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Annual Cycle of the Seminiferous Epithelium of *Myotis macrodactylus*

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The annual cycle of *Myotis macrodactylus* seminiferous epithelium and morphological features of spermatids during spermiogenesis were observed using a light microscope. Seminiferous tubule diameters gradually began to increase from April (60–63 μm) in the early arousal period, attained a peak in July (172–175 μm), and decreased rapidly to the size in October (70–75 μm), and gradually decreased to March (48–50 μm) the next year. *M. macroducylus* spermatogenesis begins in April, peaks in mid summer (July) and is suspended from October to March in the next year. Spermatocytogenesis have occurred from April to June only. Spermiogenesis occurred from July to September. The transformation of spermatids into spermatozoa *M. macrodactylus* was divided into six stages. When the mating periods were finished, from October, the redundant spermatogenic cells in the seminiferous tubules were engulfed by phagocytosis of Sertoli cells, and phagocytosis was continually observed from November to March the next year. During the phagocytosis, the seminiferous tubule contains only Sertoli cells and spermatogonia. The lumen of the seminiferous tubules was opened from July to September, and closed from October to June the next year. Therefore, the male reproductive pattern of the Korean bat, *M. macroductylus* belong to "*Pipistrellus* pattern" according to the opinion of Gustafson (1979). These results suggest that the adaptive strategy for long hibernation serves as the mechanism to regulate the breeding cycle.

INTRODUCTION

Generally, the mammalian spermatogenesis, including that of humans, can be distinguished into three major stages: spermatocytogenesis, meiosis and spermiogenesis. The spermatogenesis is the cycle of the spermatogenic cells and is a highly ordinate process in which diploid spermatogonia differentiate into mature haploid spermatozoa. Therefore, spermatogenesis is one of the most interesting processes in male reproduction.

The Chiroptera consist of two suborders, the Old World Megachiroptera and Microchiroptera. In this study species, *M. macroducylus* belong to the Microchiroptra, and are widely distributed in East Asia.

The mammalian reproductive patterns can be broadly classified into two groups: the seasonal reproductive pattern and the non–seasonal reproductive pattern. The male reproductive types of hibernating bat consist of three types of reproductive patterns–*Pipistrellus* pattern, *Myotis* pattern and the *Miniopeterus* pattern–based on spermatogenesis and changes in the stem cell and their accessory organs (Gustafson, 1979).

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In the female bat reproductive pattern, there are three basic types of reproduction: the general mammalian reproductive type in the non-hibernating bat, and the sperm storage type (Mōri *et al.*, 1982; Son *et al.*, 1987) and delayed implantation type in hibernating bats (Kimura and Uchida, 1983).

A great many of studies have been reported on bat spermatogenesis (Wilson and Findley, 1971; Singwi and Lall, 1983), spermiogenes (Uchida and Mōri, 1972; Lee *et al.*, 1992; Son *et al.*, 1995; Son *et al.*, 1997) and the seminiferous epithelium cycle and reproductive cycle (Racey, 1974; Racey and Tam, 1974; Krutzsch, 1979; Beasley and Zucker, 1984; Gustafson, 1987; Krutzsch and Crichton, 1987; Bernard and Hodgson, 1989; Heideman *et al.*, 1992; Bernard and Cumming, 1997; Morigaki *et al.*, 2001). Except for seasonal changes in the seminiferous epithelium in *Rhinolophus ferrumequinum* korai (Lee *et al.*, 1993) and *Rhinolophus cornutus* (Kurohmaru *et al.*, 2002), the annual cycle of the seminiferous epithelium in the Korean bat, *Myotis macroducylus* in bats, is unknown.

The aim of the present study, therefore, is to investigate the annual cycle of the seminiferous epithelium of *M. macrodactylus*.

MATERIALS AND METHODS

A total of 25 adult males (*Myotis macroductylus*) used in this study were collected at an abandoned mine in Kyungnam Province from October 2001 to September 2002. Testes were excised surgically under ether anesthesia, cut into smaller pieces and prefixed in cold 3% glutaraldehyde in 0.1 M Milloing's buffer (pH 7.4) for 4 h. After being thoroughly rinsed with the same buffer, they were postfixed in 1.3% osmium tetroxide in the same buffer for 2 h, and dehydrated in a graded series of aceton and embedded in Epon 812. Thick sections (1 μm) cut with glass knives were mounted on glass slides, stained in 0.5% toluidine blue and observed with the aid of a light microscope. The diameter was determined from 20 seminiferous tubules cross-sections for each of the 25 specimens utilized for this analysis.

RESULTS

Monthly changes in the seminiferous tubule diameter and the annual cycle of the seminiferous epithelium, as well as morphological features of spermatids during spermiogenesis in *M. macrodactylus*, were described (Table 1, Figs. 1–3). The annual cycle of the development of spermatogenic cells and the relationship between the seminiferous tubules diameter and the development of spermatogenic cells in the seminiferous epithelium throughout the year in the *M. macrodactylus* were also described (Fig. 4).

Monthly changes in the seminiferous tubule diameter

The diameter of the seminiferous tubules was found in April (60–63 μm; Mean: 61.65 μm), May (70–74 μm; Mean: 71.65 μm), June (90–95 μm; Mean: 92.5 μm), July (172–175 μm; Mean: 173.3 μm), August (165–168 μm; Mean: 166.3 μm), September (156–162 μm; Mean: 159.8 μm) and October (70–75 μm; Mean: 72.75 μm) during non-hibernation, and November (66–69 μm; Mean: 67.45 μm), December (63–66 μm;

Mean: 64.45μm), January (58–62μm; Mean: 60.55μm), February (54–57μm; Mean: 56.25μm) and March (48–50μm; Mean: 49.25μm) during hibernation (Table 1, Upper in Fig. 4).

Table 1. The seminiferous tubules diameter and degree of appearance of spermatogenic cell types in *Myotis macroductylus*.

Month	Diameter of the seminiferous tubules (μm)	Degree of appearance of spermatogenic cell					
		Spermatogonia			Spermatocytes		Spermatids and sperm
		Ad	Ap	B	Primary	Secondary	
1	58–62* (Mean: 60.55)	▽					
2	54–57 (Mean: 56.25)	—					
3	48–50* (Mean: 49.25)	—	—				
4	60–63* (Mean: 61.65)	—	—	—			
5	70–74* (Mean: 71.65)	—	—	—			
6	90–95* (Mean: 92.9)	—	—	—	—		
7	172–175* (Mean: 173.3)	—	—	—	—	—	—
8	165–168* (Mean: 166.3)	—	—	—			—
9	156–162* (Mean: 159.8)	—	—	—			—
10	70–75* (Mean: 72.75)	—		▼			
11	66–69* (Mean: 67.45)	—	▼				
12	63–66* (Mean: 64.45)	—	▼				

Ad, Ap and B is dark, pale and B types of spermatogonium, respectively. *, The diameter was determined from 20 seminiferous tubules cross-sections for each of the 25 specimens utilized for this analysis. ▼, degenerating spermatogenic cells. ▽, a few of spermatogenic cells are degenerated.

Monthly changes in the seminiferous epithelium

In the April sample, the basal lamina in the seminiferous tubule was found to be extremely prominent. Many of the Ad and Ap spermatogonia and a few of the B spermatogonia were observed in the seminiferous tubule. Both the type Ad and Ap cells were of an oval shape and had extensive attachments to the basal lamina of the epithelium. The B type cell and the nucleus were large and globular. Many lipofuscin were distributed throughout the closed lumen of the seminiferous tubule (Fig. 1d).

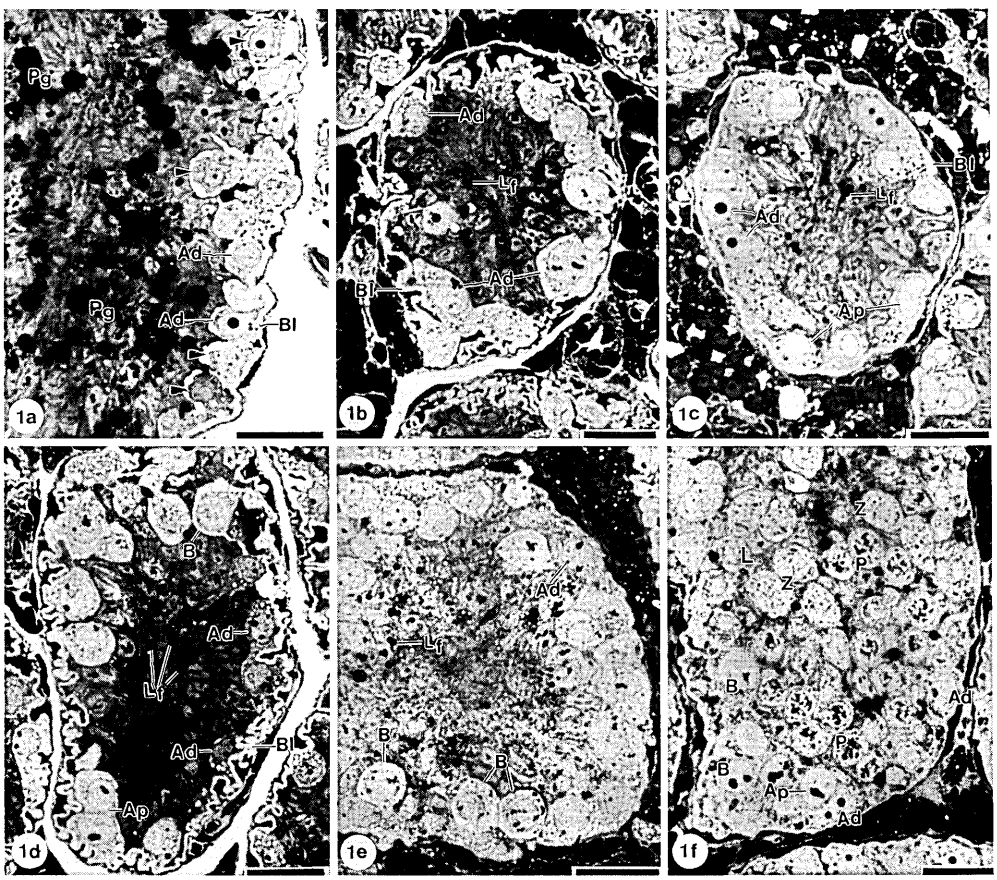
In the May sample, a few B spermatogonia and a great many Ad spermatogonia were observed in the seminiferous tubule. Many lipofuscin were distributed throughout the lumen of seminiferous tubule, and the lumen was closed as it was in April (Fig. 1e).

In the June sample, the basal lamina has expanded in comparison to its state in April and May. Moreover, leptotene zygotene, pachytene of primary spermatocytes in interphase of meiosis I and A and B spermatogonia were observed in the seminiferous tubule, a few of lipofuscins were distributed in the seminiferous tubule (Fig. 1f). Lumen was closed as it was in the April and May seminiferous tubules.

In the July sample, the pirmary round spermatis and elongating spermatids, including pachytene primary spermatocytes in interphase of meiosis I, were observed in the seminiferous tubule. The lumen was opened, and a great many sperm existed in the lumen. The characteristic aspect of this stage was that elongated spermatids bundles had moved toward the lumen of the seminiferous tubule. A few Sertoli cells nuclei and type A spermatogonia were present near the basal lamina (Fig. 1g).

In the August sample, a few A and B spermatogonia and many mature spermatids in the spermiation phase were observed in the seminiferous tubule, and elongated spermatid bundles were dissociated and located near the seminiferous tubule lumen (Fig. 1h).

In the September sample, the most notable feature was the location of elongated spermatids just being released from the seminiferous epithelium (Fig. 1i).



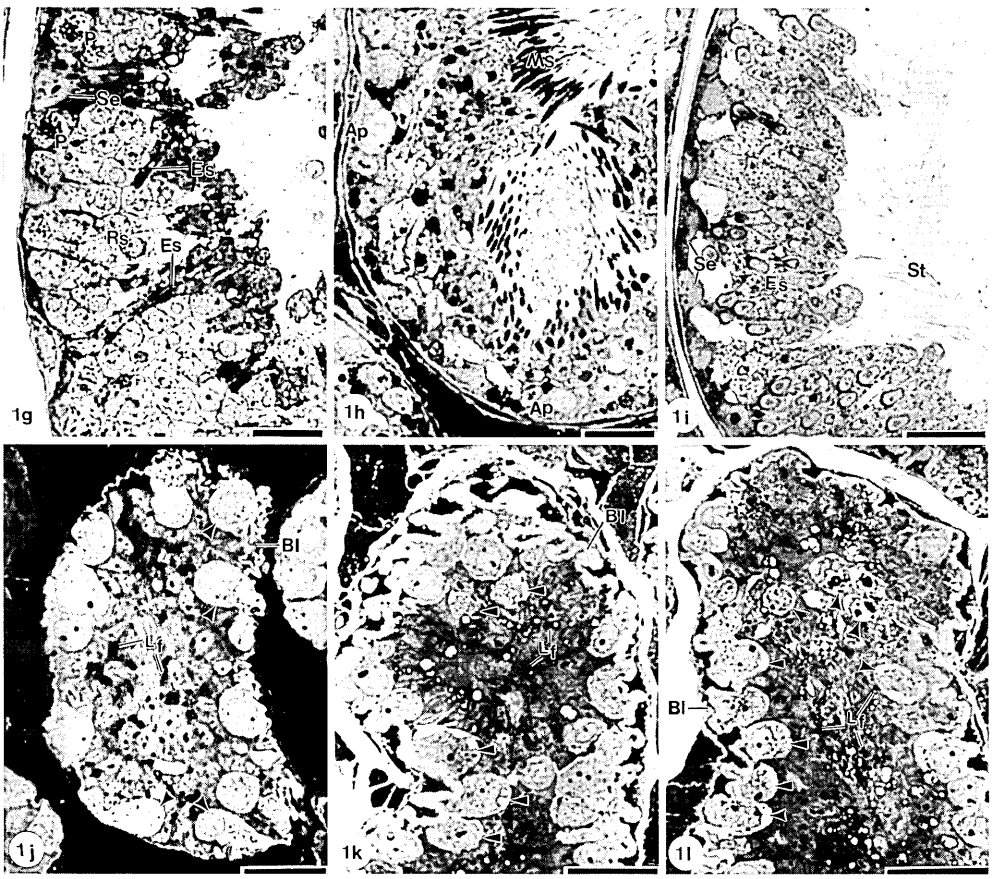


Fig. 1. Light micrographs showing the annual cycle of the seminiferous epithelium in *Myotis macrodactylus*. a, January sample; b, February sample; c, March sample; d, April sample; e, May sample; f, June sample; g, July sample; h, August sample; i, September sample; j, October sample; k, November sample; l, December sample. The lumen of the seminiferous tubules is open from July to September, and is closed from October to June of the next year. Spermatocytogenesis occurs from April to June. Spermiogenesis occurs from July to September. Redundant spermatogenic cells in the seminiferous tubules are engulfed by the phagocytosis of Sertoli cells in October. During the phagocytosis, the seminiferous tubule contains only Sertoli cells and spermatogonia (Figs. 1k, 1l and 1a–c). Numerous lipofuscin granules are observed from November to March in the next year (Resting stage). Ad and Ap, dark and pale types of spermatogonia; B, B type of spermatogonia; Bl, basal lamina; Es, elongated spermatids; L, leptotene primary spermatocyte; Lf, lipofuscin; Ms; mature spermatid; P, pachytene primary spermatocyte; Se, Sertoli cell; St, sperm tail; Z, zygotene primary spermatocyte; Arrowheads, degenerating spermatogenic cells. All scale bars=20μm .

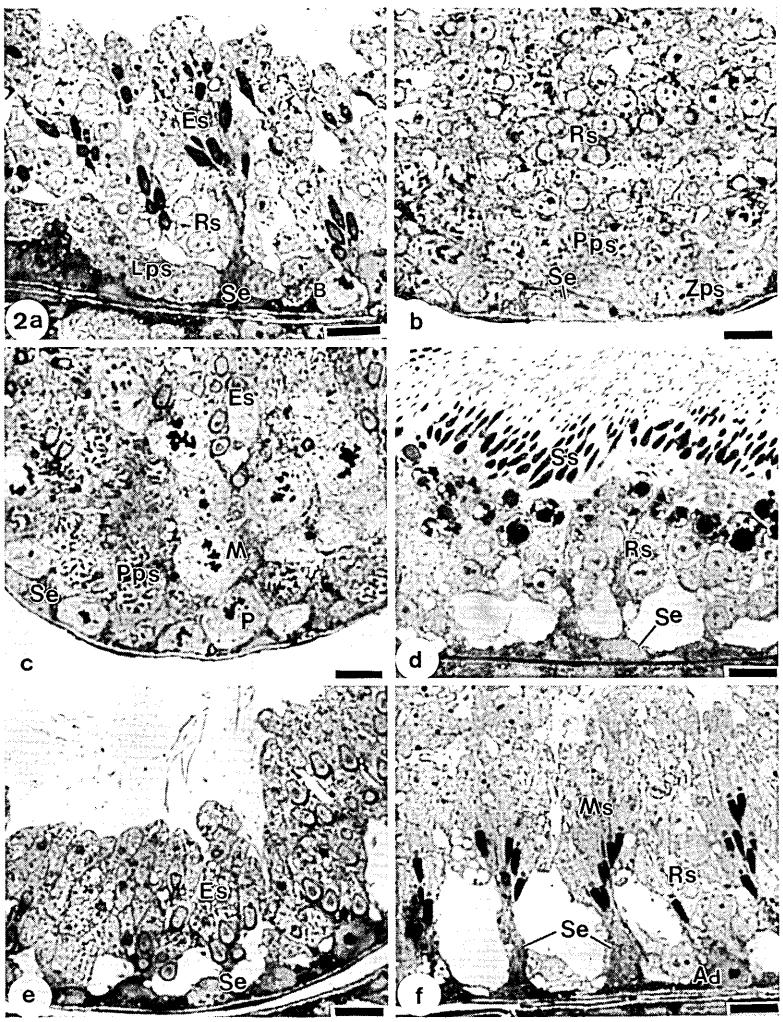


Fig. 2. Light micrographs showing stages 1 to 6 of the seminiferous epithelium cycle during spermiogenesis. a, Stage I shows type A spermatogonia; leptotene primary spermatocytes (Lps); round spermatids (Rs); elongating spermatids (Es) and Sertoli cells (Se). b, Stage II contains zygotene primary spermatocytes (Zps); pachytene primary spermatocytes (Pps); round spermatids (Rs) and Sertoli cells (Se). c, Stage III presents pachytene primary spermatocytes (Pps); prophase (P) and metaphase (M) of primary spermatocytes in meiosis I; elongated spermatids (Es) and Sertoli cells (Se). d, Stage IV shows round spermatids (Rs); spermatids of spermiation phase (Ss) and Sertoli cells (Se). e, Stage V show round spermatids (Rs); maturing spermats (MS) and Sertoli cells (Se). f, Stage VI contains elongating spermatids (ES) and Sertoli cells (Se). All scale bars = 10 μm.

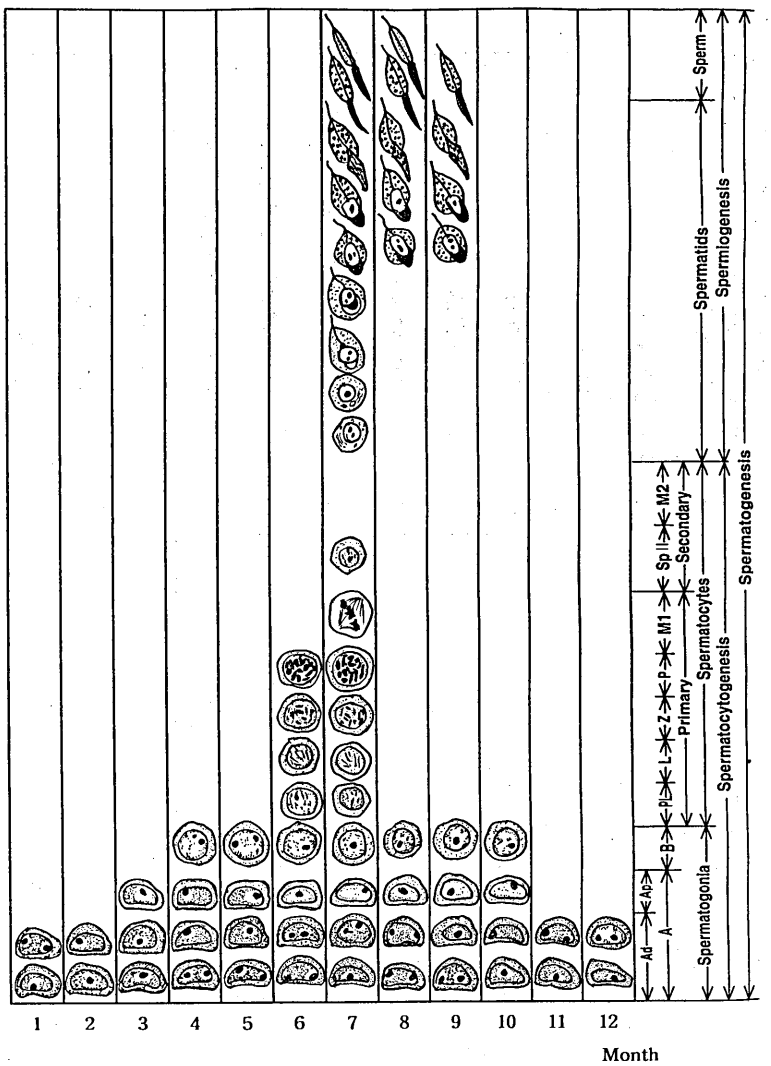


Fig. 3. The annual cycle of the spermatogenic cells in the seminiferous tubules. B spermatogonium begins to appear from April. Spermatogenesis begins in April, peaks in mid summer (July) and finishes in September. Spermatocytogenesis occurs from April to June only. Spermiogenesis occurs from July to September, and peaks in July. Individual spermatogonia, A dark (Ad) and A pale (Ap) and B, and spermatocyte preleptotene (PL), leptotene (L), zygotene (Z) and pachytene (P) are shown, in addition to spermatocytes undergoing the division of meiosis (M1), and secondary spermatocytes (Sp II). The progressive steps of spermatid development (S_{1-9}) are shown. *, A great many redundant spermatogenic cells (spermatids) are engulfed by the phagocytosis of Sertoli cells or else degenerate in September. **, Many redundant spermatogenic cells (B spermatogonia) are engulfed by the phagocytosis of Sertoli cells or degenerate in October. ▼, A few redundant A pale (Ap) spermatogonia degenerate in November and December. ▽, A few redundant A dark (Ad) spermatogonia degenerate in January.

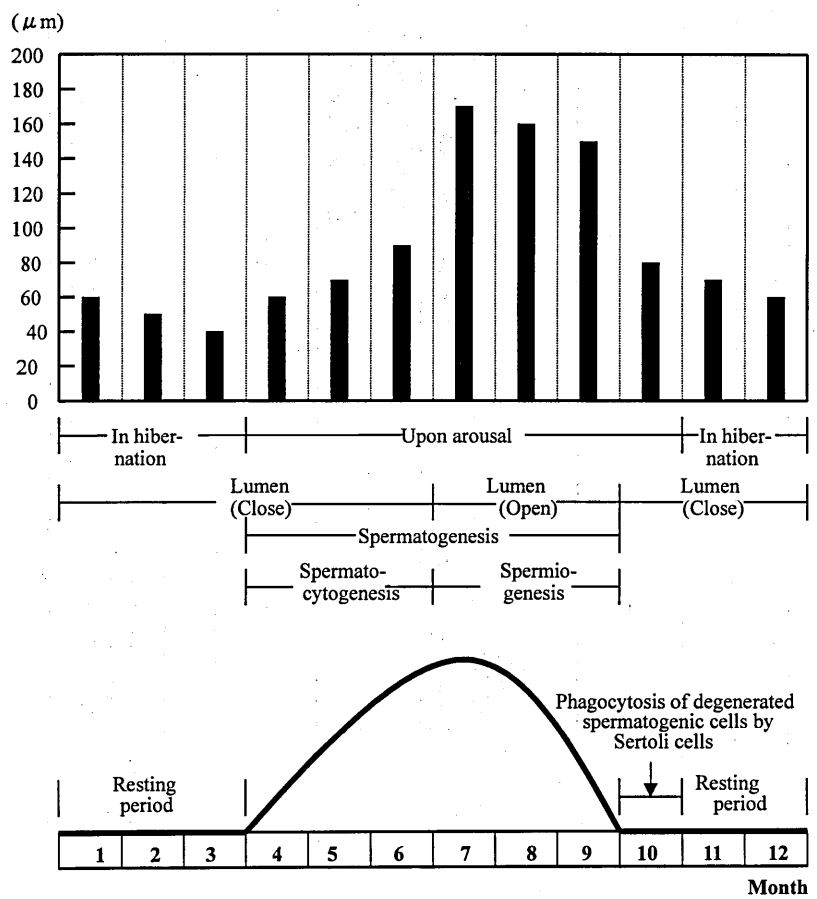


Fig. 4. The relationship between the monthly changes in the seminiferous tubule diameter (upper) and the annual cycle of the seminiferous epithelium (lower). The Seminiferous tubule diameters gradually begin to increase from April in the early arousal period, peak in July, and decrease rapidly to the size in October, before gradually decreasing to March of the next year. Spermatogenesis begins in April, peaks in mid summer (July) and is suspended from October to March of the next year. Spermatocytogenesis occurs from April to June only. Spermiogenesis occurs from July to September, peaking in July. From October, the redundant spermatogenic cells are engulfed by the phagocytosis of Sertoli cells. During the phagocytosis, the seminiferous tubule contains only Sertoli cells and spermatogonia (Resting period: from November to March).

In the October sample, immature spermatogenic cells in the seminiferous tubule were engulfed by the phagocytosis of Sertoli cells, and the lumen of the seminiferous tubule was closed (Fig. 1j). In this period, the basal lamina began to be prominent, and lipofuscin granules were observed in the seminiferous cytoplasm.

From November to March of the next year, A type spermatogonia cells existed in the seminiferous tubules (Figs. 1k, 1l, 1a, 1b and 1c). During these periods, the lumen was closed.

Spermiogenesis

Spermiogenesis of *M. macrodactylus* occurred from July to September. Based on the development of the acrosomic system and changes in the nuclear morphology, the transformation of spermatids into spermatozoa in *M. macrodactylus* from the July sample was divided into six stages (Fig. 2). The following cellular characteristics were noted.

Stage I. Type B spermatogonium attached to the basal lamina. Leptotene primary spermatocytes located near the basal lamina. Spermatids presented elongating nuclei with their heads oriented toward the Sertoli cell nucleus at the base of the tubule (Fig. 2a).

Stage II. Zygotene primary spermatocytes located close to the basal lamina. Round spermatids and pachytene spermatocytes were also observed (Fig. 2b).

Stage III. Pachytene primary spermatocytes located close to the basal lamina. The prophase and metaphase of primary spermatocytes in meiosis and elongated spermatids were observed (Fig. 2c). In particular, spermatogonial mitosis was observed at stage III only; mitosis is shown labeled in Fig. 2. The progressive steps of spermatids development ($S_{1\text{-}9}$) are shown in Figs 3, 4.

Stage IV. Round spermatids and spermatids of spermiation phase were observed in the seminiferous tubule (Fig. 2d).

Stage V. Only elongating spermatids were observed in the seminiferous tubule (Fig. 2e).

Stage VI. Round spermatids and maturing spermatids were observed in the seminiferous tubule (Fig. 2f).

DISCUSSION

In most seasonally breeding mammals, the endocrine and exocrine functions of the testis and the differentiation of spermatogenic cells are closely synchronized. Hibernating bats are exceptional in that they store sperm, have active accessory glands, and mate for many months after the seminiferous tubules have involved.

The temperate hibernating bat, *Myotis macroducylus* have very different reproductive patterns. First, cell differentiation of the seminiferous epithelium shows a number of monthly stages in the degree of appearance of spermatogenic cells in seminiferous tubules. Second, during hibernation, spermatogenesis does not occur, and redundant spermatogenic cells in the seminiferous tubules are engulfed by the phagocytosis of Sertoli cells. The phagocytosis continues throughout the period of hibernation, for example in *Rhinolophus ferrumequinum korai* (Lee *et al.*, 1993). *M. macroducylus* spermatogenesis begins in April, and peaks in mid summer (July). Spermatocytogenesis occurs from April to June only. Spermiogenesis reaches a peak in July (Figs. 2 and 4). The lumen of the seminiferous tubules is open in the active period (from July to September, see Figs. 1g, 1i and 2), and closed in the inactive period (during hibernation; October to June in next year, see Figs. 1j–1l and Figs. 1a–1f). These results were very

similar that of *Pipistrellus pipistrellus* (Racey, 1973a, b; Racey and Tam, 1974) and *Myotis lucifugus lucifugus* (Gustafson, 1987). The results suggest that the adaptive strategy for a long hibernation serves as a mechanism to regulate the breeding cycle.

In the noctule bat *Nyctalus noctula*, the time for the start of spermatogenesis is similar to that of *M. macroductylus*, but the finish of spermatogenesis takes place at a different time compared to that which runs until winter (Racey, 1974). According to Krutzsch and Crichton (1987), in Southeast Australia, the little mastiff bat *Mormopterus planiceps* displays a single annual spermatogenic cycle that commences in spring (September/October) and culminates in spermiogenesis in autumn (February–May). This result suggests that temperature may be a proximate influence on the reproductive cycle. In comparison, in the neotropical bat *Myotis nigricans*, the spermatogenic activity occurs continually from December through August, and from September through November most males are in a resting stage with regard to spermatogenesis (Wilson and Findley, 1971). In particular, spermatogenesis slows down or stops during September, October and November. In tropical Australia, for the sheath–tail bat *Taphozous georgianus*, spermatogenesis begins in early summer, peaks in autumn and declines with the onset of winter (Jolly and Blackshaw, 1987). Jolly and Blackshaw (1988) reported on the relationship between testicular migration and spermatogenesis and environmental facts. These studies suggest that temperature may be a proximate influence on reproduction in tropical Australia for the sheath–tail bat, *Taphozous georgianus*. Heideman *et al.* (1992) reported on the photoperiod treatments that might affect reproductive timing or reproductive cycle. Beasley and Zucker (1984) suggested photoperiod influences the reproductive physiology in male pallid bat, *Antrozous pallidus* by affecting the endrogenous circannual reproductive rhythm.

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