



Original Article

Ultrastructure of the secretory epithelial cells of the Cowper's gland in the Indian fruit bat, *Rousettus leschenaulti* (Desmarest) during the reproductive cycle



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ABSTRACT

The present paper describes the ultrastructural characteristics of the Cowper's glands of the Indian fruit bat, *Rousettus leschenaulti* during its sexually inactive-breeding cycle. The functional significance of the secretions of the Cowper's gland in reproduction is discussed. In *Rousettus*, Cowper's glands are small, pear-shaped, bilaterally symmetrical and are situated on either side of the base of the penis. Each lobule is made up of secretory acini lined by columnar or pyramidal cells. Ultrastructurally, the secretory epithelial cells are characterized by well-developed rough endoplasmic reticulum, extensively developed Golgi complex, and mitochondria. Three different types of secretory granules can be identified on the basis of electron density. These granules represent the different stages of granule maturation. The secretory products are released into the lumen both by apocrine and merocrine modes. The secretory material synthesized by the Cowper's gland may be involved in various male reproductive processes of this species of bat.

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1. Introduction

Considering the worldwide distribution and diversity exhibited by members of the order Chiroptera, remarkably little attention has been given to reproduction in the males. 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 [1]. 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 [2,3]. The male accessory organs show great plasticity in form and species distribution. These glands contribute various substances such as fructose, citric acid, sialic acid, proteins, and zinc to the ejaculate [4–6]. The light microscopic morphology of the accessory sex glands of Chiroptera [1] remains poorly understood, and only a single report is available on the ultrastructure of the accessory sex glands of chiropteran bats, *Artibeus planirostris* [7] and *Molossus molossus* [8]. The aim of the present study was to document the ultrastructural changes in the Cowper's gland of *Rousettus* as it passes from the sexually quiescent into the sexually active phase. This will facilitate future physiological studies and functional interpretations of this accessory gland in reproduction in bats.

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2. Materials and methods

2.1. Animals

The Indian fulvous fruit bat, *R. leschenaulti* (Desmarest) was selected for the present study because of its unique reproductive habits. This bat experiences two pregnancies in the year in quick succession, the first conception occurring in November–December and deliveries in March–April and the second pregnancy commencing immediately after parturition with deliveries occurring in July. Males are sexually active from October to April and the testes show two peak periods of spermatogenetic activity, one in October–November and another during February–March, separated by slight regression in January. Spermatogenesis ceases completely after April in the next breeding season. Male reproductive organs are inactive from May to September [9].

Animals were netted during daytime from an unused tunnel of a manganese mine at Kandri, about 60 km from Nagpur, Maharashtra State, India throughout the year, representing different reproductive states. Adult males were trapped alive, held in plastic cages and transported to the laboratory with minimum stress and constant supply of food such as apples and bananas and glucose water. The sexually active males were distinguished from non-sexually active males by observing the position of testes. The testes are abdominal in position in the immature and nonsexually active males and descend into temporary scrotal pouches in sexually active males [9]. In each collection, four animals were chloroformed and the testes and accessory sex glands were separated, cleaned of fat and connective tissues, and fixed in alcoholic Bouin's fluid. The tissues were dehydrated through a graded series of ethanol, cleared in xylene, embedded in paraffin wax, and sectioned at 5–6 μm thickness using a Leica 2417 microtome (Leica, Jena, Germany). The sections were stained with Ehrlich's hematoxylin and eosin for microscopic observation [10].

2.2. Ultrastructural study

For electron microscopic study, four animals were killed from each sexually quiescent, prebreeding and sexually active periods. The Cowper's glands were sliced into 1-mm pieces and fixed in fresh ice-cold 3% glutaraldehyde for 3 hours and then 4 hours in 0.1 M cacodylate buffer, and then postfixed for 1–2 hours in 1% 0.067 M cacodylate-buffered osmium tetroxide. After dehydration with a graded series of alcohol, the tissues were cleared in propylene oxide solution, and embedded in Araldite resin that was polymerized at 60 °C. Ultrathin sections from selected blocks were cut with a glass knife and picked up on 400-mesh copper grids. Sections were double stained with 10% alcoholic uranyl acetate for 20 minutes and for 10 minutes in Reynold's lead citrate and examined under a JEM Jeol-100s electron microscope (Jeol, Tokyo, Japan) at 80 kV accelerating voltage, and photographed.

3. Results

Microscopic study of Cowper's glands of *Rousettus* revealed that they were small, pear-shaped, bilaterally symmetrical and situated on either side of the base of the penis. They were enclosed in a fibromuscular capsule and separated into lobules by connective tissue and skeletal muscle septae that entered the gland from the capsule. Each lobule was made up of acini. The acini were secretory and each acinus was lined by columnar or pyramidal cells. During the sexually quiescent period (July), Cowper's glands were regressed. The acini were lined by low columnar cells with darkly stained, basal to central nuclei. The cytoplasm and lumen showed basophilic secretion. During the prebreeding (September) and active breeding (October) periods, the acini were enlarged and lined by tall columnar epithelial cells. The acinar lumen contained a homogeneous secretion. Regressive changes in the Cowper's gland were evident from May. The acini and cells underwent gradual hypotrophy as the quiescent period approached June.

3.1. Sexually quiescent period

Bats collected in July were sexually inactive and the testes were aspermatogenic. The acini were secretory and each acinus was lined by pyramidal or low columnar cells with basally situated nuclei. The nuclei were round to oval or elongated and contained one or two nucleoli. Heterochromatin clumps were distributed in the nucleoplasm and some chromatin material was seen adherent to the inner surface of the nuclear envelope. The Golgi apparatus was well developed and placed juxtannuclear in position. It consisted of five or six Golgi lamellae, arranged in parallel, forming a semicircle. The Golgi lamellae were curved and dilated. Associated with Golgi lamellae, small secretory vesicles with electron-lucent material and vacuoles containing electron dense material were present in the Golgi zone. Large numbers of coated vesicles were present near the Golgi lamellae as well as in apical cytoplasm. Rough endoplasmic reticulum (RER) was present, associated with secretory vesicles and in the form of short and long lamellate cisternae dotted with ribosomes. Mitochondria were numerous and distributed throughout the cytoplasm. They showed lamellar cristae. Some mitochondria were vacuolated and showed cristae at the periphery. The apical surface of the cells showed many microvilli that were thick, short or elongated. These microvilli projected into the lumen. Some microvilli were seen lying free in the lumen. Junctional complexes were seen between two adjacent cells. Desmosomes were present on the lateral membranes of two adjacent cells. In some cells, basal plasma membranes showed interdigitations.

The secretory granules were numerous and distributed throughout the cytoplasm. They showed various degrees of electron density. They varied in shape and size. Secretory granules were oval to round in shape. Three types of secretory granules were identified, based on their electron density. The Type 1 secretory granules were few in number and contained electron-lucent material; Type 2 secretory granules contained a small amount of

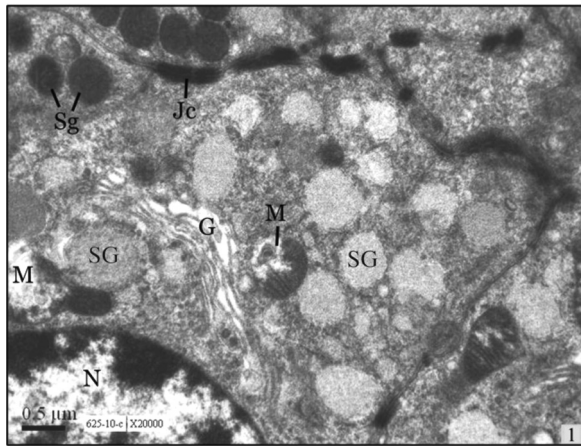


Fig. 1. High power electron micrograph of acinar cells during the sexually inactive period showing well-developed Golgi apparatus (G), mitochondria (M) near the Golgi, and a large number of electron-lucent secretory granules (SG) and electron-dense secretory granules (Sg) in the supranuclear part of the cytoplasm and Golgi zone. Junctional complexes (Jc) are prominent between the two adjacent cells. 20,000 \times magnification.

electron-dense material; and Type 3 secretory granules contained a high amount of electron-dense material. In the cytoplasm, large vacuoles were observed, which contained electron opaque to electron-lucent materials. The electron-dense secretory granules were seen just below the apical plasma membrane. Some of these fused with the apical plasma membranes and released their contents into the lumen, showing a merocrine mode of secretion. The lumen showed scanty homogeneous secretion. Thus, the secretory apparatus was well developed in the acinar cells of Cowper's gland during the inactive period (Figures 1 and 2).

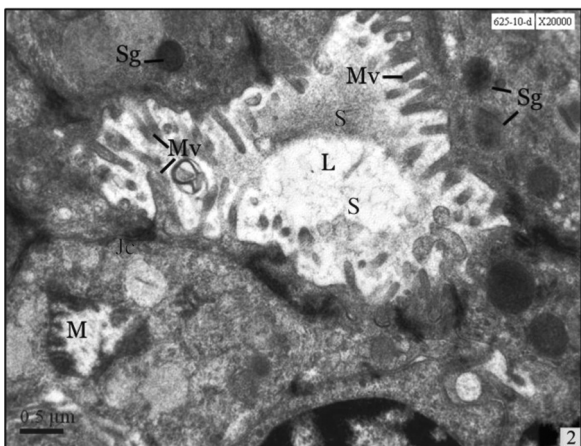


Fig. 2. High power electron micrograph of acinar cells showing mitochondria (M) with lamellar cristae and junctional complexes (Jc). The electron-dense secretory granules (Sg) lie below the apical plasma membrane and fuse with the apical plasma membrane, releasing their content into the lumen. The luminal surface of acinar cells shows long and thick microvilli (Mv) projecting into the lumen (L). The lumen content homogeneous electron-dense and electron-opaque secretion (S). 20,000 \times magnification.

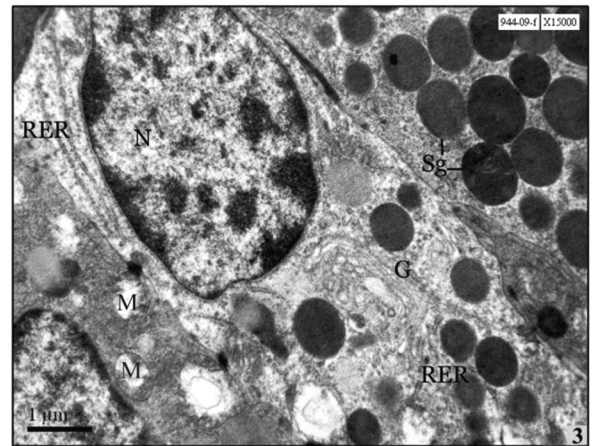


Fig. 3. Electron micrograph of Cowper's gland during prebreeding period showing acinar cell containing large basal nucleus (N). Golgi apparatus (G) is well developed. Coated vesicles are also seen in the Golgi zone. Mitochondria (M) with lamellar cristae are seen near the Golgi. Apical cytoplasm is filled with moderately electron-dense and highly electron-dense secretory granules (Sg) in the supranuclear cytoplasm. Rough endoplasmic reticulum (RER) in the form of short tubular and lamellar cisternae dotted with ribosomes is observed near the perinuclear part of the cytoplasm. 15,000 \times magnification.

3.2. Prebreeding period

Bats collected in September were in recrudescence. The testes showed initiation of spermatogenesis and various stages of spermatogenesis up to spermatid formation. During this stage, acini of the Cowper's gland were lined by tall columnar or pyramidal cells. Nuclei were oval or round. Heterochromatin clumps were distributed in the nucleoplasm. Some of the chromatin material was attached to the inner surface of the nuclear membrane. The Golgi apparatus was well developed and consisted of 8–10 Golgi lamellae that were dilated and arranged in parallel stacks. These lamellae were associated with large vac-

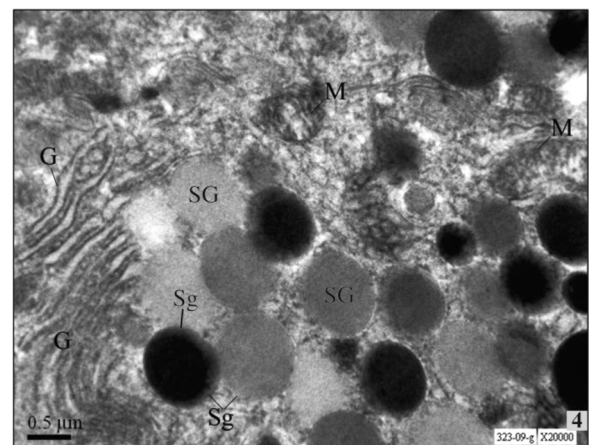


Fig. 4. Electron micrograph of the acinar cells of Cowper's gland during the prebreeding period shows well-developed Golgi apparatus (G). Mitochondria (M) are vesicular and elongated with lamellar cristae. Secretory granules with various electron densities are seen in the cytoplasm. 20,000 \times magnification.

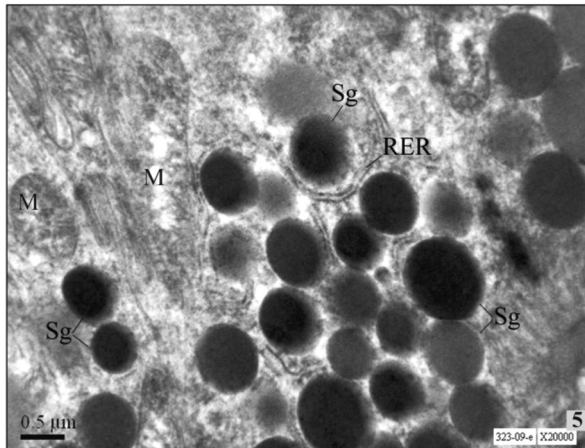


Fig. 5. Electron micrograph of the acinar cells of Cowper's gland during the prebreeding period showing large number of electron-dense granules (Sg) of various sizes and electron densities surrounded by cisternae of rough endoplasmic reticulum (RER). 20,000 \times magnification.

ules containing electron-lucent material (Figures 3 and 4). Mitochondria were round to rod-shaped with lamellar cristae distributed throughout the cytoplasm. Some mitochondria showed vacuolations and cristae at the periphery. RER was in the form of lamellar cisternae that were arranged in parallel. Cisternae surrounded the secretory granules in the cytoplasm. In some cells, lamellar cisternae were dilated and showed round to oval shapes dotted with ribosomes containing electron-lucent material (Figures 5 and 6).

The secretory granules of varying shapes and sizes were distributed throughout the cytoplasm. Three different types of secretory granules were observed as described in the sexually quiescent period. They were seen below the

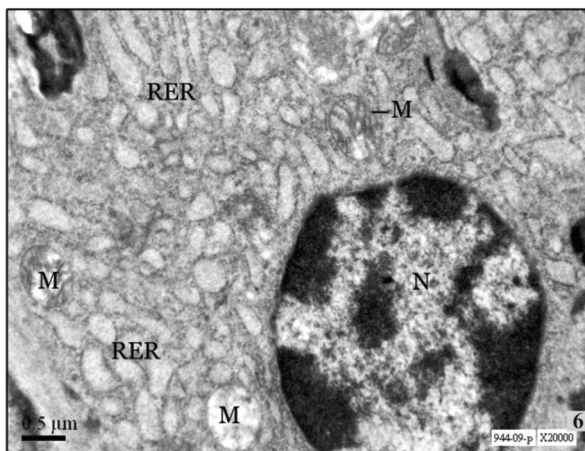


Fig. 6. Electron micrograph of the acinar cells during the breeding period showing large nucleus (N) and mitochondria (M) with lamellar cristae. Note that the cytoplasm is filled with dilated cisternae of rough endoplasmic reticulum (RER) containing electron-lucent material. 20,000 \times magnification.

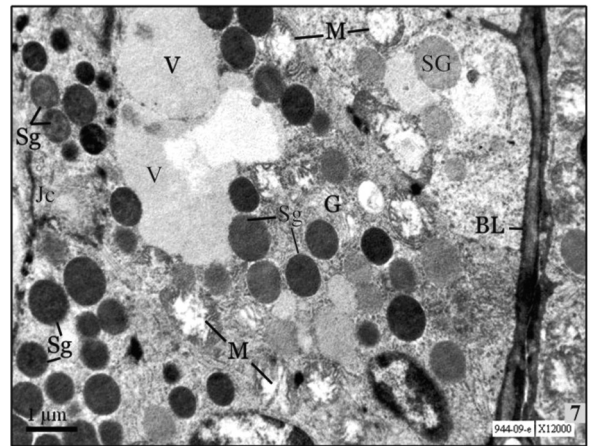


Fig. 7. High-power electron micrograph of acinar cell during the breeding period showing moderately developed Golgi apparatus (G). Mitochondria (M) are numerous with lamellar cristae distributed throughout the cytoplasm. Two to three secretory granules fuse and form large vacuoles (V) containing electron-lucent secretory material. Large numbers of secretory granules (Sg) containing secretory material of various densities are seen. Junctional complexes (Jc) are seen on lateral side of adjacent cells. 12,000 \times magnification.

apical plasma membrane and some were seen releasing their content. Vacuoles containing amorphous material were seen in the cytoplasm. The lumen was filled with homogeneous secretory material and some vacuoles containing amorphous material were seen in the lumen. In some cells, the apocrine blebs were protruding into the lumen containing cytoplasmic matrix. Thus, secretory material was released into the lumen by apocrine and merocrine modes. The luminal surface of the cells showed short microvilli protruding into the lumen. Junctional complexes were observed between the two adjacent cells.

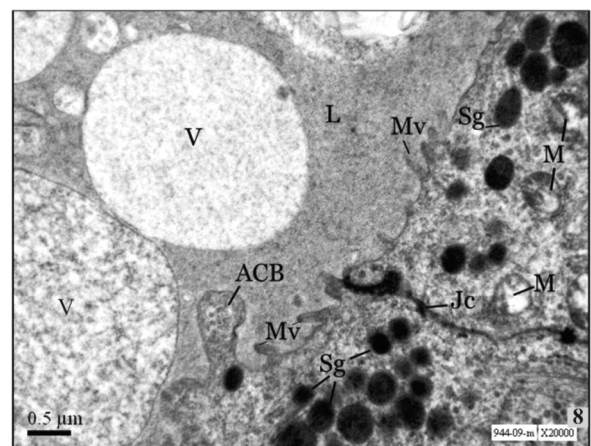


Fig. 8. Electron micrograph the acinar cells of Cowper's gland during the breeding period showing short and thick microvilli (Mv) on the apical surface. Electron-dense secretory granules (Sg) are seen below the apical plasma membrane releasing their content into the lumen (L). Apical cytoplasmic blebs (ACB) containing cytoplasmic matrix are seen projecting into the lumen. Lumen contains vacuoles (V) of various sizes bounded by membranes containing flocculent material and homogeneous electron-dense secretions. Junctional complexes (Jc) are seen between lateral membranes of adjacent cells. 20,000 \times magnification.

3.3. Active breeding period

Bats collected in October and November were sexually active and their testes showed vigorous spermatogenesis. There were no marked differences in ultrastructural features of Cowper's gland during the active breeding period as compared to the Cowper's gland during the prebreeding period. As in the prebreeding period, the acinar cell showed well-developed Golgi apparatus, large numbers of mitochondria, and lamellar as well as dilated cisternae of RER. Three types of secretory granules were observed within the cytoplasm of acinar cells, as observed in the acinar cells of the prebreeding period. The secretory granules containing a high amount of electron-dense material were maximum in the acinar cells during the active period as compared to the other two stages of the reproductive cycle. The secretory material was released into the lumen by merocrine and apocrine modes (Figures 7 and 8). Thus, the ultrastructural features of acinar cells of Cowper's gland showed no marked differences in the secretory apparatus during the different phases of the sexual cycle.

4. Discussion

Cyclic histological changes in Cowper's gland have been studied in few species of bats. They become hypertrophied and secretory with granular columnar epithelium in synchrony with the elevated testicular steroid cycle, and involuted with cuboidal epithelium and agranular cytoplasm in the inactive testicular state in several bat species: *Nactyla noctula* [11], *Pipistrellus dormeri* [12], *Taphozous melanopogon*, *Miniopterus* [13], *Brachyphylla cavemarum* [14], *Hipposideros lankadiva* [15], and *Eidolon helvum* [16]. Similar cyclical changes in the histology of the Cowper's gland of *Rousettus* have been reported in the present study, conforming the observations on Cowper's glands of other species of bats.

There are no marked differences in ultrastructural characteristics of the secretory epithelial cells of Cowper's gland of *Rousettus* during different stages of the reproductive cycle. Ultrastructural differences between these secretory cells thus reflect a secretory cycle in different phases of cellular activity, and this may also result in the observed differences in the amount of secretion within the lumen. The secretory epithelial cells of acini of Cowper's gland are characterized by the well-developed RER, with lamellate parallel profiles and dilated cisternae distributed throughout the cytoplasm. Extensive development of the Golgi complex and large numbers of mitochondria with lamellate cristae and numerous secretory granules of various electron densities are dispersed in the cytoplasm. Similar secretory apparatus containing mitochondria, Golgi apparatus, endoplasmic reticulum and secretory granules has been reported in the secretory cells of Cowper's gland of rats [17], humans [18], water buffalo [19], and boar [20,21], supporting the present observations in bats. Thus, the secretory cells of Cowper's glands of *Rousettus* show the ultrastructural characteristics that indicate that this gland is involved in synthesis and secretion of proteinaceous materials during the different phases of the reproductive cycle.

Membrane-bound secretory granules of various sizes and densities are seen in the cytoplasm. Three different types of granules can be identified on the basis of electron density. It appears that these granules are not of different types but they represent the different stages of granule maturation. Immature granules are associated with the Golgi complex and they are mostly seen during the inactive period. As they mature, they are carried towards the apical plasma membrane during prebreeding and breeding periods of bats. Secretion of the granules from cells into the acinar lumen takes place by exocytosis. In some acini, the apical surface of the secretory cells shows apical bulges that protrude into the lumen containing secretory material. This secretory material is then released into the lumen. In Cowper's gland of *Rousettus*, secretory products are released into the lumen by both apocrine and merocrine modes. The mode of secretion is merocrine in many mammals. However, apocrine and merocrine modes are reported in few mammals [15,17–20].

The luminal surfaces of the secretory cells of Cowper's gland in *Rousettus* show microvilli. Junctional complexes, desmosomes, and interdigitations are found between adjacent cells. The basal plasmalemma is usually straight, but folding of this surface is occasionally observed. The presence of microvilli may represent a form of microapocrine secretion. Similar observations are reported in buffalo [19].

The plasma testosterone concentration was assessed by radioimmunoassay in *R. leschenaulti* during different phases of reproductive cycle in our laboratory [22]. During the sexually inactive period, plasma testosterone concentration was 0.2 ng/mL and Cowper's glands were regressed but the ultrastructural features indicated that the secretory cells of Cowper's glands are synthetically active and synthesize secretory products. The concentration of plasma testosterone increased during prebreeding (0.6 ng/mL) and the highest concentration of plasma testosterone was reported during the active breeding period in November (1.0 ng/mL). During these periods, secretion from the Cowper's glands is active, as indicated by the ultrastructural features of the gland, which synthesizes large quantities of secretory products. Thus, it indicates that even a small amount of testosterone during the sexually inactive period stimulates synthesis of secretory products in Cowper's glands of *Rousettus* [22].

Fructose, sialic acid, and citric acid have been demonstrated in the secretory cells of Cowper's glands of *Rousettus* [6], *Pteropus giganteus* [4] by biochemical method and neutral, sialic acid and sulfomucin in *Pipistrellus dormeri* [12], *T. melanopogon* and *Miniopterus schreibersii* [13] and *H. lankadiva* [15] by histochemical methods. Ultrastructural features of secretory cells of Cowper's gland of *Rousettus* indicate the synthesis of these metabolites as reported in other species of bats. From the above discussion, it is suggested that secretory materials synthesized by Cowper's gland of *Rousettus* may be involved in various male reproductive processes, such as maturation, and survival and metabolism of spermatozoa [23–25].

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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