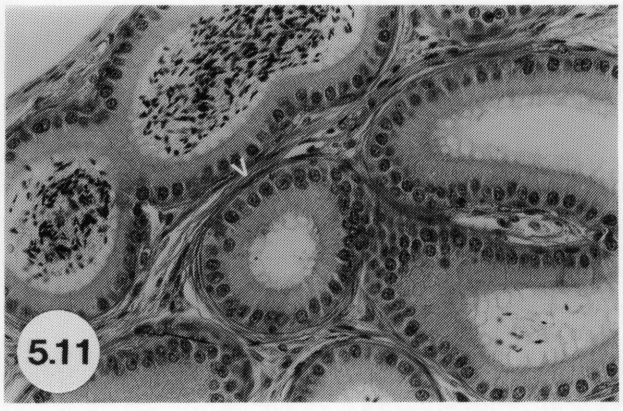
The ductuli efferentes joined to form a single convoluted ductus epididymis. The epididymis was lined by pseudostratified columnar epithelium which showed a gradual, proximo-distal decrease in height. The cauda epididymis contained low columnar epithelial cells (Figure 5.4), whereas the epithelium of the caput epididymis consisted of tall columnar cells (Figure 5.10). Two types of epithelial cells were distinguished, viz principal cells and basal cells (Figure 5.10). Principal cells bore tufts of long stereocilia. During the breeding season, their nuclei were located predominantly

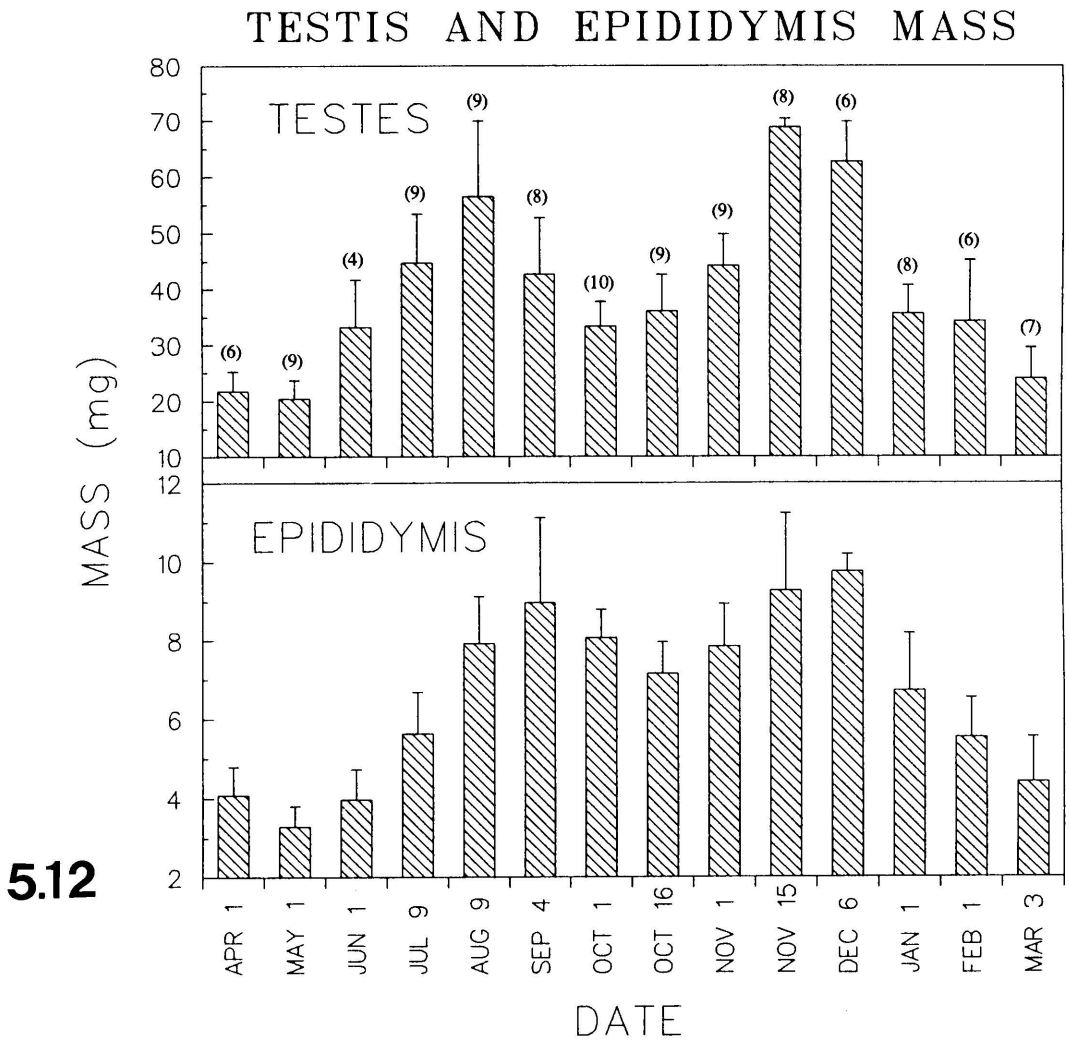
Figure 5.10. Caput epididymis showing the tall columnar epitelium with principal cells (P), basal cells (arrow-head) and thin muscularis (double arrow-head). x 400.

Figure 5.11. Distal part of the cauda epididymis showing the thick muscularis (arrow-head). x400.

Figure 5.12. Mean testis and epididymis mass (± 1 SD) in male *T. condylura* (sample sizes indicated in brackets; sample sizes for epididymis mass as indicated for testis mass).







5.12

at the base of the cell, whereas during spermatogenic quiescence, the nuclei were more centrally located. Basal cells were small angular or pyramidal shaped cells lodged between the bases of the principal cells.

The ductus epididymis was surrounded by smooth muscle tissue which showed a gradual proximodistal increase in thickness. In the caput epididymis, the cells were long and slender and constituted only one circumferential layer (Figure 5.10), but where the corpus epididymis transcended to the cauda epididymis, larger smooth muscle cells appeared. In the distal part of the cauda epididymis, the two-layered muscle coat changed to a characteristic, rough three-layered coat (Figure 5.11).

5.2.3 MALE REPRODUCTIVE PATTERN

5.2.3.1 REPRODUCTIVE PATTERN

The male *T. condylura* in the Eastern Transvaal is a bimodally seasonal breeder displaying a nine month breeding season extending from early June to February, closely following the bimodal pattern of reproductive activity displayed by the female. The reproductive pattern is characterised by testicular recrudescence in late May/early June, followed by two definite peaks in activity in August/early September and November/early December. Gradual testicular involution occurred from late December to February, with the testes reaching baseline activity in March.

5.2.3.2. SEASONAL CHANGES IN TESTIS AND EPIDIDYMIS MASS

Statistical analysis (One-way ANOVA) of the data on testis and epididymis mass collected during 1989 showed a definite bimodal seasonal pattern (Figure 5.12). The testis remained

involuted from early March to May, reaching its minimal size during early May (20.3 ± 1.1 mg). Recrudescence began in May and the testis showed a rapid increase in size from late May through June and July, resulting in a peak during mid-August (54.2 ± 4.8 mg). Testis mass then decreased during September and early October (33.4 ± 1.4 mg), but again increased significantly through October and early November (44.1 ± 1.9 mg, $P<0.02$, d.f. = 16), resulting in a second peak during mid-November (69.7 ± 5.3 mg, $P<0.001$, d.f. = 15). Testicular involution began during December, with the testis decreasing significantly in size through early January (35.6 ± 1.9 mg, $P<0.02$, d.f. = 11), reaching baseline values during March (23.9 ± 2.9 mg).

Although showing seasonal changes in size similar to that of the testis, the epididymis only attained maximum proportions about 3 weeks after the testis (Figure 5.12). This was because young spermatozoa were only released into the epididymis at an advanced stage of development. The epididymis remained involuted from February to early June, reaching its minimal size in early May (3.3 ± 0.2 mg). Recrudescence started in June with the epididymis increasing significantly in size during June (5.6 ± 0.4 mg, $0.05>P$, d.f. = 11) and August (7.6 ± 0.4 mg, $P<0.001$, d.f. = 16), reaching a peak in early September (10.1 ± 0.4 mg, $P<0.002$, d.f. = 18). The epididymis then showed a decrease in mass during late September through mid-October (7.1 ± 0.3 mg, $0.05>P$, d.f. = 17). Renewed recrudescence began during late October, resulting in a second peak in early December (9.8 ± 0.2 mg). This was followed by a rapid decrease in mass during December and January.

5.2.3.3 SHORT TERM MOVEMENTS OF THE TESTIS

In all ten males used in the experiment, it was found that both testes had changed from a scrotal to an abdominal position over a 24 hour period. This was also observed in

Figure 5.13. Seminiferous tubules in May, showing abundant spermatids. x200.

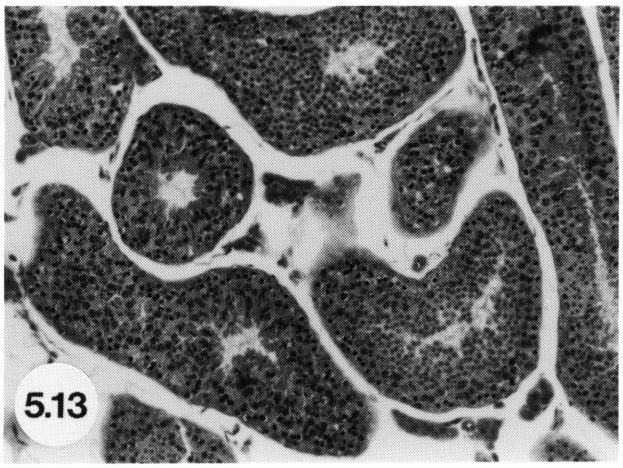
Figure 5.14. Seminiferous tubules in July, showing abundant spermatids and clusters of developing spermatozoa attached to Sertoli cells (arrow-head). x200.

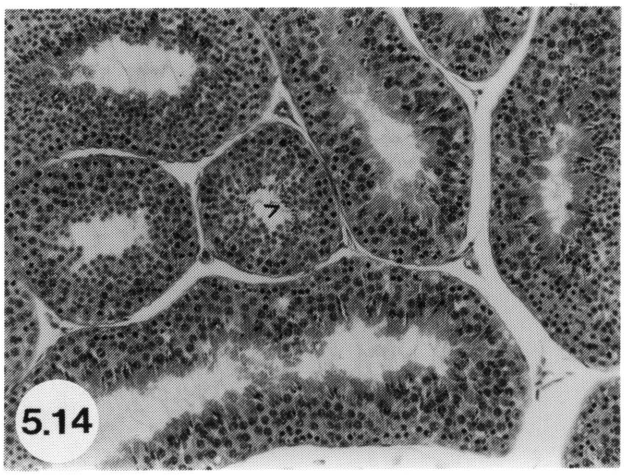
Figure 5.15. Seminiferous tubules in August showing peak spermatogenic activity with premature spermatozoa clustered around the lumen (arrow-head). x200.

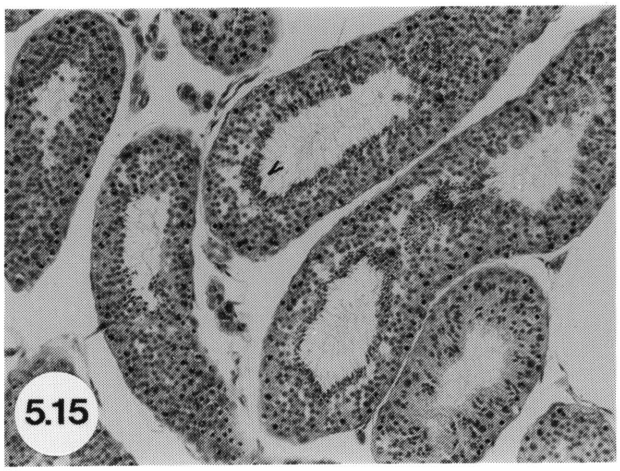
Figure 5.16. Testis and epididymis in August with the caput epididymis (CP) filled to capacity with spermatozoa. x40.

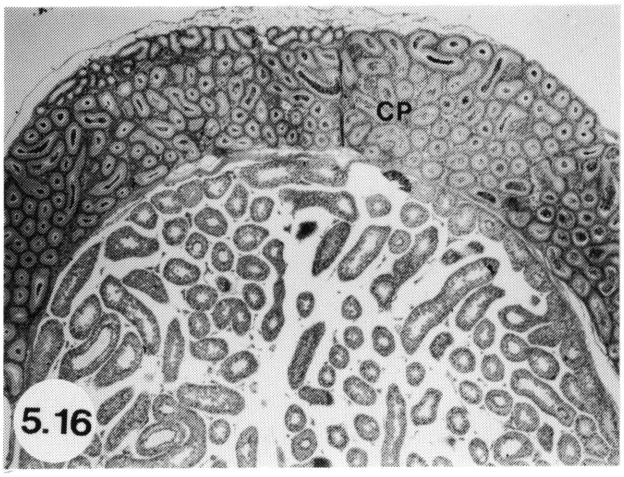
Figure 5.17. Testis in February still showing many spermatids and developing spermatozoa although spermatogenic activity had decreased considerably. x200.

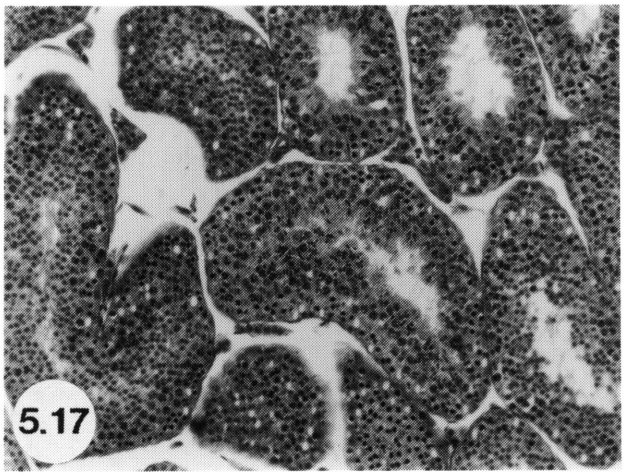
Figure 5.18. Testis of a specimen collected during March displaying very low spermatogenic activity, although round spermatids (RS) and meiotic figures (M) were still observed. The Leydig cells (LC) are small with little cytoplasm and small, tightly packed nuclei. x400.

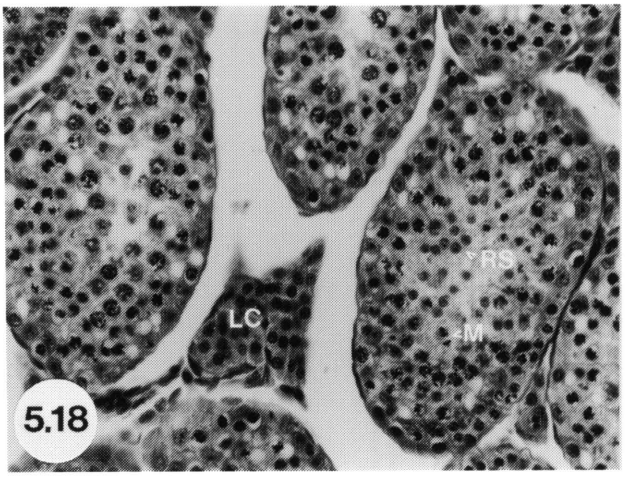












another male collected during mid-October, in which both testes were scrotal when captured, but became abdominal about one minute after capture. The testes of males collected during the breeding season and examined within six hours after capture, were invariably found to be in the scrotal position (100%, n = 30). In males examined after six hours in captivity 43% of the left testes and 53% (n = 30) of the right testes had become abdominal.

5.2.3.4. CYCLIC CHANGES IN TESTIS POSITION

Histological examination of the testes indicated considerable variation in the regression of the testes during spermatogenic quiescence. Testes which were inactive or which showed little spermatogenic activity, were invariably abdominal, whereas testes that showed little regression and remained active, were found to be scrotal. Adult males examined during early April were all found to be spermatogenically inactive, and their testes were all abdominal (100%, n = 10). The testes of one specimen examined during early March were scrotal and contained large numbers of developing spermatozoa. The remaining males from the same sample all had inactive, abdominal testes. In juvenile bats, the testes were invariably abdominal.

5.2.4 SPERMATOGENESIS

5.2.4.1. SPERMATOGENIC ACTIVITY

Throughout the year, the testes of all specimens examined showed abundant type A and B spermatogonia and primary spermatocytes. During the period of spermatogenic quiescence, i.e. from February to the end of May, seminiferous tubules were characterised by abundant mitotic and meiotic activity, spermatogonia and primary spermatocytes, particularly zygotene and pachytene stages. Some specimens also showed abundant round spermatids.

Spermatogenic activity in males from both localities began to increase in late May, during which time most testes were characterised by increasing numbers of primary spermatocytes, abundant round and elongating spermatids, and some premature spermatozoa attached to Sertoli cells (Figure 5.13). Some specimens already showed a few spermatozoa in the lumen of the seminiferous tubules and the caput epididymis, with the corpus and cauda epididymis still empty.

In early July, seminiferous tubules were dominated by round and elongating spermatids forming columns often consisting of six to seven layers of cells. Large numbers of developing spermatozoa were attached in distinct clusters to the luminal ends of Sertoli cells (Figure 5.14), while many spermatozoa were also present in the lumen of some seminiferous tubules and in the caput epididymis. In some males, spermatozoa were also present in the corpus and cauda epididymis.

Spermatogenic activity reached a peak in August. Seminiferous tubules were packed with spermatozoa, attached to Sertoli cells, around the edge of the lumen (Figure 5.15). Spermatids were still present in large numbers, with the majority undergoing spermiogenesis. Primary spermatocytes had decreased in number relative to the number of spermatids. The caput epididymis had become satiated with spermatozoa (Figure 5.16), whereas the corpus and cauda epididymis were still relatively empty (Figure 5.16).

During early September, seminiferous tubules were filled with spermatozoa. Most spermatids were undergoing spermiogenesis and the epididymis had become completely filled with spermatozoa.

During October, seminiferous tubules were characterised by a reduction in spermatogenic activity, showing a decrease in the numbers of developing spermatozoa. Abundant round sperma-

tids were still observed, many of which were undergoing spermiogenesis. The majority of spermatozoa were clustered around the lumen of the seminiferous tubules. Although the epididymides were still filled with spermatozoa, a marked decrease in sperm abundance was observed in the caput epididymis relative to the cauda epididymis.

Renewed spermatogenic recrudescence occurred during late October and early November. By mid-November, spermatogenic activity had again reached pre-copulatory levels as observed during August, and seminiferous tubules were dominated by large concentrations of developing spermatozoa attached in clusters to Sertoli cells and free-lying spermatozoa in the tubule lumina. The epididymides were extended showing abundant spermatozoa, especially in the caput epididymis.

By early December, seminiferous tubules were dominated by large concentrations of spermatozoa in the tubule lumina, while elongating spermatids and clusters of developing spermatozoa attached to Sertoli cells were also abundant. The epididymides of two specimens collected in early December were filled to capacity. In two specimens examined at the same date, only the caput epididymides were packed with spermatozoa, while the corpus and cauda were only partially full. This suggested that the latter two specimens had already copulated, resulting in the distal part of the epididymides being temporarily emptied.

From mid-December onwards, spermatogenic activity gradually decreased with some specimens already reaching baseline activity in early February. However, most testes examined in early January still contained large numbers of spermatozoa. Elongating spermatids and developing spermatozoa were still present at that stage, although in greatly reduced numbers. The epididymides also still contained large numbers of spermatozoa, the majority of which occurred in the corpus and cauda epididymides. In some cases, however, the seminiferous

tubules were already empty, showing only small numbers of premature spermatozoa.

As previously mentioned, the extent of testicular regression during spermatogenic quiescence showed considerably variation. Some specimens showed a complete cessation of sperm production, whereas others maintained spermatogenesis and sperm production at a low level. In early February, the seminiferous tubules and epididymides of two specimens collected at Komatipoort, still showed abundant spermatozoa, whereas the seminiferous tubules of two others were almost depleted of spermatozoa. However, all of these males still contained spermatids undergoing spermiogenesis and premature spermatozoa attached to Sertoli cells, indicating that spermatogenesis was maintained at a reduced level (Figure 5.17).

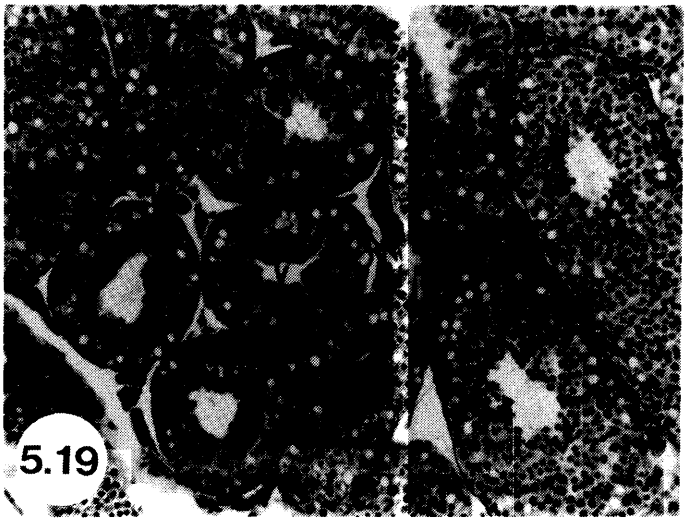
In some of the males examined during the period early March to early May, only solitary round spermatids were present in the testes, although mitotic and meiotic figures were still observed (Figure 5.18). The testes of other males collected during the same period were, however, characterised by abundant spermatids and individual premature spermatozoa (Figure 5.19). The epididymides also ranged during this period from being completely depleted of spermatozoa, to some still containing a few remaining spermatozoa in the cauda epididymis or individual young spermatozoa in the caput epididymis.

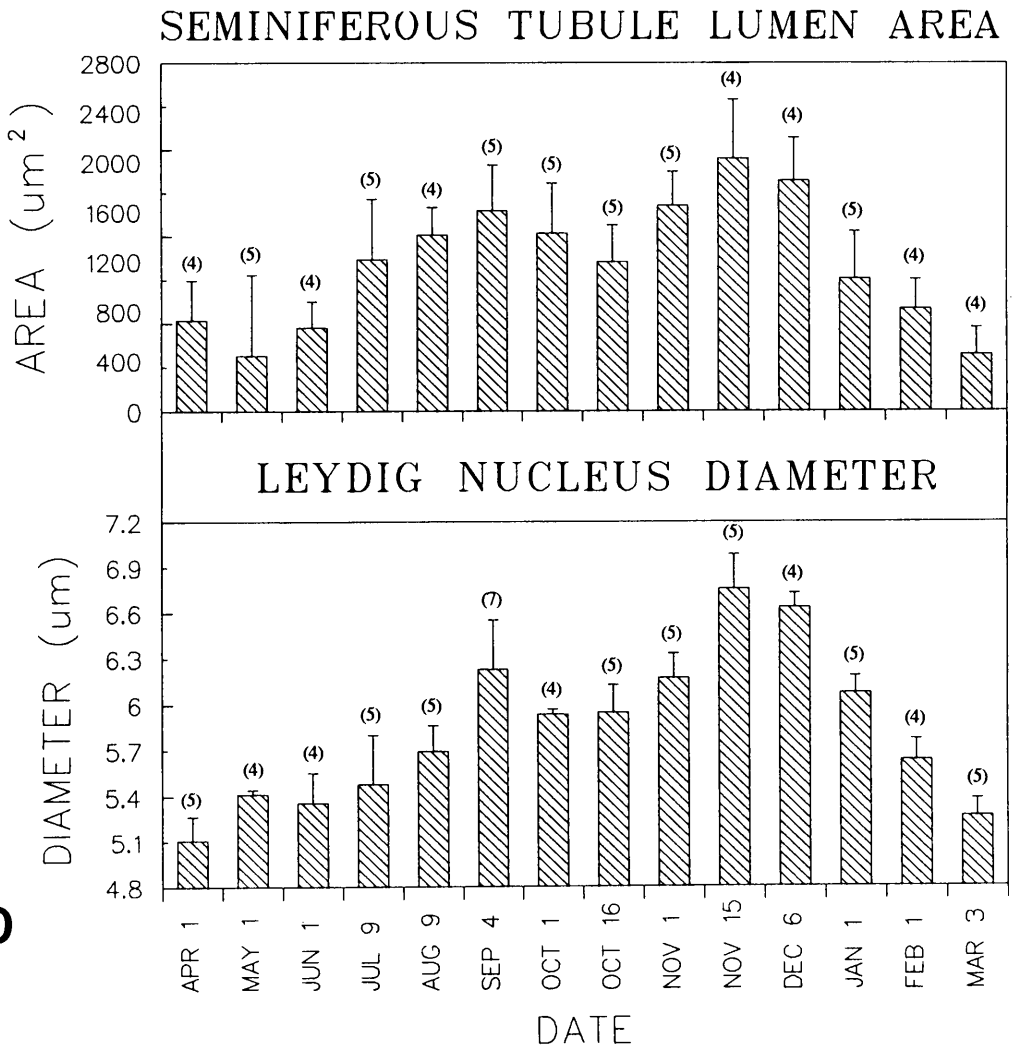
5.2.4.2. SEASONAL CHANGES IN SEMINIFEROUS TUBULE AREA

Seminiferous tubule area displayed a seasonal bimodal pattern similar to that observed in spermatogenic activity and testis and epididymis mass (Figure 5.20). Although the sample size was small ($n = 4-6$), one way ANOVA revealed significant seasonal variations ($F = 6.7$, $P<0.001$) in seminiferous tubule area, characterised by peaks in early September and November and a mid-season low in mid October.

Figure 5.19. Testis of a specimen also collected during March displaying a higher level of spermatogenic activity than the previous example. Seminiferous tubules still show abundant spermatids and a few developing spermatozoa, although some tubules only contain spermatogonia and primary spermatocytes (arrow-head). Leydig cells display more cytoplasm and the nuclei are small but not tightly packed (double arrow-head). x200.

Figure 5.20. Mean monthly seminiferous tubule lumen area and Leydig cell nucleus diameter (± 1 SD) in male *T. condylura* (sample sizes indicated in brackets).





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During spermatogenic quiescence, seminiferous tubules remained involuted for three months, attaining minimum size during the period March (10365 ± 974 μm^2) to May (9595 ± 472 μm^2). Tubule area increased gradually from late May through June, July and August, resulting in a peak in early September (15864 ± 1155 μm^2). This was followed by a significant decrease during early October, resulting in a mid-season low in mid October (11065 ± 555 μm^2, $P<0.001$, d.f. = 8). Following a gradual increase during late October and early November, tubule area increased significantly to reach a second peak in mid-November (17200 ± 836 μm^2, $P<0.01$, d.f. = 7). From early December through February, seminiferous tubules gradually became involuted to reach minimum values in March.

5.2.4.3. CYCLIC CHANGES IN LEYDIG CELL MORPHOLOGY.

Leydig cell nucleus diameter was used as an estimate of Leydig cell activity as it changed concurrent with seasonal variations in Leydig cell morphology (Figure 5.20).

Leydig cell morphology and nucleus diameter closely followed spermatogenic activity, with Leydig cells attaining maximal size during peak testicular activity. During the period of testicular quiescence, i.e. early March to early June, Leydig cells remained involuted and tightly packed in the interstitial spaces between seminiferous tubules (Figures 5.18 & 5.19). Cell nuclei, reaching minimal size in early April (5.11 ± 0.07 μm), were small and condensed, showing a distinct rough outline. Cells contained very little cytoplasm, resulting in the nuclei becoming very tightly packed (Figure 5.19).

From early July, a gradual increase in nucleus diameter indicated an increase in Leydig cell activity. Leydig cells became progressively larger with the nuclei spaced further apart, primarily as a result of the more abundant cytoplasm. Nuclei also became more prominent, loosing the rough uneven

appearance (Figure 5.6).

By mid-August, Leydig cells were hypertrophied displaying abundant finely granular cytoplasm. The nuclei of the Leydig cells showed a significant increase in size during August reaching a peak in early September (6.23 ± 0.12 μm, $P<0.05$, d.f. = 11). By early September the finely granular cytoplasm in the majority of cells had become unevenly dispersed and the cell attained a typical vacuolated appearance (Figure 5.21). In most cases, the cytoplasm had become condensed in a loop around the nucleus, forming a distinct ring-vacuole around the periphery of the cell (Figure 5.21). Cell borders had become very clear, which is in contrast to the situation found during spermatogenic quiescence, where cell borders were vague and normally obliterated by the tightly packed nuclei. Nuclei showed distinct centrally located nucleoli. This condition persisted throughout the remainder of the breeding season.

Although nucleus diameter decreased slightly during early October concurrent with the decline in spermatogenic activity, Leydig cells remained hypertrophied. Following a gradual increase during late October, nucleus diameter increased significantly during early November (6.17 ± 0.07 μm, $P<0.05$, d.f. = 8) to reach a second peak during late November and early December (6.75 ± 0.1 μm). Leydig cells were very large and prominent during November and early December with the cytoplasm characterised by large ring-vacuoles. This was followed during late December through February by a general decrease in the size of the Leydig cells. By early March Leydig cells had again become involuted showing smooth cytoplasm and small and densely packed nuclei (Figure 5.19).

The degree of regression of the Leydig cells often varied between individuals from the same sample. Regression of the Leydig cells were in general closely related to the degree of spermatogenic activity observed in the testes. The two speci-

mens examined in early February with abundant spermatozoa in the seminiferous tubules and epididymides, contained well developed Leydig cells with prominent nuclei. Two other specimens of the same sample had only a few spermatozoa in their epididymides and their testes were exhausted. The Leydig cells in these males were small with densely packed nuclei. Four of the five specimens examined during early March contained testes depleted of spermatozoa, while only a few remained in the epididymides. The Leydig cells of these males were regressed showing tightly packed nuclei with a characteristic rough outline. The testes of the fifth specimen still contained abundant spermatozoa and its Leydig cells were large with prominent nuclei and abundant cytoplasm.

5.2.4.4. GEOGRAPHIC VARIATIONS IN THE DURATION OF SPERMATOGENESIS

Specimens collected at Skukuza (24°59'S) during 1988 and at Komatipoort (25°26'S) during 1989, differed slightly with regard to the duration of spermatogenesis. The testes of males collected at Skukuza during mid-February were inactive i.e. the testes were small with no spermatozoa and hardly any spermatids present in the seminiferous tubules. Contrastingly, males from Komatipoort only became quiescent during late February as some collected during early February still had relatively large numbers of elongated spermatids and spermatozoa in the seminiferous tubules with the epididymides still crowded with spermatozoa.

5.2.4.5. TESTICULAR REGRESSION

The testes of adult males never regressed to prepubertal conditions during spermatogenic quiescence as is the case in many seasonal breeding species, but rather maintained reduced levels of spermatogenic activity. Total regression of the testes (defined as testes characterised by the absence of primary spermatocytes and spermatids, with seminiferous

tubules lined by type A spermatogonia and Sertoli cells only) was not found in any of the adult males examined. Of all specimens collected and examined between early February and early June, the testes of only one individual from each locality (n = 23 for Komatipoort; n = 18 for Skukuza) were regressed to the level of containing only spermatogonia and primary spermatocytes (Figure 5.22), which indicated cessation of spermatogenic activity, but not complete regression of the spermatogenic epithelium. The testes of the remainder of males (95%, n = 41) examined during this period were all characterised by the presence of varying numbers of round spermatids (Figures 5.18 & 5.19). Elongating spermatids and a few premature spermatozoa were, however, also recorded in a number of testes (43%, n = 23), thus indicating spermatogenic activity (Figure 5.19).

The degree of regression of the testes varied considerably between individuals of the same sample and to a certain extent between samples from Skukuza and Komatipoort.

The sample collected at Komatipoort in early March consisted of five adult males, of which one showed no spermatids or spermatozoa in the seminiferous tubules, two had no spermatozoa but large numbers of round spermatids, and the remaining two contained active testes with large numbers of round spermatids, many elongating spermatids and a few premature spermatozoa in the seminiferous tubules. The testes of specimens from most other samples collected during spermatogenic quiescence, i.e. from February to early June, displayed similar variations, ranging from those with no spermatozoa and only a few round spermatids in the seminiferous tubules to those having varying numbers of elongating spermatids and premature spermatozoa in the seminiferous tubules.

The testes of specimens collected at Skukuza from February to early May appeared slightly more regressed than those from Komatipoort. Only 25% (n = 12) of the adult males from Skuku-

za examined between mid-February and the end of April showed any developing spermatozoa in the seminiferous tubules, compared to 47% (n = 15) for those from Komatipoort. For the same period, only 33% (n = 12) of the males from Skukuza had elongating spermatids in their testes, compared to 60% (n = 15) for those from Komatipoort.

5.2.5. SPERMATOGENIC CYCLE

The spermatogenic cycle displayed by the testes of male *T. condylura* can be divided into eleven stages on the basis of germinal cellular associations and nuclear morphology. As a result of the staining technique used during histological preparation, only cellular associations during spermatocytogenesis and meiosis could be distinguished. It was not possible to identify the different stages of spermatid differentiation during the process of spermiogenesis.

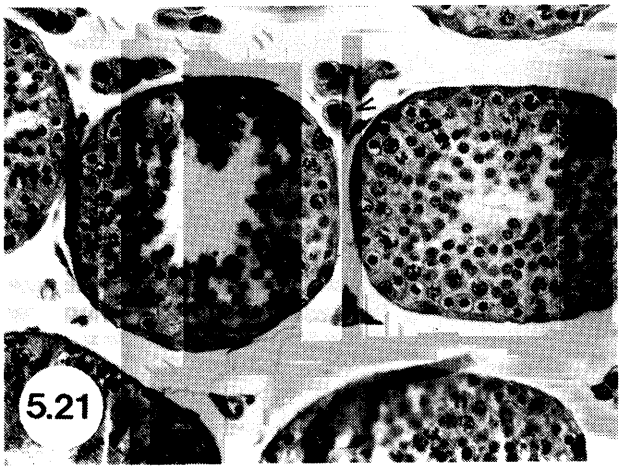
The eleven different stages of the various nuclear associations observed during the spermatogenic cycle are schematically illustrated in Figure 5.23. Each of these stages will be individually described and discussed.

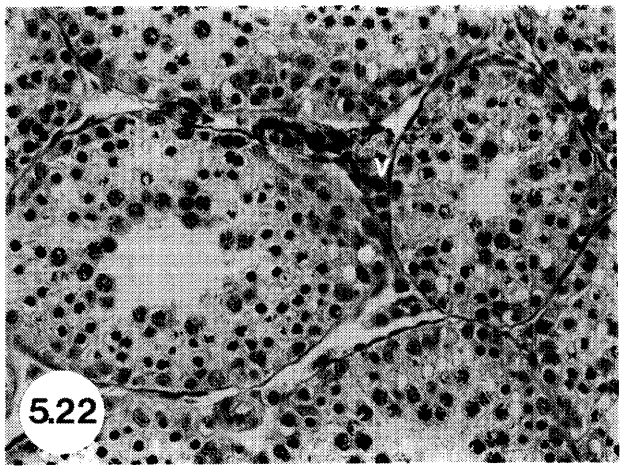
Stage 1: This stage represented the onset of a new spermatogenic cycle, being the first stage immediately after the release of spermatozoa into the lumen of the seminiferous tubules. As in all other stages, abundant type A spermatogonia formed the peripheral layer of cells adjacent to the basal lamina. This stage was characterised by abundant mitotic figures and by a single layer of preleptotene primary spermatocytes. This was followed by a few layers of large pachytene primary spermatocytes, which were separated from the lumen by several layers of small spherical spermatids. The lumen was either empty or contained a few individual spermatozoa. Some residual bodies were also present early in this stage.

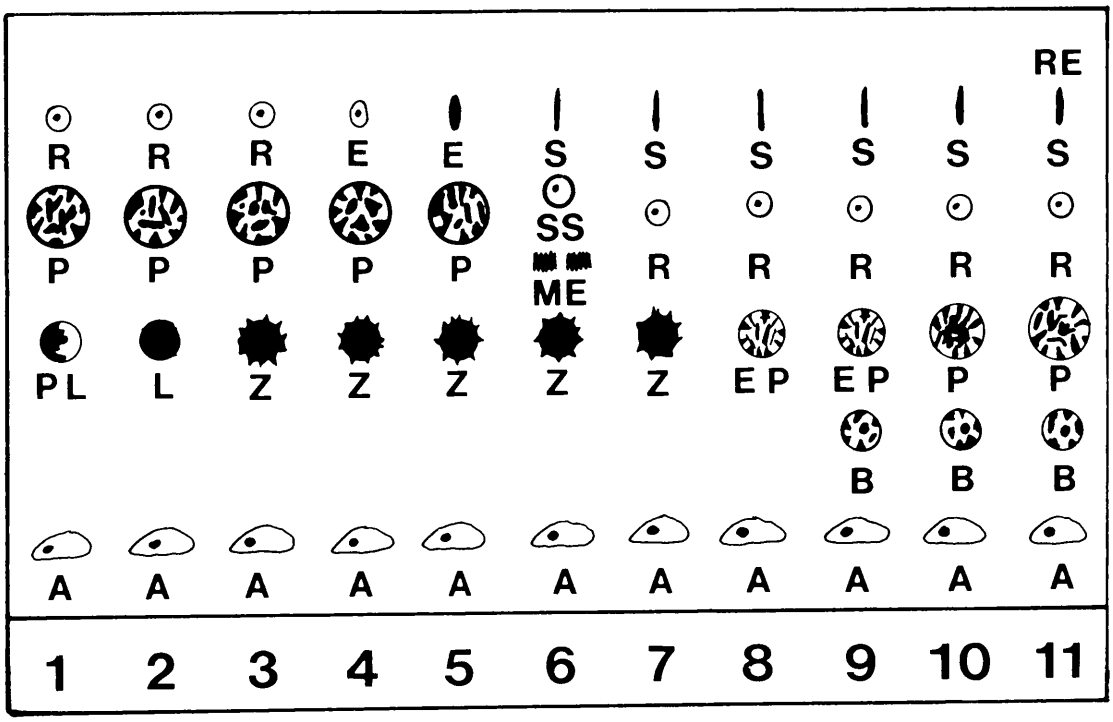
Figure 5.21. Seminiferous tubules during the breeding season with well developed interstitial cells of Leydig showing abundant cytoplasm concentrated around the nucleus, resulting in a ring-vacuole around the perimeter of the cell (arrow-head). x400.

Figure 5.22. Seminiferous tubules of the most regressed testis found during reproductive quiescence, showing baseline spermatogenic activity. Seminiferous tubules are lined with spermatogonia and primary spermatocytes only and no spermatids or developing spermatozoa are observed. Leydig cells are small with little cytoplasm and the nuclei are condensed and tightly packed (arrow-head). x400.

Figure 5.23. Schematic representation of the eleven stages of the spermatogenic cycle in the testis of T. condylura, as determined through cellular associations and nuclear morphology. Type A spermatogonia (A); Type B spermatogonia (B); Elongating spermatids (E); Early pachytene primary spermatocytes (EP); Leptotene primary spermatocytes (L); Meiosis with meiotic figures (ME); Pachytene primary spermatocytes (P); Preleptotene primary spermatocytes (PL); Round spermatids (R); Spermatozoa released in tubule lumen (RE); Developing spermatozoa (S); Secondary spermatocytes (SS); Zygotene primary spermatocytes (Z).



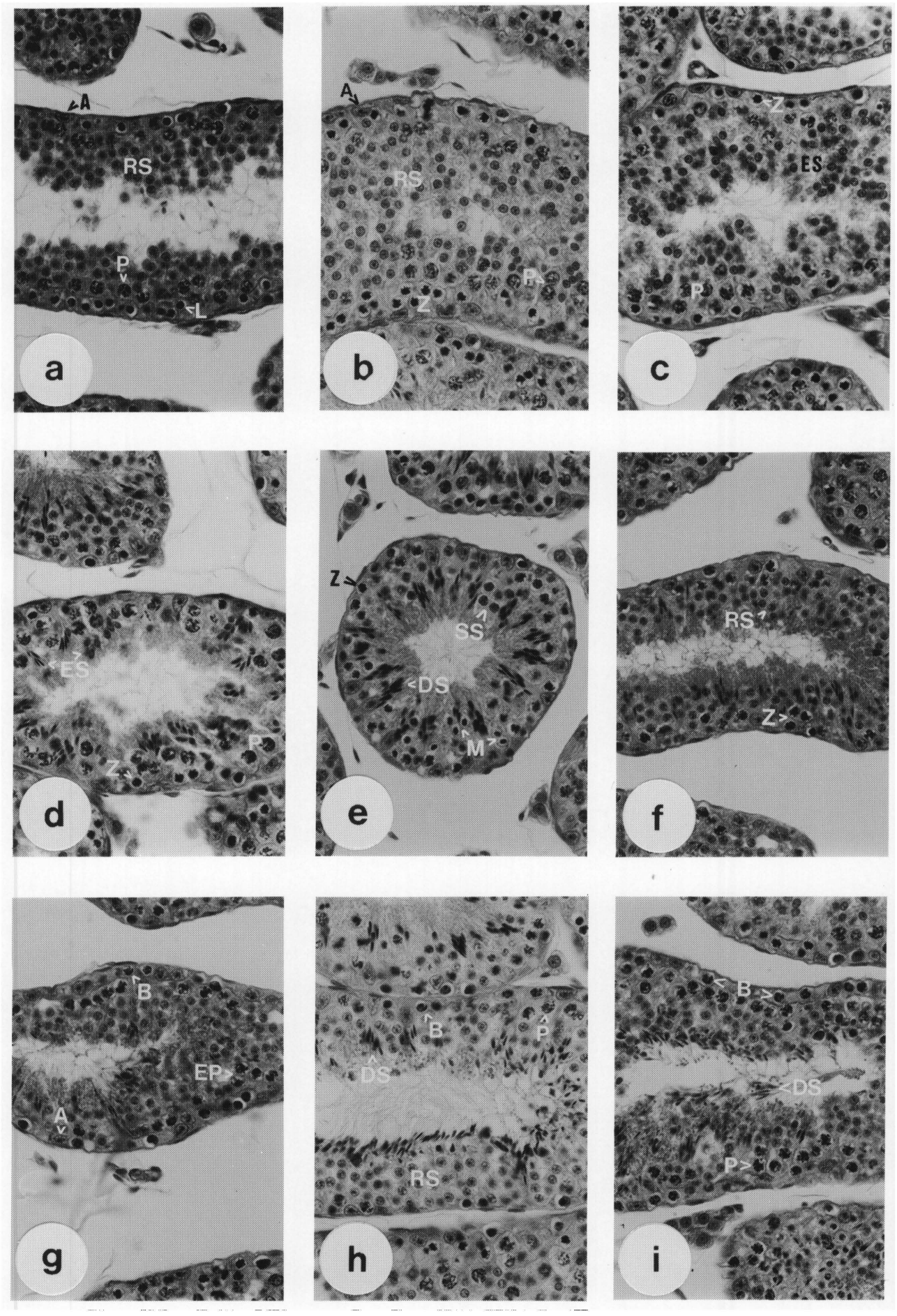




5.23

Figure 5.24. Spermatogenic cycle in *T. condylura*:

a) Stage 2 showing Type A spermatogonia (A), leptotene primary spermatocyte (L), pachytene primary spermatocyte (P) and round spermatids, (RS).
b) Stage 3 showing type A spermatogonia (A), pachytene primary spermatocyte (P), round spermatids (RS) and zygotene primary spermatocytes (Z).
c) Stage 4 showing spermatids at the start of elongation (ES), pachytene primary spermatocytes (P) and zygotene primary spermatocytes (Z).
d) Stage 5 showing further elongation of thethe spermatids (ES), pachytene primary spermatocytes (P) and zygotene primary spermatocytes (Z).
e) Stage 6 showing meiosis with secondary spermatocytes (SS) and meiotic bodies (M). Zygotene primary spermatocytes are still present and developing spermatozoa (DS) are clustered on the luminal ends of Sertoli cells.
f) Stage 7 showing only type A spermatogonia, zygotene primary spermatocytes (Z) and round spermatids (RS).
g) Stage 9 showing type A spermatogonia (A), type B spermatogonia (B), early pachytene primary spermatocytes (EP) and abundant round spermatids.
h) Stage 10 showing type A spermatogonia (A), type B spermatogonia (B), pachytene primary spermatocytes (P), round spermatids (RS) and developing spermatozoa (DS), some of which are ready to be released from the Sertoli cells.
i) Final stage showing abundant developing spermatozoa (DS) in the seminiferous tubule lumen, type B spermatoatogonia (B) and pachytene primary spermatocytes (P). x400.



Stage 2: Stage 2 differed from the previous stage by the absence of mitotic figures and by having the preleptotene primary spermatocytes replaced by a single layer of leptotene primary spermatocytes. The type A spermatogonia, pachytene primary spermatocytes and round spermatids remained unchanged (Figures 5.23 & 5.24a). As a result of the relative short lifespan of preleptotene and leptotene primary spermatocytes, these last two stages were found to be less common than the majority of stages.

Stage 3: Stage 3 could be distinguished from the previous stage by having one or more layers of distinct zygotene primary spermatocytes replacing the leptotene primary spermatocytes (Figures 5.23 & 5.24b). The type A spermatogonia, pachytene primary spermatocytes and round spermatids again remained unchanged.

Stage 4: Stage 4 was characterised by the onset of spermiogenesis with numerous spermatids starting to elongate (Figures 5.23 & 5.24c). By the end of this stage, spermatids were grouped as clusters of condensed elongated nuclei. Type A spermatogonia and zygotene and pachytene primary spermatocytes were unchanged.

Stage 5: Further elongation occurred during this stage. Elongated spermatids had become more congregated, forming distinct clusters of developing spermatozoa attached to the luminal ends of Sertoli cells (Figures 5.23 & 5.24d). Type A spermatogonia and zygotene and pachytene primary spermatocytes remained unchanged.

Stage 6: This stage was characterised by the occurrence of meiosis. In most tubular cross-sections, meiotic divisions were not always synchronised, often resulting in all representative forms of meiosis, i.e. diplotene primary spermatocytes, meiotic figures, secondary spermatocytes and newly formed spermatids, all in the same cross-section. Secondary

spermatocytes were short-lived and they were not commonly observed in the seminiferous tubules. Groups of small, dark, rectangular meiotic figures were characteristic of this stage. Type A spermatogonia and zygotene primary spermatocytes were unchanged and developing spermatozoa were still firmly attached to Sertoli cells (Figures 5.23 & 5.24e).

Stage 7: This stage appeared to be short-lived and therefore not common. During this stage only zygotene primary spermatocytes were observed. Type A spermatogonia, several layers of round spermatids and clusters of developing spermatozoa, still attached to Sertoli cells, constituted the remainder of the germinal layer (Figures 5.23 & 5.24f).

Stage 8: During this stage, zygotene primary spermatocytes were transformed to young pachytene primary spermatocytes, which appeared to have relatively small nuclei with more condensed chromosomal strands. Type A spermatogonia, round spermatids and developing spermatozoa remained unchanged, although some mitotic divisions were already observed in the peripheral layer of type A spermatogonia (Figure 5.23).

Stage 9: This stage differed from the previous stage due to the presence of numerous mitotic divisions and type B spermatogonia in the peripheral tubular region. Pachytene primary spermatocytes formed several layers above the spermatogonia, with the nuclei showing more distinct strands of chromatin. Round spermatids were unchanged. Elongating spermatids were still clustered to the Sertoli cells (Figures 5.23 & 5.24g).

Stage 10: Mitotic figures and type B spermatogonia were again characteristic of this stage, although fewer mitotic divisions were observed. Clusters of premature spermatozoa were starting to break up with some spermatozoa being released into the tubule lumen. Pachytene primary spermatocytes and round spermatids remained unchanged (Figures 5.23 &

5.24h).

Stage 11: The final stage of the spermatogenic cycle was characterised by the release of spermatozoa into the lumen of the seminiferous tubules, resulting in a dark ring of spermatozoa and residual bodies along the edge of the lumen. Type B spermatogonia formed an almost continuous ring around the periphery of the tubule and spermatogonial mitosis was also frequently observed. Pachytene primary spermatocytes and round spermatids remained unchanged in position and appearance (Figures 5.23 & 5.24i).

Circular cross-sections of seminiferous tubules often revealed more than one stage of the spermatogenic cycle, indicating that spermatogenic development did not occur in a simple wave along the seminiferous tubule, but advanced in certain parts of the seminiferous tubule more rapidly than in others. This was particularly evident when meiosis occurred, as stage 5 and 6 were frequently observed in the same cross-section (Figure 5.23 & 5.24e). Meiosis also did not occur simultaneously in all parts of cross-sections, and different forms of meiotic figures were often observed in the same section (Figure 5.24e). Stages 10 and 11 were sometimes observed in the same section, with the spermatozoa still attached to the germinal layer in one part of the section, while released spermatozoa and abundant residual bodies characterised the remaining part of the section (Figure 5.24h).

5.7 DISCUSSION

The reproductive pattern displayed by male *T. condylura* in the Eastern Transvaal appears to be unique among males of the family Molossidae, since a bimodally seasonal reproductive pattern has never before been recorded in any other male molossid for which information is available (Table 5.1). Temperate molossid species seem to be characterised by unimodal seasonal spermatogenic cycles which conform to the seasonal monoestrous pattern observed in the female (Sherman 1937; Krutzsch 1955a, b; Davis *et al* 1962; Krutzsch & Crichton 1987; Heideman *et al* 1990). Of the three tropical species studied to date, one displays a unimodal seasonal spermatogenic cycle (Mutere 1973a), while the remaining two species both display aseasonal continuous patterns (Marshall & Corbet 1959; Mutere 1973b; Krutzsch & Crichton 1990a; see Table 5.1).

Although the information regarding the reproductive pattern of male *T. condylura* is very limited and based almost entirely on general and macroscopical observations, it does indicate that males from the tropics are aseasonal continuous breeders (Mutere 1973b), whereas males from subtropical regions display a seasonal pattern (Happold & Happold 1989). In Malawi (14°02' - 16°13'S), Happold and Happold (1989) observed seasonal changes in the size and position of the testes, which became scrotal and attained maximum size during the breeding season (November - February). Males from Uganda (0°6'N), however, remained reproductively active throughout the year, with the testes showing no significant seasonal variation in size (Mutere 1973b). Although the information presented by Happold and Happold (1989) is meagre, their observations are concurrent with results from the present study, indicating a seasonal reproductive pattern for males throughout the entire subtropical region.

With regard to the bimodal seasonal pattern observed during the present study, it would be reasonable to suspect that this pattern is not unique to males from the Eastern Transvaal, but that it would also be found in males from other subtropical regions once more detailed information becomes available. This is supported by the fact that the reproductive pattern displayed by female *T. condylura* from Malawi was very similar to the bimodal pattern observed in the Eastern Transvaal (Happold & Happold 1989).

The anatomical organisation and structure of the testes and epididymides of male *T. condylura* conformed to the general molossid pattern (Sherman 1937, Krutzsch 1955a, b, 1979; Marshall & Corbet 1959; Davis *et al* 1962; Mutere 1973a, b; Krutzsch & Crichton 1987; Happold & Happold 1989; Heideman *et al* 1990; Krutzsch & Crichton 1990a). The composition of the accessory glands were not investigated, and as slight differences in the reproductive tracts of other molossids were generally attributed to the accessory glands (Krutzsch & Crichton 1987, 1990a), a viable morphological comparison of the reproductive tract of the male *T. condylura* to that of other species could therefore not be made.

As in most other molossids, the fascia enclosing the testes and epididymides was not pigmented. Although the absence of external fascia pigmentation in molossids and some phyllostomatid and pteropid species appears to be characteristic of bats that do not store spermatozoa, *Mormopterus planiceps* seems to be the exception as this temperate molossid was found to store spermatozoa during winter (Krutzsch & Crichton 1987). Sperm storing bats are generally characterised by pigmentation of the fascia (Krutzsch & Crichton 1987).

The seasonal variations in testes and epididymides mass and seminiferous tubule area recorded in male *T. condylura* during the present study is not unexpected in view of the

bimodal spermatogenic activity pattern observed in this species. In seasonal non-hibernating species, testes, epididymides and seminiferous tubule size generally closely follow spermatogenic activity, reaching maximal proportions during highest spermatogenic activity, and minimal proportions during spermatogenic quiescence (Sherman 1937; Krutzsch 1955a, 1979; Davis *et al* 1962; Mutere 1973b; Krishna 1985; Jolly & Blackshaw 1987; McGuckin & Blackshaw 1987a; McWilliam 1988a, 1988b). *Pipistrellus minus*, an Old World tropical vespertilionid, displays three annual breeding cycles, and Krishna (1985) recorded three definite corresponding peaks in testis, epididymis and seminiferous tubule size.

The temporal delay between maximum testes and epididymides size can be explained by considering the passage of spermatozoa from the seminiferous tubules into the caput epididymis. Although some spermatozoa can be found in the epididymis during the early stages of the breeding season, the majority of spermatozoa are released into the epididymis only at the peak of the breeding season when the seminiferous tubules and testes have already reached maximum proportions. In male *T. condylura*, this delay is approximately three weeks, with the seminiferous tubules and testes becoming fully developed in mid August, while the epididymides only become fully engrossed in early September (Figure 5.12).

Changes in Leydig cell morphology have been recorded in a number of seasonal breeding bats including *Pipistrellus pipistrellus* (Krutzsch 1975), *Myotis velifer* (Krutzsch 1961), *Pteropus poliocephalus* (McGuckin & Blackshaw 1987a), *M. lucifugus lucifugus* (Gustafson 1987), *Rhinolophus capensis* (Bernard 1986) and also in a seasonal breeding molossid species, *Mormopterus planiceps* (Krutzsch & Crichton 1987). In all of these species, variations in interstitial cell morphology was related to steroidogenic activity, and the interstitial cell cycle occurred concomitant with the spermiogenic cycle, reaching maximal proportions during peak spermatogen-

esis.

Hibernating species of the families Vespertilionidae and Rhinolophidae are, however, characterised by a distinct asynchrony between primary and secondary reproductive events. In these species, Leydig cells are either active during both spermatogenesis and hibernation (*Pipistrellus* pattern), or during spermatogenesis alone (*Myotis* pattern) (Bernard 1986; Gustafson 1979, 1987; McGuckin & Blackshaw 1987a; Krutzsch & Crichton 1990b). In most other hibernating species and in non-hibernating seasonal breeders, reproductive events are more synchronised and developmental changes in Leydig cells closely follow the testicular cycle (Gustafson 1987).

Based on structural changes recorded during the breeding season, Leydig cells in *T. condylura* exhibited a definite seasonal pattern of activity that closely parallelled that of the seminiferous tubule epithelium (Figure 5.20), reaching maximal proportions during highest spermatogenic activity. During testicular quiescence, Leydig cells were typically inactive with little cytoplasm. The nuclei changed from large with conspicuous nucleoli during the breeding season to small, densely packed nuclei during spermatogenic quiescence. In *Pteropus poliocephalus*, McGuckin and Blackshaw (1987a) recorded definite seasonal variations in Leydig cell nuclei diameter which closely followed the spermatogenic cycle. They suggested this to be related to the steroidogenic pattern observed in the species. *Taphozous georgianus* was the only subtropical seasonal breeding species which displayed no seasonal interstitial cell changes. This could perhaps be attributed to an underlying continuous breeding pattern which this species may display in the tropics (Jolly & Blackshaw 1988a). *Pipistrellus pipistrellus* also shows no Leydig cell cycle (Racey & Tam 1974).

The bimodal pattern in interstitial morphology observed during the present study has so far never been recorded in any other Old World tropical species. It is, however, expec-

ted that as more information regarding Old World tropical species becomes available, similar patterns will appear.

As has been shown in many other mammalian species, the position of the testes changes concurrently with the onset of the breeding season from an abdominal to a scrotal position (Krutzsch 1955a; Krutzsch & Crichton 1990a). However, although numerous authors have reflected on the position of the testes with regard to the breeding condition of male bats, none of these authors have ever mentioned how long after capture such observations were made. In collecting *T. condylura* for histological examination during the present study, it was often observed that both testes were scrotal immediately after the bats were captured, but when examined a few hours later, most males had one or both testes in the abdominal position. When the effect of capture and handling on the short term movement of the testes was investigated, it was found that the testes of all ten males investigated had changed from scrotal to abdominal within 24 hours. In one individual, the testes changed from scrotal to abdominal within one minute after capture.

Seasonally breeding species generally exhibit seasonal migration of the testes, with the testes and epididymides descending into the scrotum for the duration of the breeding season (Jolly & Blackshaw 1988b). In male *T. condylura*, seasonal variations in the position of the testes and epididymides followed the general pattern characteristic of seasonally breeding chiropterans (Krutzsch 1955a, b; Krutzsch & Crichton 1987, 1990a; Jolly & Blackshaw 1988b). The migration of the testes may be a temperature regulating mechanism, allowing the testes to adjust to seasonal temperature fluctuations. As illustrated by *T. condylura*, testicular migration in a subtropical environment is, however, not only related to temperature fluctuations, but in this species, the position of the testes was found to be related to the degree of testicular regression. During testicular quiescence,

spermatogenically inactive testes were invariably found to be abdominal, whereas testes remaining active were recorded in the scrotal position. In *Molossus fortis* (Krutzsch & Crichton 1990a), an aseasonally continuous breeding tropical molossid, the testes were never recorded in the abdominal position, but remained in the inguinal canal during periods of least testicular activity.

Seasonally breeding chiropteran species display a wide range of testicular regression during winter. Some species such as *Miniopterus schreibersii* (Bernard, Bojarski & Millar 1991), *Pipistrellus pipistrellus* (Racey & Tam 1974) and *Macrotus californicus* (Bradshaw 1962) and most other hibernating chiropterans (McGuckin & Blackshaw 1987a), are characterised by complete cessation of spermatogenesis with the testes regressing to prepubertal conditions. On the other end of the scale are some species which remain spermatogenically active with the testes merely showing a reduction in sperm production outside the breeding season (McGuckin & Blackshaw 1987a). In aseasonally breeding species such as *Molossus fortis* (Krutzsch & Crichton 1990a) and *Tadarida pumila* (Mutere 1973b), the testes remain active throughout the year, showing no significant seasonal variation in size or activity. In *T. condylura* in the Eastern Transvaal, the regression of the testes conformed to a typical non-hibernating seasonal activity pattern, never regressing to the prepubertal state, but generally maintaining very low levels of spermatogenic activity. Marked intraspecific variation in regression of the testes was found in this species. In some specimens, sperm production halted completely, whereas others maintained sperm production, but at a much reduced level. The significance of this variation is not clear, since males from the same sample often differed considerably with regard to regression of the testes. It is suggested, however, that a difference in social dominance among males could lead to the observed variation in testicular regression, with the most dominant males being spermatogenically most active during and

outside the breeding season. McWilliam (1988a) found evidence of a harem system in *Tadarida pumila* in Ghana, in which only the largest males were reproductively active.

The significance of the geographical variation in spermatogenic activity and testicular regression observed during the present study is not clear, since the two sampling sites are only approximately 60 km apart. Both sites are situated in the same biotic zone at corresponding altitudes, which means that differences in climatic conditions between the two sites will be almost negligible. In seasonal breeding animals, however, it has been shown that changing climatic conditions can cause considerable variation in animal reproductive patterns (Kitchener 1973; McWilliam 1988a, 1988b). Unfortunately, no rainfall or temperature figures for Komatipoort are available, but when the mean monthly rainfall and temperature figures for Skukuza for 1988 and 1989 are compared, it is apparent that temperature figures for the two years were much the same. The rainfall figures, however, showed an interesting pattern in that precipitation during late winter/early spring in 1989 was much lower than in 1988, but almost twice as much rain fell during the summer of 1989 compared to 1988. McWilliam (1988a, 1988b) stressed the importance of rainfall as a major determinant of the timing of reproduction in tropical bats through its effect on the food supply. The lower precipitation in 1989 probably delayed matings and ovulation, while the higher precipitation in summer could possibly have prolonged reproductive activity, resulting in males only becoming quiescent later in the breeding season. It is suggested, therefore, that the variations observed in male reproduction between the two sites are caused by inter-annual climatic changes and not geographical variations in reproductive patterns.

The spermatogenic cycle exhibited by male *T. condylura* differs little from that found in most other mammalian species (Clermont & Leblond 1959). The number of cellular asso-

ciations (n = 11) falls within the range of eight to fifteen described for other species (Singwi & Lall 1983). Intermediate type spermatogonia could, however, not be distinguished, contrary to what has been described in two chiropteran (McGuckin & Blackshaw 1987b; Singwi & Lall 1983) and other mammalian species (Clermont & Leblond 1959; Oakberg 1956a). Surprisingly, the spermatogenic cycle observed in *T. condylura* is very similar to that displayed by the grey-headed fruit bat, *Pteropus poliocephalus* (McGuckin & Blackshaw 1987b), whereas it differs markedly from that found in the insectivorous bat, *Rhinopoma kinneari* (Singwi & Lall 1983). Although the spermatogenic cycle in *P. poliocephalus* consists of eight stages, compared to eleven for *T. condylura*, the general pattern of development appears to be the same (McGuckin & Blackshaw 1987b). Also, the cellular associations in both *T. condylura* and *P. poliocephalus* more closely resemble those found in the rat than those in *R. kinneari*. These results seem to contradict the evidence presented by McGuckin and Blackshaw (1987b) for the theory on the phylogenetical separation of the two suborders of bats.

T. condylura, however, differs from the other two species in that spermatogenesis in these species occurs in a wave which runs perpendicular to the seminiferous tubules, whereas in *T. condylura*, circular cross-sections often displayed more than one stage (McGuckin & Blackshaw 1987b; Singwi & Lall 1983). In this respect, therefore, *T. condylura* more closely resembles the human than other chiropterans and rodents (Heller & Clermont 1963).

6. CONCLUSION

Although female *T. condylura* displayed a bimodally seasonal reproductive pattern both in tropical (Mutere 1973b) and subtropical (Happold & Happold 1989; present study) regions, reproductive events in this species in the Eastern Transvaal was confined to a relatively short summer rainy season, whereas in the tropics, the breeding season lasted most of the year and reproductive cycles were almost six months apart (Mutere 1973b). Male *T. condylura* displayed a more pronounced geographical variation in their reproductive pattern, showing a bimodally seasonal pattern in the Eastern Transvaal (present study) in contrast to being an aseasonal breeder in the tropics (Mutere 1973b). *T. condylura* seems to be unique in this respect, since this is the first molossid species in which both male and female display a bimodally seasonal reproductive pattern.

The cost of reproduction is of significance to animals inhabiting seasonal environments. This is particularly true for small mammals such as bats in which the energy requirement of reproduction is disproportionately greater than in larger mammals (Kurta & Kunz 1987). In the female, late pregnancy, lactation and weaning can be regarded as the most demanding period in the reproductive cycles of mammals (Heideman 1988), and reproductive success may be increased if these events occur during optimum periods (Heideman 1988; Bronson 1985). Seasonal variations in daylength, temperature and rainfall become increasingly more pronounced at higher latitudes, all of which result in shortening the optimal period for bearing and raising offspring. At the equator, rainfall and temperature show little seasonal variation, which means that food is available all year round, allowing species inhabiting these regions to reproduce throughout the year (Mutere 1973b). At higher latitudes, however, seasonal fluctuations in food availability are more severe, forcing

resident species to adjust their reproductive patterns in order to avoid periods of adverse conditions (Rautenbach *et al* 1988; Wolda 1988). A tropical species such as *T. condylura* inhabiting a subtropical region like the Eastern Transvaal, has, therefore, become adapted to a shortened seasonal reproductive pattern as a result of constraining environmental conditions.

The bimodally seasonal reproductive pattern of the male Angolan free-tailed bat in the Eastern Transvaal differs from the aseasonal continuous reproductive pattern observed in this species in equatorial Africa (Mutere 1973b). This can be regarded as is an indication of the extent of intraspecific variation which can be expected in the reproductive pattern of animals inhabiting both equatorial and subtropical regions. Although geographic intraspecific variations in the timing of reproductive events is common in many chiropteran species (Dwyer 1970; Medway 1971; Racey 1982), only a few species show a complete alteration in their breeding pattern at different latitudes (Jolly & Blackshaw 1988; Krishna 1985; La Val & La Val 1977; Van der Merwe *et.al.* 1986; Wilson 1979).

T. condylura conformed to the typical pattern displayed by non-hibernating chiropterans in that reproductive events in males and females were synchronised throughout the breeding season (Jerret 1979; Krutzsch 1979). Spermatogenic activity and interstitial cell development in the male parallelled ovarian follicle and uterine endometrium development in the female.

T. condylura can be regarded as a typical molossid with regard to the histology of its genital tracts. The complete dextral dominance observed in the female reproductive system is characteristic of this family (Wimsatt 1979).

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