Reproduction in the Cape horseshoe bat (*Rhinolophus capensis*) from South Africa

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Structure of the reproductive organs and the processes of spermatogenesis and follicular development are described and are similar to those described for other members of the genus *Rhinolophus*. The reproductive cycle of the Cape horseshoe bat is characterized by spermatogenesis between October and May (spring to autumn) with sperm released to the cauda epididymis in April and May. At this time the females are in oestrus or submaximal oestrus but copulation and ovulation are delayed until August and September (the end of winter hibernation). Between May and September spermatozoa are stored in the cauda epididymis where they show no positive association with the epididymal epithelium. Parturition occurs in November and December after a three to four month gestation.

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Die struktuur van die voortplantingsorgane en die prosesse van spermatogenese en follikulêre ontwikkeling word beskryf en is soortgelyk aan die van ander lede van die genus *Rhinolophus*. Die voortplantingsiklus van die Kaapse saalneusvlermuis word gekenmerk deur spermatogenese wat tussen Oktober en Mei (lente en somer) plaasvind met saad wat teenwoordig is in die cauda epididimis gedurende April en Mei. Die wyfies is in estrus of submaksimale estrus gedurende hierdie tydperk maar paring en ovulasie word tot Augustus en September (die einde van die winterslaap) vertraag. Tussen Mei en September word spermatozoa in die cauda epididimis gestoor waar hulle geen positiewe verbintenis met die epididimale epiteel vertoon nie. Geboorte vind plaas gedurende November en Desember na 'n dragtyd-perk van drie tot vier maande.

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Reproduction of members of the genus *Rhinolophus* in northern and southern subtropical and temperate latitudes is characterized by spermatogenesis and follicular development in late summer, copulation at the beginning of winter and a period of sperm storage, by the female, during winter hibernation. Ovulation is delayed until spring when the secondary oocyte is fertilized by stored sperm or, in some cases, by sperm from late winter or spring copulations (Rollinat & Trouessart 1897; Matthews 1937; Gaisler & Titlbach 1964; Gaisler 1965; Dwyer 1966; Gaisler 1966; Bernard 1983). In tropical India the reproductive cycle of *Rhinolophus rouxi* may include a period of delayed implantation (Ramakrishna & Rao 1977), while in tropical Africa (Zaire) the reproduction of members of this genus is typically mammalian (Anciaux de Faveaux 1978).

At least nine species of *Rhinolophus* occur in South Africa (Hayman & Hill 1971) and details of the reproductive processes are available for *Rhinolophus clivosus* only (Harrison & Clancey 1952; Laycock 1976; Bernard 1983). Information concerning the remaining species is limited to capture records of pregnant or lactating females which indicate that parturition occurs between October and December (Herselman 1980; Smithers 1983).

The aim of this paper is to describe the structure of the reproductive organs, the processes of gametogenesis, and the cyclical nature of reproduction in *R. capensis*.

Materials and Methods

Specimens of the Cape horseshoe bat were collected, on a monthly basis, over a two-year period (January 1982 to December 1983) from a tunnel on Table Farm (33°17′S/26°25′E) in the Cape Province of South Africa. The colony was relatively small with seasonally variable numbers and for these reasons the monthly samples were kept low. A minimum of two and maximum of four males and females were collected each month.

Specimens were killed by asphyxiation with CO_2, the reproductive tracts removed, fixed in Bouin's fluid and thereafter stored in 70% alcohol. All mass measurements were made from 70% alcohol to the nearest 1,0 mg.

Following routine embedding and sectioning at 5 μm, sections were stained with Ehrlich's haematoxylin and eosin.

Changes in seminiferous tubule diameter were quantified by measuring two diameters at right angles in cross sections of 10 tubules per testis. Ovarian activity was quantified by plotting mean monthly diameters for secondary and Graafian follicles. Mean diameters were calculated from two measurements, at right angles, for all secondary and Graafian follicles

from both ovaries. All diameters were measured with an ocular micrometer.

Throughout this report, where sample size was more than 10, the mean value has been given plus or minus the standard deviation, where the sample was less than 10, the mean value alone has been given.

In this study two age groups have been recognized, immatures (less than one year old) and adults (more than one year old), the classification being based on coloration, ossification of the epiphyses and degree of wear of the canines.

Transmission electron microscopy

Small pieces of epididymis from two specimens collected in April, May, July, August and October 1983 were fixed in cold (4°C) 5% glutaraldehyde in 0,1 mol dm^{-3} phosphate buffer (pH 7,3). After primary fixation, tissues were washed in the buffer, secondarily fixed in 1% osmium tetroxide for 90 to 100 min and washed again. After rapid dehydration through a graded alcohol sequence, tissues were embedded in Taab 812. Ultrathin sections were stained with aqueous uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) and examined under a Jeol JEM-1I transmission electron microscope.

Results

Both male and female (adult and immature) Cape horseshoe bats are present in the study tunnel throughout the year with males always many times (2 – 10) more numerous than females. Hibernation occurs between May and August but is not continuous, being interrupted by periods of activity during warm spells.

Female reproductive anatomy

The uterus of *R. capensis* is bicornuate with the right uterine horn longer and wider than the left. In anoestrous adult females the mean dimensions of the uterine horns are: right, 2,0 × 0,9 mm and left, 0,9 × 0,7 mm ($n = 9$).

The ovaries are ellipsoidal and in section comprise a wide outer cortex and thin central medulla. The cortex includes numerous developing follicles surrounded by interstitial tissue, while the medulla comprises blood and lymph vessels, connective tissue and some interstitial tissue. The interstitial tissue of the cortex and medulla is mostly of the undifferentiated thecal-type with some stromal-type in the region of the hilus (Figure 1). Small patches of gonadal adrenal-type interstitial tissue occur in the region of the hilus, particularly associated with the ovarian rete and epoophoron (Figure 1). During two periods of the year (March – April and September – November) the cells of the interstitial tissue become hypertrophied.

Figure 1 Section through the ovary of *R. capensis* showing thecal-type (T), stromal-type (S), and gonadal adrenal-type (G) interstitial tissue (× 114).

Follicular development

Follicular development in *R. capensis* is typically mammalian. The primordial follicles are numerous and located at the periphery of the ovary. The primary oocyte of such a follicle is surrounded by between five and seven fusiform follicular cells and the mean diameter of the follicle is 19,1 ± 0,9 µm. Primary follicles have a mean diameter of 38,6 ± 1,7 µm and the follicular cells are typically cuboidal. Secondary follicles range in diameter from 97,0 ± 3,4 µm (with two layers of follicular cells) to 120,3 ± 9,4 µm (with between six and eight layers of follicular cells). Secondary follicles are characterized by the development of a thin theca folliculi, which is entirely fibrous, and the zona pellucida.

Mean diameter of the Graafian follicle varies from 150,5 ± 10,8 µm (at an early stage of antral development) to the largest preovulatory Graafian follicle with a diameter of 448,1 µm. The preovulatory Graafian follicle is characterized by a cumulus oophorus of about four cell layers and division of the theca folliculi into a cellular theca interna and fibrous theca externa.

The corpus luteum of *R. capensis* comprises a single type of secretory cell and has a life of between $3\frac{1}{2}$ and $4\frac{1}{2}$ months.

Follicular atresia occurs during all months but is most common between September and December (during gestation). Two types of atresia occur: Type 1 which occurs in multilaminar follicles and in which the follicular cells degenerate before the oocyte, and Type 2 which occurs in primary and small secondary follicles and in which the oocyte and follicular cells degenerate at about the same time. In some instances Type I atresia is associated with formation of corpora atretica by the hypertrophy of thecal cells.

Biovular and polyovular follicles (Figure 2) occur regularly in the ovaries and ovarian cysts were recorded on two occasions, in both cases located in the region of the hilus (Figure 3).

Cyclical nature of female reproduction

The Cape horseshoe bat is a monoestrous, monotocous seasonal breeder.

The vagina first appears cornified in February although at this time the endometrium is thin and the ovaries contain only primary and secondary follicles. During March and April the endometrium remains undeveloped but small Graafian follicles appear in the ovaries. Between May and July the endometrium increases in thickness, becomes glandular and vascularized and there is an increase in the diameter of the Graafian follicles in the ovaries. During the period from February to July the cornification of the vagina is such that the lumen is blocked or considerably narrowed (Figure 4). During August and early September the right ovary contains a single very large Graafian follicle while both ovaries contain several smaller Graafian follicles. During this period the endometrium is thicker, more glandular and more vascularized than in previous months.

Copulation, ovulation and fertilization occur in August and September (spermatozoa were present in the vagina and uterus of a female collected in late August and all females collected in late September were pregnant) and parturition occurs in November and December after a gestation of between three and four months. Ovulation was from the right ovary only, and in all cases examined (17) implantation was in the right

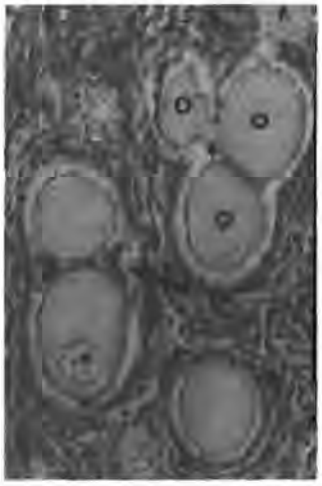

Figure 2 A biovular primary follicle (arrow) and polyovular follicle with three oocytes (o) from the ovary of *R. capensis* (× 220).

Figure 3 Section through the ovary, in the region of the hilus, showing an ovarian cyst (× 110).

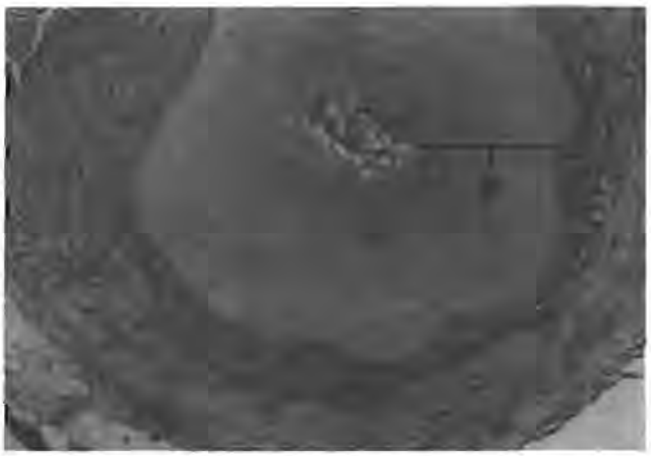

Figure 4 Section through the vagina of *R. capensis* showing the plug of epithelial cells (P) (× 90).

uterine horn.

Lactation occurs in December and January and the reproductive tract is characterized by a thin vaginal epithelium, and primary and secondary follicles in the ovaries. Monthly changes in follicular diameter are shown in Figure 5C.

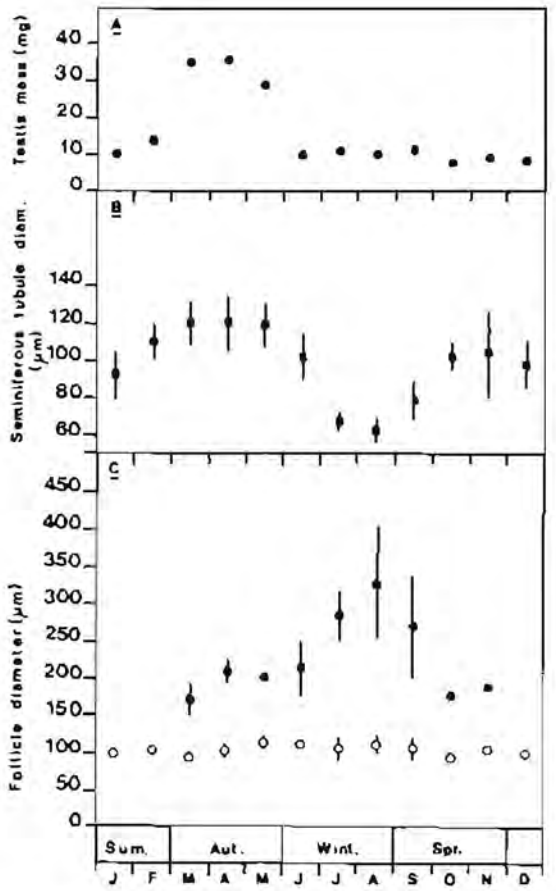

Figure 5 Monthly changes in the testis and ovary of the Cape horseshoe bat. Changes in testis mass (A) and seminiferous tubule diameter (B) indicate spring and summer activity, while changes in follicular diameter (C) indicate ovulation in early spring.

Age at sexual maturity

Nine immatures were banded in December 1982 and of these seven were recaptured during November and December 1983. None of the recaptured specimens was pregnant or lactating.

Male reproductive anatomy

The accessory gland complex of the Cape horseshoe bat is located at the base of the bladder and runs posteriorly along the urethra. The complex comprises anterior, paired ampullary glands, a medial prostate, and large, single, posterior urethal gland (Figure 6). The ampullary gland comprises numerous oval and spherical vesicles lined by a simple cuboidal epithelium; the prostate comprises semi-flattened vesicles lined by a

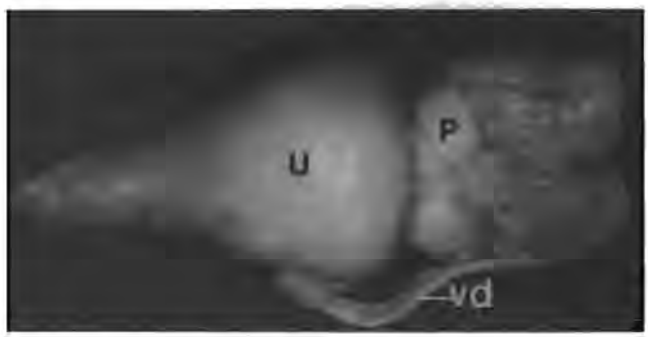

Figure 6 Ventral view of the accessory gland complex of *R. capensis* showing urethral (U), prostate (P), and ampullary (A) glands. The vas deferens (vd) can be seen entering the ampullary gland (× 8).

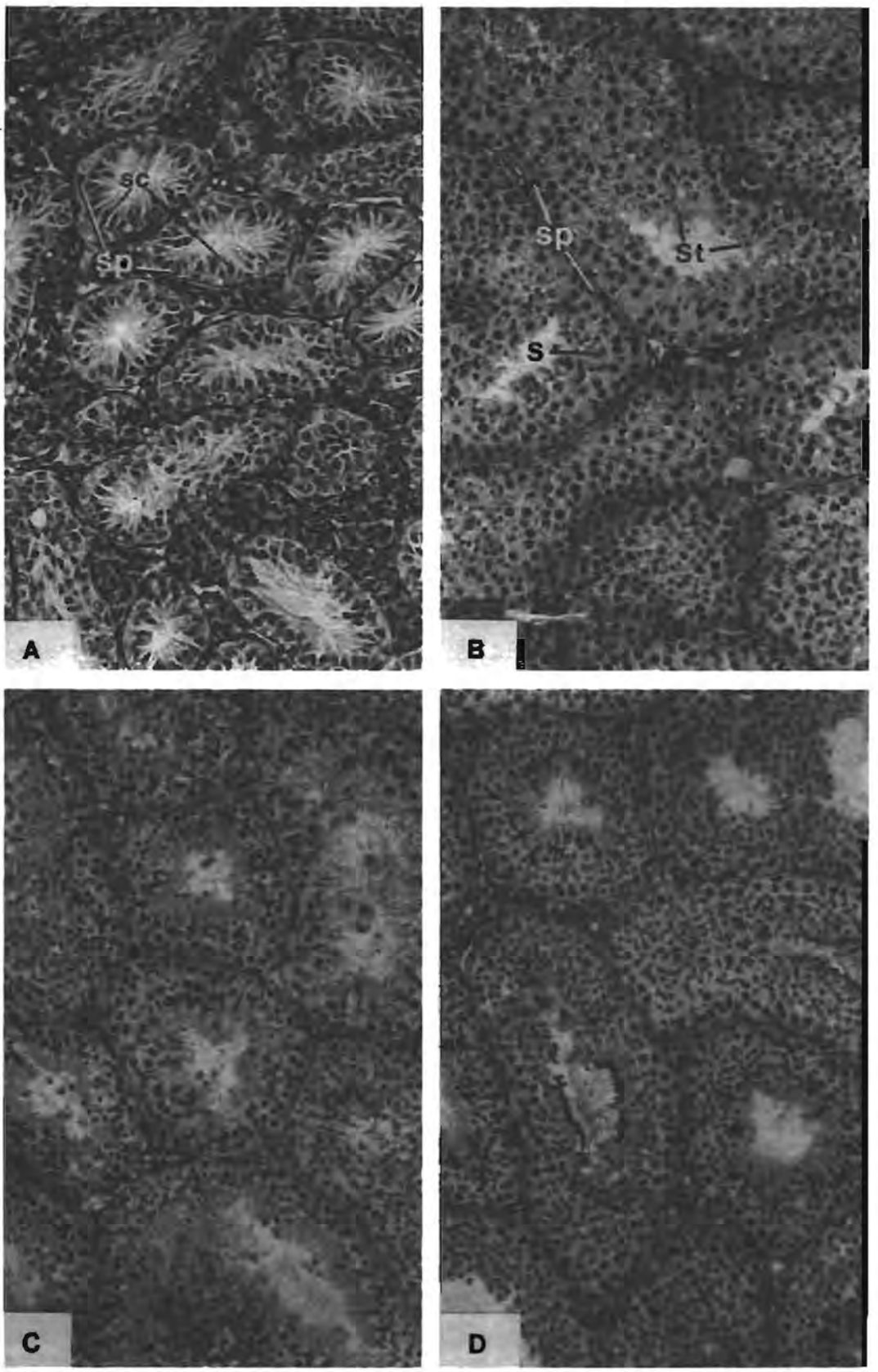

Figure 7 Sections through the testis of *R. capensis* showing stages in the spermatogenic cycle. A. Section showing the appearance of an inactive testis (July – September). Note the spermatogonia (sp) and Sertoli cells (*sc*) lining the seminiferous tubule (× 250). B. Specimen collected in January showing spermatogonia (sp), spermatocytes (s), and spermatids (st) in the walls of the seminiferous tubules (× 250). C & D. Stages in spermiogenesis from specimens collected in March (C) and May (D) (× 250).

simple columnar epithelium; and the urethral gland, flattened vesicles with a simple columnar epithelium.

The testes of *R. capensis* are ellipsoidal, non-scrotal and located sub-dermally. The cauda epididymis, during the non-breeding season, is short and located in close proximity to the testis. However, from April to September it is lengthened and located in parapenial pouches.

Cyclical nature of male reproduction

Between July and September, approximately coinciding with

winter, the testes are inactive and the seminiferous tubules lined by a single layer of Sertoli cells and spermatogonia. Division of the spermatogonia occurs in October and November and in December and January primary spermatocytes undergo a process of maturation. Spermiogenesis occurs in February and March and sperm are first released to the cauda epididymis in April. Spermiogenesis and sperm release continue in May and by June the seminiferous tubules are inactive. The general pattern described above is masked by differences in the timing of onset of spermatogenesis between specimens and between seminiferous tubules. As a result of this, most stages of the spermatogenic cycle can be found between January and June. Stages in this spermatogenic cycle are illustrated in Figure 7. The cycle of spermatogenesis mirrors changes in testis mass and seminiferous tubule diameter (Figure 5A, B).

During October and November a few of the seminiferous tubules of three specimens were lined by hypertrophied cells (Figure 8).

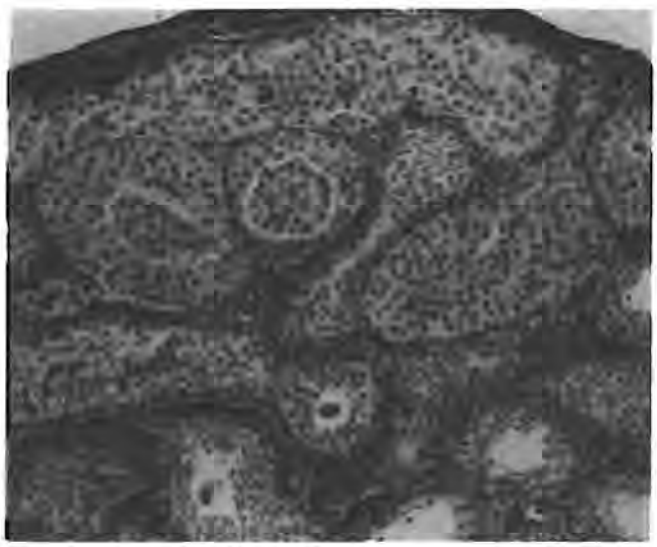

Figure 8 Section through the testis of a specimen from November showing the hypertrophied cells lining some of the seminiferous tubules (× 110).

Once released to the epididymis, spermatozoa are stored there for about four months prior to copulation and during this period they form extensive masses within the epididymal lumen (Figure 9). There is no indication that the sperm heads either burrow into, or are surrounded by epididymal cells during this period (Figure 9). After the termination of copulations large numbers of spermatozoa remain in the cauda epididymis and most of these excess sperm are removed by luminal macrophages (Bernard 1984).

Discussion

The structure of the reproductive organs of *R. capensis* is similar to those described for other members of the genus *Rhinolophus* (*R. ferrumequinum*, Matthews 1937; *R. hipposideros*, Gaisler 1965, 1966; *R. rouxi*, Gopalakrishna & Ramakrishna 1977; *R. clivosus*, Bernard 1983). The dextral functional reproductive asymmetry is the most widely encountered type of asymmetry in the Chiroptera and appears to be a characteristic of the genus *Rhinolophus* (Matthews 1937; Ramakrishna 1950; Wimsatt 1979; Bernard 1983).

The amount and types of interstitial tissue in the ovaries of Microchiroptera are variable. There is no comparable information for the Rhinolophidae, but the Vespertilionidae are characterized by much stromal and thecal and varying amounts of gonadal adrenal-type interstitial tissue, while the Phyllostomatidae have little thecal, no stromal and little or no gonadal adrenal interstitial tissue (Mossman & Duke 1973). The well-differentiated interstitial gland tissue described in *R. capensis* is a feature of the ovaries of most mammals during proestrus, oestrus and the first half of pregnancy (Mossman & Duke 1973). The differentiated interstitial tissue is endocrine in nature producing a steroid hormone, probably an oestrogen (Mossman & Duke 1973).

The two types of follicular atresia described in the present study are similar to those described for other Microchiroptera (Guthrie & Jeffers 1938, for *Myotis lucifugus* and *M. grisescens*; Van der Merwe 1979, for *Miniopterus schreibersi*;

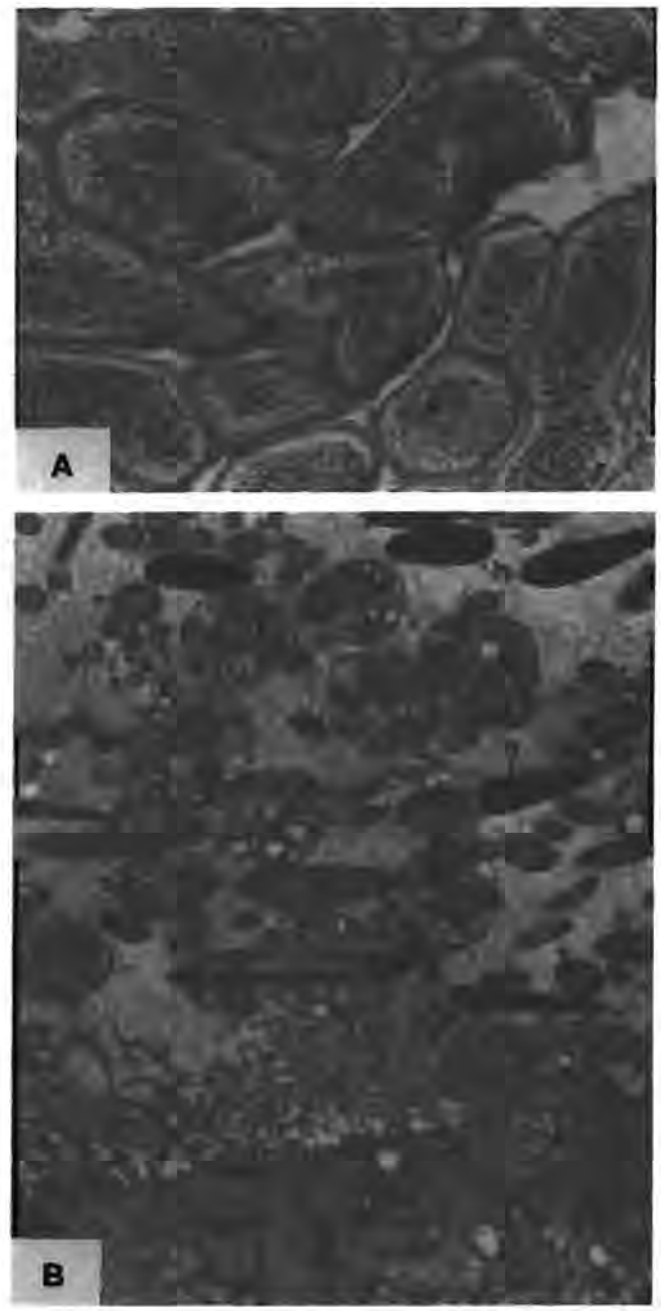

Figure 9 A. Section through the cauda epididymis of a specimen from June showing the typical appearance of this organ during the period of sperm storage (× 110). B. Electronmicrograph of a section through the cauda epididymis of a specimen from June showing the apparent lack of any association between the sperm heads (h) and epididymal epithelium (E) (× 7200).

Bernard 1980, for *Miniopterus fraterculus*). The development of corpora atretica, by hypertrophy of thecal cells during follicular atresia, is common in some Microchiroptera (members of the genus *Myotis* Mossman & Duke 1973; Bernard 1982) and absent in others (Bernard, unpublished data). Corpora atretica were not observed in *R. ferrumequinum* (Matthews 1937) nor in *R. clivosus* (Bernard 1983) and their relative scarcity in *R. capensis* would suggest that their development is rare in members of this genus.

Biovular and polyovular follicles have been reported in the ovaries of *R. ferrumequinum* (Matthews 1937) and from at least 37 mammalian genera (Mossman & Duke 1973). Gopalakrishna & Ramakrishna (1977) have reported a case of monozygotic twins in *R. rouxi* and it is possible that this may have resulted from the ovulation of a biovular follicle.

The accessory gland complex of *R. capensis* is typical for members of this genus (Krutzsch 1979) but the location of the cauda epididymis in parapenial pouches, during the period of sperm storage, is unusual. It is unlikely that the movement of the cauda epididymis into the pouches is simply a response to an increase in epididymal length as there is plenty of room elsewhere and it seems more likely that this may be related to the temperature requirements of the stored sperm during winter hibernation.

The age at sexual maturity in the Rhinolophidae is highly variable with males reaching sexual maturity at ages varying from 15 months to $4\frac{1}{2}$ years, and females at ages from three months to $3\frac{1}{4}$ years (Tuttle & Stevenson 1982). Data from the present study indicate that female *R. capensis* do not become reproductively mature in their first year, and that although the testes of males are active after 11 or 12 months, they would be able to mate for the first time in only their second year.

The occlusion or near occlusion of the vagina of *R. capensis* by cornified epithelial cells has not previously been recorded for this genus. A vaginal plug, of male accessory gland origin, has been widely reported for rhinolophid bats (Rollinat & Trouessart 1897; Matthews 1937; Gaisler 1966), and Racey (1979) has described occlusion of the vagina of *Pipistrellus pipistrellus* (Vespertilionidae) by cornified epithelial cells. Racey (1979) has suggested that vaginal plugs may serve to ensure fertilization by a single male but this cannot be the case in *R. capensis* where vaginal occlusion occurs prior to copulation. A similar situation is seen in *Hipposideros caffer* where the vagina is occluded (between May and October) by a plug of epithelial cells. In this case the plug is formed after fertilization and so cannot play a role in ensuring fertilization by a single male (Bernard 1983).

The origin of the hypertrophied cells in the seminiferous tubules is unknown. However, it is unlikely that they are significant in terms of reproduction since the majority of seminiferous tubules were normal.

The cycles of spermatogenesis and follicular development described for *R. capensis* are similar to those of north temperate members of this genus (Rollinat & Trouessart 1897; Matthews 1937; Dwyer 1966; Gaisler 1966; Gustafson 1979; Oxberry 1979) and *R. clivosus* from Natal, South Africa (Bernard 1983). However, it is typical for hibernating members of the Rhinolophidae to copulate prior to winter and for the females to store sperm during the winter (Gustafson 1979; Oxberry 1979; Racey 1979, 1982; Bernard 1983). *R. capensis* differs from this pattern and although spermatozoa are released to the cauda eipididymis prior to winter, and the females are in oestrus or submaximal oestrus at this time (as indicated by cornification of the vaginal epithelium) copulations do not occur until the end of winter. As such, *R. capensis* is a species where the onus of sperm storage falls entirely on the male. It should be noted, however, that in many of the north temperate rhinolophids and vespertilionids in which most copulations occur prior to hibernation, the males retain fertile spermatozoa in the cauda epididymis during winter and that fertile copulations may occur during winter and spring also (Aubert 1963; Racey 1979).

In many of the species of Vespertilionidae in which females store sperm, there is some form of contact between sperm head and epithelium lining the storage organ (Racey 1979). The absence of this type of association in the cauda epididymis of *R. capensis* is not necessarily unexpected since the glands of the accessory complex could supply the required environment for prolonged sperm survival.

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