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Histological Investigation of Cyclic Variation in Secretory Activity of Seminal Vesicles in Emballonurid Bat, *Taphozous kacchensis* (Dobson)

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Abstract: The present study describes the cyclical changes in the secretory activity of the seminal vesicle in the emballonurid bat, *Taphozous kacchensis*. The secretory activity in the seminal vesicles during the quiescence cycle was not well marked in *T. kacchensis*. During the sexually quiescent period (May to August), seminal vesicles were regressed, the tubules were lined by cuboidal to low columnar epithelial cells with round to elongated darkly stained basally to centrally placed nuclei. Cytoplasm was basophilic containing fine or coarse secretory granules in the cytoplasm. The lumina of the tubules were devoid of secretion. During the pre-breeding (September to January) period, the tubules were enlarged and were lined by tall columnar epithelial cells with large spherical basally situated nucleus. The secretory blebs were seen arising from the apical surface of the cells and were seen releasing into the lumen. Lumina of the tubules were filled with homogenous eosinophilic secretion. During the breeding period (February to March), the seminal vesicle showed hypertrophy resulting in the increase in the tubular diameter as compared to that of quiescent period. The epithelial lining of the tubules was cuboidal with centrally placed nuclei. The tubular lumina were full of homogenous secretion during active pre-breeding and breeding period as the secretory material released into the lumen by both apocrine and merocrine modes. Regressive changes in the seminal vesicle were evident from April. The tubules showed gradual hypotrophy as the quiescent period approaches in May.

Keywords: Chiroptera, Bat, *Taphozous kacchensis*, Seminal vesicle, Epithelial cells, Tubule, Apocrine, Merocrine

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Introduction

Bats (Chiroptera) are among the most diverse and widely distributed group of mammals and can be

found in most continents. Bats are the only flying mammals and they have a wide range of feeding

and roosting habits, social behaviours, and reproductive strategies. They are second only to rodents in numbers of living genera and species, which represents nearly a quarter of all the species of mammals on earth, and the majority live in tropical and semitropical regions (Riede, 2004). Bat is a unique animal exhibiting variety of patterns of reproduction. They are important models to understand many interesting problems in reproduction, which have direct relevance to human beings both academically and for application (Krutzsch, 1979). The details of the structure of the reproductive system are generally not described. Even less is known about the function and physiological control of reproduction in the male (Krutzsch, 2000; Danmaigoro, *et al.*, 2014). Male bats also exhibit diversity in the timing and frequency of their reproductive cycles annually (Beguelini, *et al.*, 2013). In some species this may be expressed in unique functional (dysynchronous) timing between primary and accessory sex glands (Krutzsch, 1979; Gopalakrishna and Sapkal, 1986). Several authors have reviewed the progress of research on chiropteran reproduction (Bernard, 1989; Araujo *et al.*, 2013). Most of the reviews in the past dealt mainly with temperate zone bats. Later, attempts were made to provide more complete information about the patterns of reproduction in bats taking into consideration of both temperate and tropical zone species. Nonhibernating bats display a variety of reproductive patterns even though environmental cues often seen subtle and seasonality is poorly marked (Krutzsch, 1979; Zortea, 2003). Non-hibernating bats demonstrate reasonable synchrony between male and female reproductive processes. Spermatozoa are produced in testis and accessory sex glands are secretorally active at a time consistent with onset of the estrous cycle in the female. Breeding biology of some Indian bats was reviewed by Gopalakrishna and Sapkal (1986). They concluded autumn as the basic breeding season in the bats and only a few species have adopted spring as a breeding season. The protected storage of inseminated spermatozoa in the genital tract of

the female in autumn breeders of temperate regions appears to be an adaptation to bring forth the young ones in a season when there is abundant supply of food both for the mothers in lactation and the newly weaned young ones. The spring breeding in tropical bats is a modification of the same mechanism because even in these bats the time of delivery is so adjusted as to be most advantageous to the adults and the juveniles. The gross and microscopic structure of the primary (paired testes) and secondary (accessory glands) reproductive organs in bats follows the normal mammalian pattern, Pteropodidae (Pal, 1984a); Rhinopomatidae (Banerjee and Karim, 1986); Emballonuridae (Kitchener, 1973, 1976; Mokkapati and Dominic, 1976, 1977; Bhardwaj and Lall, 1979; Jolly and Blackshaw, 1988); Megadermatidae (Pal, 1984b); Rhinolophidae (Bernard, 1983, 1985; Krutzsch *et al.*, 1992); Phyllostomidae (Mokkapati and Dominic, 1976) and Vespertilionidae (Gopalakrishna, 1948; Gustafson, 1976, 1977; Richardson, 1977; Bernard, 1980; Krutzsch and Crichton, 1986, 1990a). Puga, *et al.* (2013) studied the structure, histochemistry and ultrastructure of the male reproductive accessory glands in the neotropical flat-faced fruit-eating bat, *Artibeus planirostris* (Chiroptera: Phyllostomidae).

Considering the worldwide distribution and immense diversity exhibited by members of the order Chiroptera, remarkably limited attention has given to reproduction in the male. The details of the structure of the reproductive system are generally not described. Even less is known about the function and physiological control of reproduction in the male. Male bats also exhibit diversity in the timing and frequency of their reproductive cycles annually. In some species this may be expressed in unique functional (dysynchronous) timing between primary and accessory sex glands. There are many gaps in our basic knowledge of the morphology and physiology of the male reproductive system of Chiroptera. Only a few taxa have been examined over an extended period of time in which seasonal

changes in the system have been recorded and related to organ structure and physiological function. A very limited number of species of bats have seasonal reproductive variations correlated with male reproductive events. The aim of the present study was to study secretory process at the histo-architectural level in accessory sex organ (seminal vesicle) of *Taphozous kacchensis* during the reproductive cycle as it passes from the sexually quiescent phase to sexually active phase. This will facilitate the future physiological studies and functional interpretations of this accessory gland in reproduction in bats.

Materials and Methods

Specimens of *Taphozous kacchensis* were collected from Kampa-Tempa (20°40'42.7"N 79°36'41.1"E), Chandrapur (Maharashtra State), 100 km away from Nagpur, India. The specimens were collected in such a way that every calendar month will represent by one or more collections or coincide with the key time of the reproductive cycle. The specimens were netted at random with the help of butterfly net and brought alive to the laboratory with minimum stress and constant supply of food and glucose water. Live animals were at once anesthetized with chloroform and weighed immediately and then they were dissected and fixed in various fixatives like aqueous Bouins, Calcium acetate formalin (CAF), and neutral formalin for histological studies. After fixation for 24 h, tissues were washed with 70% ethanol. For histological examination, the tissues were dehydrated through the graded series of ethanol, cleared in xylene and embedded in paraffin wax. Thin sections of 6 µm thickness were cut with the help of Leica 2417 microtome. The sections were stained with Ehrlich's haematoxylin and counter stained with eosin (HE) and mounted in DPX. The micro-measurements were taken with the help of an ocular micrometer calibrated to a stage micrometer. Activity of the seminal vesicle was quantified by measuring the height of the secretory epithelium from a minimum of 3-4 specimens of different stages of reproductive cycle. The photographs were taken with the help

of a Karl Zeiss camera attached to the microscope and enlarged to the required size.

Results

Morphometry:

The average weight of the seminal vesicle was 6.46 ± 0.22 mg in the month of May to August when animals were spermatogenetically inactive (Table 1). There was a gradual increase in the weight of the gland. The average weight of the gland was 8.6 ± 0.21 mg in the month of September to January (Table 1) when testis showed an initiation of spermatogenesis and beginning of secretory activity in the seminal vesicle. There was a further increase in the weight of the gland in the month of February and March. The weight of the gland was highest (12.5 ± 0.16 mg) when the spermatogenesis was at peak in the testis and seminal vesicles were hypersecretory in nature. During the sexually quiescent period, the lumina of the tubules were empty, small in size and their diameter decreased to 50.28 ± 0.56 µm (Table 2). The epithelial height of the cell was about 9 µm (Table 2). The cells showed no secretory activity. During the pre-breeding period the diameter of the tubules increased to 101.24 ± 0.89 µm and the height of the epithelial cells was 12.2 ± 0.5 µm (Table 2). The diameter of the tubules was highest (152.32 ± 1.18 µm) (Table 2) during the sexually active period, and lumina of the tubules were completely filled with secretory blebs and the height of the epithelial cells decreased due to the release of their content into the lumen. It was about 7µm. The relationship between weight of testis and weight of seminal vesicles during different periods of the sexual cycle is shown in Figure 1. Figure 2 illustrates relationship between weight of seminal vesicles, tubular diameter and epithelial height of seminal vesicles during different periods of sexual cycle.

Seminal vesicle during inactive period:

The seminal vesicles were found in regressed condition during the inactive period. They became hypotrophied. The regression started from the month of May when vacuolations were seen in the

Table 1: Weight of the testis and seminal vesicle during the different phases of the reproductive cycle

Month of Collection	Reproductive Phase	Weight of testis (mg) Mean $\pm$ SEM (n = 5)	Weight of the seminal vesicle (mg) Mean $\pm$ SEM (n = 5)
May-August	Inactive	12.66 $\pm$ 0.24	6.46 $\pm$ 0.22
September-January	Pre-breeding	14.46 $\pm$ 0.31	8.60 $\pm$ 0.21
February-March	Breeding	28.55 $\pm$ 0.28	12.50 $\pm$ 0.16

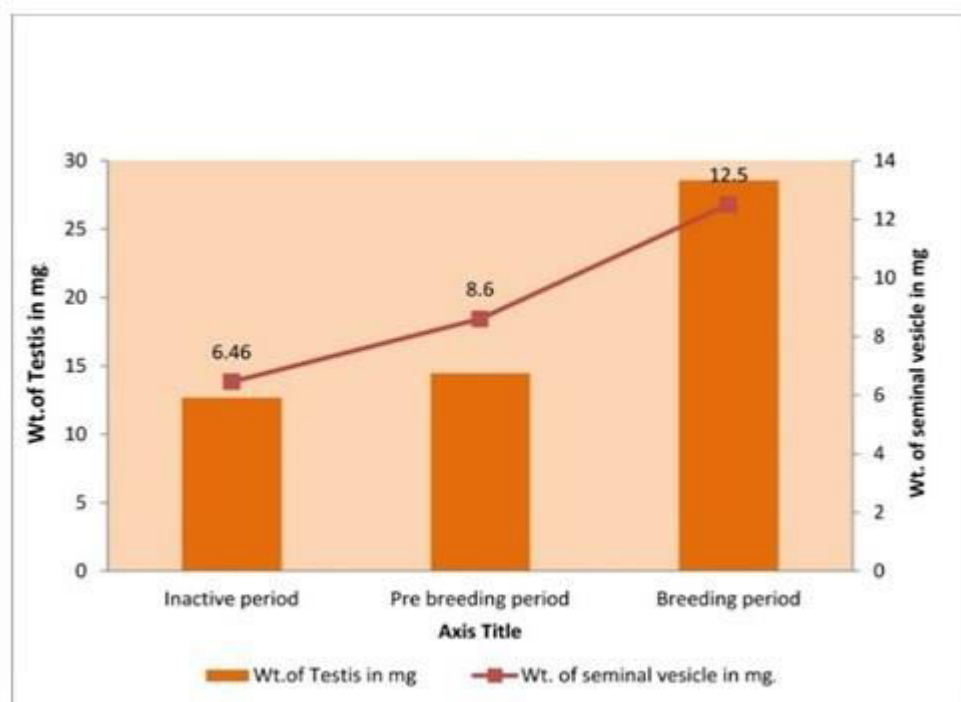


Fig. 1: Relationship between weight of testis and weight of seminal vesicle during different periods of sexual cycle.

Table 2: Weight of the seminal vesicle, diameter of the tubule and epithelial height during the different phases of the reproductive cycle

Month of Collection	Reproductive Phase	Weight of the seminal vesicle (mg) Mean $\pm$ SEM (n = 5)	Diameter of tubule (μ m) Mean $\pm$ SEM (n = 15)	Epithelial height (μ m) Mean $\pm$ SEM (n = 15)
May-August	Inactive	6.46 $\pm$ 0.22	50.28 $\pm$ 0.56	9 $\pm$ 0.5
September-January	Pre-breeding	8.6 $\pm$ 0.21	101.24 $\pm$ 0.89	12.2 $\pm$ 0.5
February-March	Breeding	12.5 $\pm$ 0.16	152.32 $\pm$ 1.18	7 $\pm$ 1

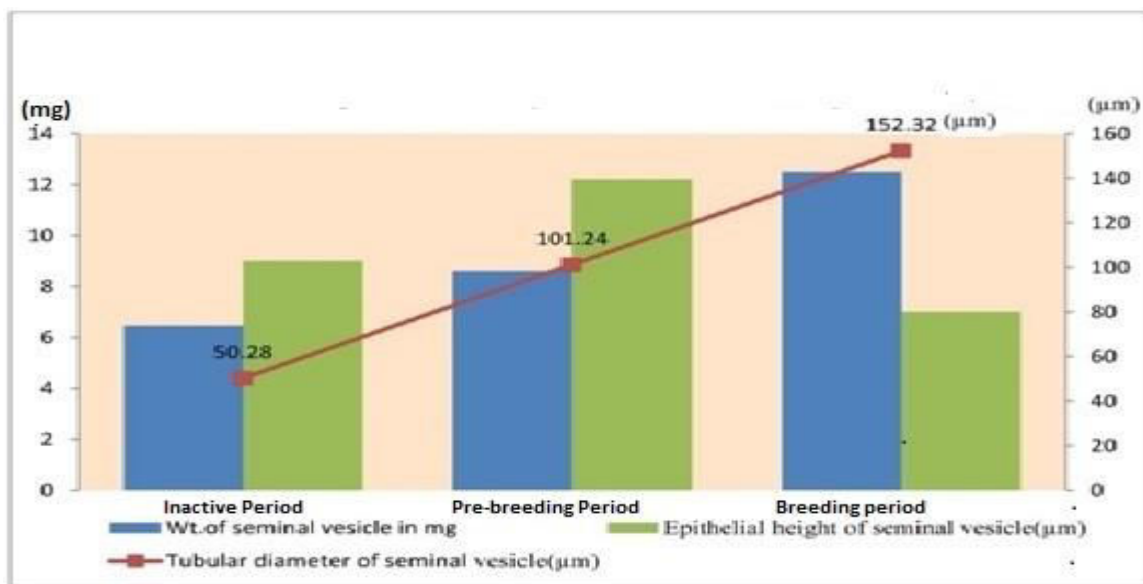


Fig. 2: Relationship between weight of seminal vesicle, tubular diameter and epithelial height of the seminal vesicle during different periods of sexual cycle.

seminal vesicles. During this regressed period, there was shrinkage of tubules resulting in the decrease in the tubular diameter. The intertubular connective tissue increased separating the tubules relatively from each other. The tubules were lined by cuboidal to low columnar epithelial cells. Nuclei were round to elongate, darkly stained and basally or centrally placed. Cytoplasm was basophilic containing fine or coarse secretory granules in the cytoplasm. The lumina of the tubules were devoid of secretion (Figs. 3-6).

Seminal vesicle during pre-breeding period:

During the pre-breeding period, seminal vesicles were increased in size as compared to previous stage. There was also an increase in the size of the tubules during this stage. These tubules were lined by tall columnar epithelial cells with large spherical basally situated nucleus. Cytoplasm was filled with basophilic granular secretions. Thus, the secretory material was packed in the form of numerous small globules. The secretory blebs were seen arising from the apical surface of the cells and were seen releasing into the lumen. Lumina of the tubules were filled with homogenous eosinophilic secretion. In early stages, the tubules showed some secretion attached to the luminal border. The mode of

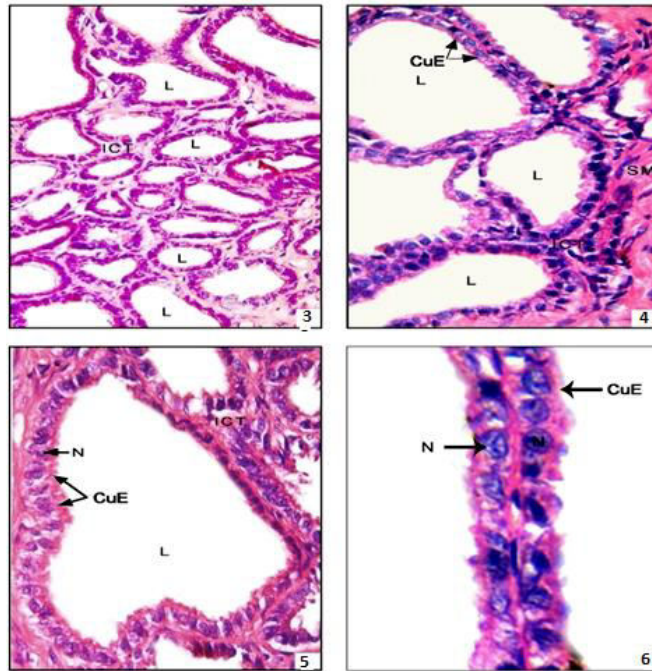
secretion in the seminal vesicle of this bat was apocrine and sometimes whole cell along with the nuclei contributing to the luminal secretion. The intertubular connective tissue stroma was reduced due to enlargement of the tubules (Figs. 7-10).

Seminal vesicle during breeding period:

During the breeding period, the seminal vesicle showed hypertrophy resulting in the increase in the tubular diameter as compared to that of quiescent period. At the height of the activity, the epithelial lining of the tubules was cuboidal with centrally placed nuclei. The tubular hypertrophy continued and culminated after attaining a maximum tubular diameter in the month of February and March when copulation occurred. The height of the epithelium decreased as a result of continuous oozing of secretory material in the form of blebs. The tubular hypertrophy resulted in the reduction of inter-acinar connective tissue. In the month of March, the tubular lumen was full of homogenous secretion (Figs. 11-14).

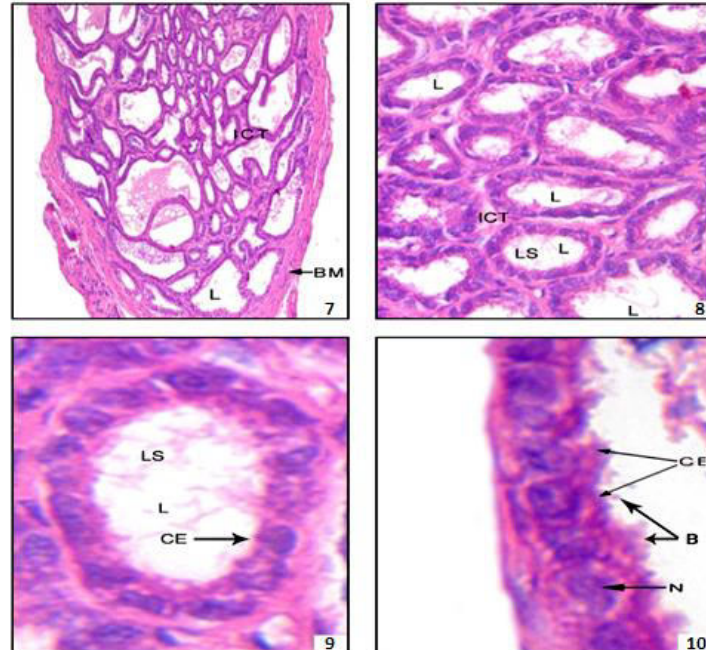
Discussion

It is well known that the efficient reproduction in male animals is dependent to a great extent on the



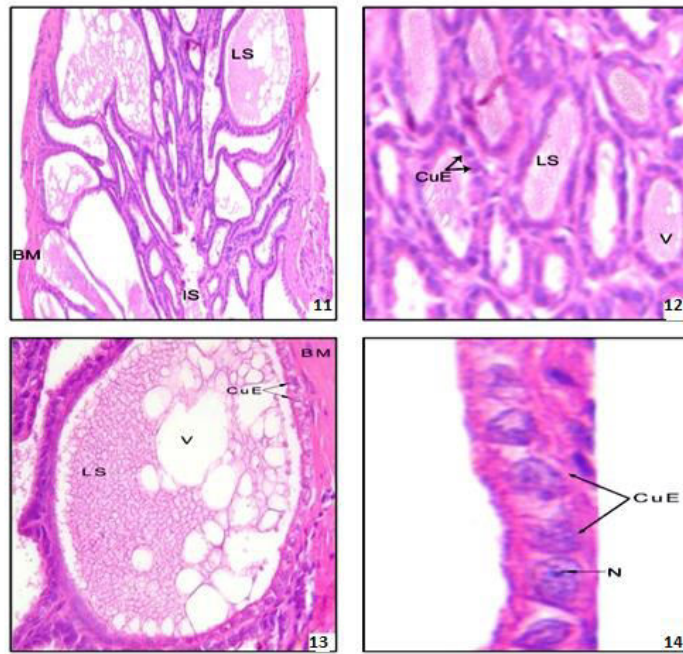
Figs. 3, 4: Transverse section of the seminal vesicle during the inactive period. Note the presence of small tubules. The tubules are lined by cuboidal epithelial cells (CuE). Cytoplasm is basophilic and narrow lumina (L) without secretion. HE x 400.

Figs. 5, 6: Magnified part of the tubule of the seminal vesicle. Note the tubules are lined by cuboidal (CuE) to columnar epithelial cells (CE). Nuclei are round darkly stained basally or centrally placed. Cytoplasm is basophilic containing fine or coarse secretory granules in the cytoplasm. Lumen is empty. HE x 800.



Figs. 7, 8: Transverse section of the seminal vesicle during pre-breeding period. Note that the tubules are having irregular shape and are lined by tall columnar epithelial cells (CE). Cytoplasm is filled with basophilic granular secretions. Lumen (L) of the tubules is filled with homogenous eosinophilic secretions. HE x 300.

Figs. 9, 10: Magnified part of the tubule of the seminal vesicle during pre-breeding period. Note the increased size of tubules during this stage. These tubules are lined by tall columnar epithelial cells (CE) with large spherical basally situated nucleus (N). Cytoplasm is filled with basophilic granular secretions. Note the apical part of the cytoplasm buldges out at some places in the form of secretory blebs (B) which are spherical and are seen releasing into the lumen (L). HE x 800.



Figs. 11, 12: Transverse section of the seminal vesicle during active breeding period. Note the increase in the size of the tubules. The intertubular connective tissue layer (ICT) also becomes thin due to increase in the size of the tubules. Lumina (L) are completely filled with homogenous eosinophilic secretions (LS). HE x 300 and x 400.

Figs. 13, 14: Magnified part of the tubule of the seminal vesicle during active breeding period. Tubules are lined by low columnar (CE) to cuboidal epithelial cells (CuE) with centrally placed nuclei (N). HE x 600 and x 800.

effective functions of the genital glands. The accessory genital glands are a series of glands situated between the vas deferens and the root of the penis. They include the ampullary, vesicular, prostate, bulbourethral and urethral glands (Banks, 1993; Davies Morel, 2003). Collectively, these glands are responsible for the secretion of the seminal plasma, which forms the major fluid fraction of semen. Seminal plasma provides the substrate for conveying the sperm to the female genital tract and ensuring final maturation. The reproductive tract of male mammals generally consists of the testes and associated epididymis, vasa deferentia, accessory sex gland complex, urethra and penis. The accessory sex gland complex consists of ampullary gland, prostate glands, urethra, Cowper's glands and seminal vesicles (Krutzsch, 1979; Danmaigoro *et al.*, 2014). However, not all glands are present in every mammalian order (Setchell and Breed, 2006). In the Chiroptera, the gross and microscopic structure of the primary (paired testis) and

secondary (accessory glands) reproductive organs in bats (Crichton, 2000) follow the normal mammalian pattern. In *Taphozous kachhensis*, the male genital tract consists of pair of testis, pair of epididymis, vasa deferentia, pair of seminal vesicles, unpaired prostate and pair of Cowper's glands. Similar reproductive structures are reported in other emballonurid bats (Kitchner, 1973, 1976; Mokkapati and Dominic, 1976, 1971; Swami and Lall, 1979; Bhardwaj and Lall, 1979; Pal, 1984b; Jolly and Blackshaw, 1987, 1988). The seminal vesicles of *Taphozous kachhensis* showed cyclic changes in weight, tubular diameter and epithelial height with relation to the reproductive cycle. The seminal vesicle was found in regressed condition during the inactive period. They became hypotrophied. The weight of the seminal vesicle, diameter of tubule and height of epithelium was low during inactive period when testes were spermatogenetically inactive. The tubules were lined by cuboidal to low columnar epithelial cells with darkly stained basally situated nuclei.

Cytoplasm was basophilic containing fine or coarse granules. The lumina were devoid of secretion. During the pre-breeding period, there was an increase in weight, diameter of tubules and height of the epithelial cells when testis showed an initiation of spermatogenesis. The tubules were lined by tall columnar cells with large basally situated nucleus. Cytoplasm was filled with basophilic granular secretion. The cytoplasmic secretory blebs were seen arising from the apical surface of the cells and were released into the lumen. Lumina of the tubules were filled with homogenous eosinophilic secretion. At the peak of the spermatogenesis in testis during the breeding period, the seminal vesicles exhibited hypertrophy resulting in an increase in weight, tubular diameter and epithelial height as compared to the previous stages. At the height of the activity, the epithelial lining became cuboidal to low columnar because of loss of cytoplasmic matrix in the form of blebs. The lumina were full of homogenous secretion. Thus in seminal vesicles of *Taphozous kachhensis*, the mode of secretion was merocrine as well as apocrine. Cyclic histological changes in seminal vesicles have been studied in other species of bats. These glands are greatly hypertrophied during the reproductively active state of bats. Their tubular lumina are filled with highly granular, eosinophilic secretion that emanate from columnar epithelial cells. Reproductively inactive glands are much reduced in size and are lined by low columnar or cuboidal epithelium. Cytoplasm shows fine granular secretory material and lumina of the tubules are empty or little secretion in *Pipistrellus herpestes* (Krutzschn, 1975); *P. dormeri* (Gadegone and Sapkal, 1983); *Taphozous longimanus* (and Dominic, 1982a); *P. giganteus* (Rajalakshmi and Prasad, 1970); *T. melanopogon* and *Miniopterus* (Gadegone *et al.*, 1994); *Macrotus californicus* (Krutzschn *et al.*, 1976) and *Hipposideros lankadiva* (Gadegone *et al.*, 2007), supporting the present observations on the seminal vesicles of *T. kachhensis*. During sexually inactive period, seminal vesicle is in regressed condition. The

secretory epithelial cells are low columnar with basally situated nuclei. A few secretory granules are seen in the apical cytoplasm and lumen contains scanty secretion. As the bat enters into the pre-breeding and sexually breeding period, the structural features of the secretory epithelial also changes. Now the cells are tall columnar with basally situated nuclei. The cytoplasm is filled with large number of secretory vesicles of varying shapes and sizes. Some secretory vesicles are empty and appear as vacuoles. The secretory vesicles contain electron dense granules surrounded by electron lucent space. These secretory vesicles release their content into the lumen by exocytosis, showing merocrine mode. The secretion of secretory epithelial cells is released by forming secretory or cytoplasmic blebs. These blebs detach from the apical surface of the cells and release into the lumen. They dissolve and form finely granular content and homogenous secretion in the lumen. Thus in *Taphozous kachhensis*, both merocrine as well as apocrine mode of secretion is observed.

Conclusion

The seminal vesicle of *Taphozous kachhensis* showed cyclic changes in weight, tubular diameter and epithelial height with relation to the reproductive cycle. The seminal vesicle was found in regressed condition during the inactive period. They became hypotrophied. The weight of the seminal vesicle, diameter of tubule and height of epithelium was low during inactive period when testis was spermatogenetically inactive. The tubules were lined by low columnar epithelial cells with darkly stained basally situated nuclei. Cytoplasm was basophilic containing fine or coarse granules. The lumina were devoid of secretion. During the pre-breeding period, there was an increase in weight, diameter of tubules and height of the epithelial cells when testis showed an initiation of spermatogenesis. The tubules were lined by tall columnar cells with large basally situated nucleus. Cytoplasm was filled with basophilic granular secretion. The cytoplasmic

secretory blebs were seen arising from the apical surface of the cells and were released into the lumen. Lumina of the tubules were filled with homogenous eosinophilic secretion. At the peak of the spermatogenesis in testis during the breeding period, the seminal vesicles exhibited hypertrophy resulting in an increase in weight and tubular diameter, epithelial height as compared to the previous stages. At the height of the activity, the epithelial lining became cuboidal to low columnar because of loss of cytoplasmic matrix in the form of blebs. The lumina were full of homogenous secretion. In seminal vesicles of *Taphozous kachhensis*, the mode of secretion was merocrine as well as apocrine.

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