DOI : 10.5958/2277-8934.2020.00013.2 *Vol 27 No 1, p 89-96*

ULTRASTRUCTURAL AND MORPHOMETRIC STUDIES ON THE BULBOURETHRAL (COWPER'S) GLAND OF CAMEL (*Camelus dromedarius*)

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ABSTRACT

The bulbourethral glands of 40 dromedary camels were studied histologically, ultrastructurally and morphometrically. Three types of secretory units were encountered in the gland designated as type A, B and C. Type A cells were pyramidal or columnar in shape containing irregular nuclei. These were characterised by a cytoplasm packed with electronlucent granules. Type B units usually had dark or light cells with spherical nuclei. Type C units consisted of both type A and type B cells. Interestingly, myoepithelial cells were distinguished in this gland. Morphometric data showed that the glandular tissue occupied the bulk of the gland (54.02%) compare to the connective tissue and muscle (35.23). The ducts, blood vessels and nerves had volume densities of 6.39% and 4.36%, respectively.

Key words: Bulbourethral gland, camel, Camel Cowper's gland, morphometry, ultrastructure

The bulbourethral glands have been studied in various species, i.e. rat (Adebayo *et al*, 2015) boar (Aitken, 1960), bull (Trotter, 1959; Salisbury and Vandemark, 1961; Faulkner, 1969; Kainer *et al*, 1969; Campero *et al*, 1988), man (Riva *et al*, 1990); ram (Aitken, 1959; Khalaf and Merhish, 2010), stallion (Bradley, 1948; Bharadwaj and Calhoun, 1962), donkey (Abou-Elhamd *et al*, 2012) and water buffalo (Abou-Elmagd and Wrobel, 1989).

Nevertheless, the gland in the camel received little attention. Apart from the work of Lesbre (1903) who reported the presence of the bulbourethral glands and absence of the seminalis in the camel, only a few studies were recorded giving brief accounts on the morphology, histochemistry or morphometry of the bulbourethral gland (El-Wishy *et al*, 1972; El-Wishy, 1988; Ali *et al*, 1976, 1977; Mosallam, 1981; Badawi and Yousef, 1982; Degan and Lee, 1982; Marroni *et al*, 1982; Abou-Ahmed *et al*, 1988; Hafez and Hafez, 2001; Luo *et al*, 2016; Abdullahi *et al*, 2016).

However, ultrastructure or morphometry of the bulbourethral gland were not reported previously in camels. Current research was thus aimed to study the ultrastructure and morphometry of the bulbourethral gland of the dromedary camel.

Materials and Methods

Bulbourethral glands were collected from 40 apparently healthy mature camels, immediately after slaughtering at Tambul Slaughterhouse, Sudan.

Light Microscopy

Samples from 11 animals were used for the preparation of histological sections. Tissue pieces from the bulbourethral glands were fixed either in Bouin's fluid, 10% formal-saline, 10% formalin or Zenker formal. Sections, 5 mm^3 thick were cut from the different levels of the gland, then processed for normal paraffin techniques. Paraffin sections at 3-5µm were cut and stained with Haematoxylin and Eosin (H&E) or Masson's Trichrome.

Electron Microscopy

Material for ultrastructural studies were obtained from 9 animals. Small pieces (1mm^3) of tissues were fixed in 2.3% glutaraldehyde in 2.14% sodium cacodylate buffer pH 7.4, for 2 hours. They were then washed in 2.14% sodium cacodylate pH 7.4, postfixed in 2% osmium tetraoxide in 2.14% sodium cacodylate buffer pH 7.4, for 2 hours. They were then washed in 2.14% sodium cacodylate buffer pH 7.4 twice for 30-60 minutes. Dehydration was carried

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out in ascending grades of acetone or ethanol 50%, 70%, 90%, 95% and 100% for 30-60 minutes each. The materials were prestained in 2% uranyl acetate and 2% phospho-tungstic acid at 4°C for 20 hrs and then embedded in resin.

Semi-thin sections (0.5-1.0µm) were cut on Reichert ultramicrotome (Germany) using glass knives, stained with toluidine blue and examined with light microscope. The desired regions for electron microscopy were then selected and ultrathin sections, pale gold to silver (70-90 nm), were cut with glass knives. The sections were mounted in uncoated copper grids, double-stained with uranyl acetate for 5 minutes, washed in distilled water and placed in lead citrate for 30-45 seconds. They were then washed dried and studied in a Zeiss EM 109 electron microscope (Germany).

Morphometry

Tissue samples were collected from 20 animals. The weight and volume of the organs were measured. The volume was determined by water displacement method (Scherle, 1970).

Each gland was cut into four slices about 5 mm thick. Since the slices were more or less identical it was necessary to analyse all of them and so every second slice was taken for histological processing. The slices from each animal were used for morphometric analysis. Sections were cut at 2-5 µm thick and stained with Masson's Trichrome.

In order to determine the optimum number of points to be counted for each component of the gland, one slice was completely analysed field by field, using a 100-point integrating eye piece (Zeiss). The sufficient number of points necessary to count from each component to keep the standard error below 5% was then determined by the plots of Weibel (1963) and Dunnill (1968). Parameters like blood vessels and nerves occupied relatively small volumes and did not fall within the scope of the plots. The objective lens of X20 was used in the analysis of sections.

The volume densities of the components of the gland were taken as means of the results of analysis of all sections. The absolute volumes of these components were calculated from their volume densities (Vv) and from the total volume (V) of the gland (i.e. Absolute volume Vv. V).

The statistical analysis of the data obtained by point-counting was restricted to determination of the means and standard deviation (Weibel, 1963).

Results

Light Microscopy

The secretory units of the glands were lined with one layer of pyramid, cuboidal or columnar cells, which extended from the basal lamina to the lumen. The average cellular height was about 21 µm. There were three types of secretory units in the bulbourethral gland designated as type A, B and C. These units have been studied ultrastructurally and an account on each together with that on the basal cells herewith follows.

Electron microscopy

Type A units

The cells which constitute type A units were tall pyramidal or columnar in shape and possessed infolded plasma membrane. The lateral membranes were straight and the apposed surfaces were held together by moderate junctional complexes (Fig 1).

The nucleus was irregular flat and pushed down towards the basal cytoplasm (Fig 1). The euchromatin was evenly distributed, but heterochromatin was concentrated around the peripheral parts. One or two nucleoli, irregular in shape, were eccentrically placed in the nucleoplasm. The nucleolus had a dense band in its outer part and a lighter central portion divided into two or three parts (Fig 1).

The cell contained a large Golgi complex which was consisting of several parallel arrays of cisternae and vacuoles of various sizes and shapes. It was observed adjacent to the nucleus (Fig 2).

Mitochondria were observed possessing various forms, i.e. oval, rounded and elongated (Fig 3), but they were scattered throughout the cytoplasm nevertheless tended to concentrate in the peripheral parts. The endoplasmic reticulum consisted of branching and anastomosing tubules distributed throughout the cytoplasm (Fig 4).

The entire cytoplasm was filled with closely packed electron lucent granules of different sizes and shapes. Many of these granules coalesced with each other to from large irregularly shaped units. Small electron dense inclusions were seen frequently inside the large secretory granules (Fig 1). Few lysosomes and fat droplet were also encountered.

Type B unit

The epithelial lining of type B units contained two types of cells; dark and light (Fig 5). Some units had basal cells wedged between the columnar cells and the basement membrane (Fig 6). Myoepithelial

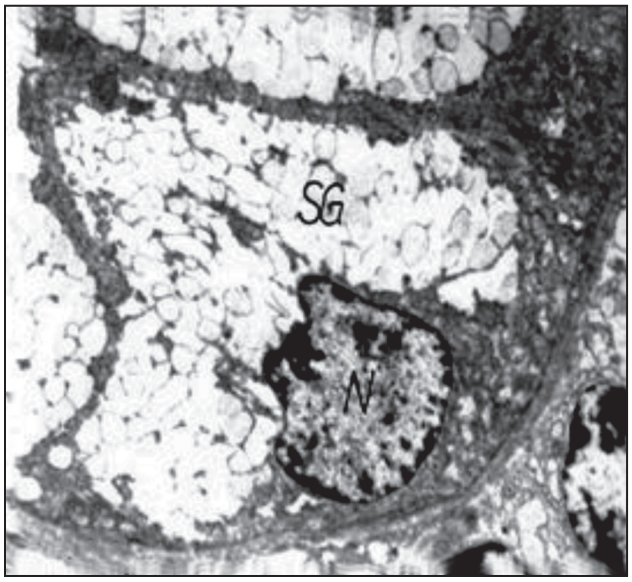

Fig 1. SEM micrograph of Bulbourethral gland. Type A unit showing pyramidal cells with flat and basal nucleus (N). Note the nucleolus. The cell is filled with electron lucent secretory granules (SG). 42200X.

Fig 3. SEM micrograph of Bulbourethral gland. Type A unit. Note the different forms of the mitochondria (M). The matrix traversed by lamellar Cristae. 60000 X.

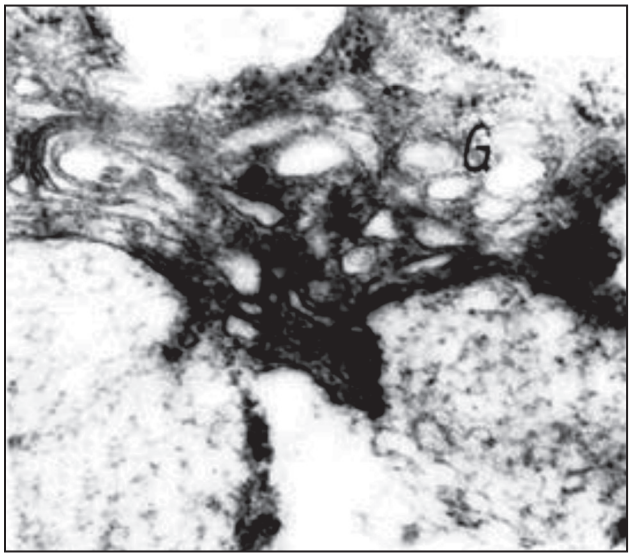

Fig 2. SEM micrograph of Bulbourethral gland. Type A unit. A large Golgi complex (G) consisting of several parallel arrays of cisternae and vacuoles of various sizes and shapes . 60000X.

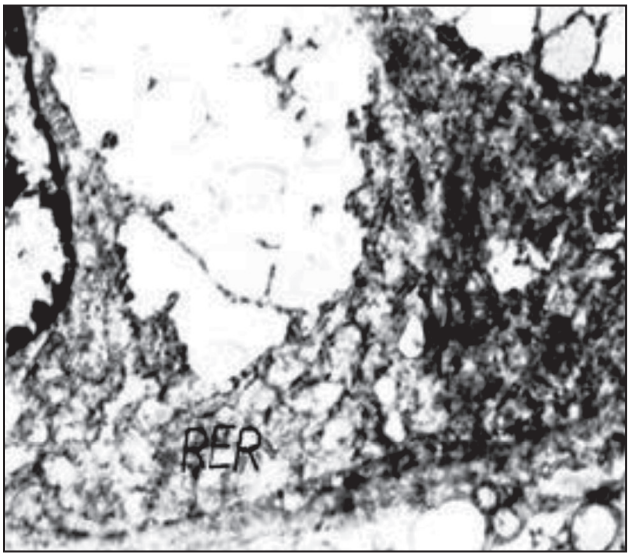

Fig 4. SEM micrograph of Bulbourethral gland. Type A unit. Rough endoplasmic reticulum (RER). 100000 X.

cells were seen in close association with outer parts of the units (Fig 7). The luminal border of the columnar cell carried long microvilli. The lateral cell membranes of epithelial cells were moderately folded and possessed desmosomes throughout their length. However, extensive folding was encountered in the basal parts.

The nucleus was spherical in shape and was disposed at different levels in the cytoplasm (Fig 8). The euchromatin was finely granular and heterochromatin was disposed peripherally. A single nucleolus could be observed and was eccentrically located in the nucleus. It has a dense band in its outer part and a lighter central portion (Fig 8).

Elements of smooth endoplasmic reticulum possibly including a Golgi complex were seen in the form of parallel arrays of cisternae and vacuoles of various sizes. They were located at different sites in the cytoplasm (Fig 9).

There were many mitochondria distributed randomly in the cytoplasm. They were in the form

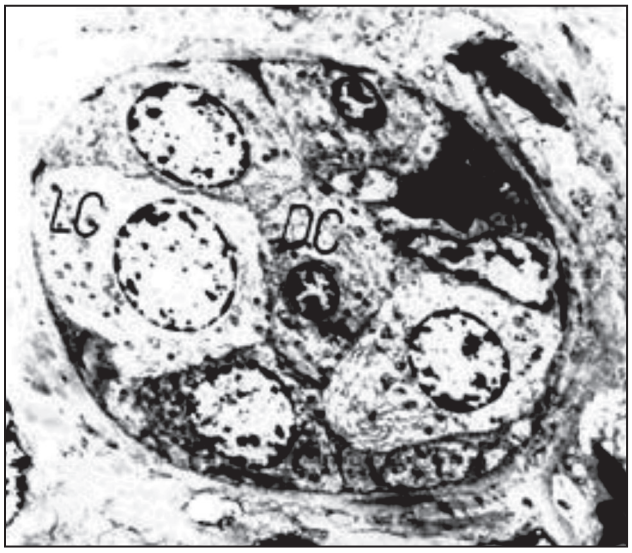

Fig 5. SEM micrograph of Bulbourethral gland. Type B unit showing dark cells (DC) and light cells (LC). 3800 X.

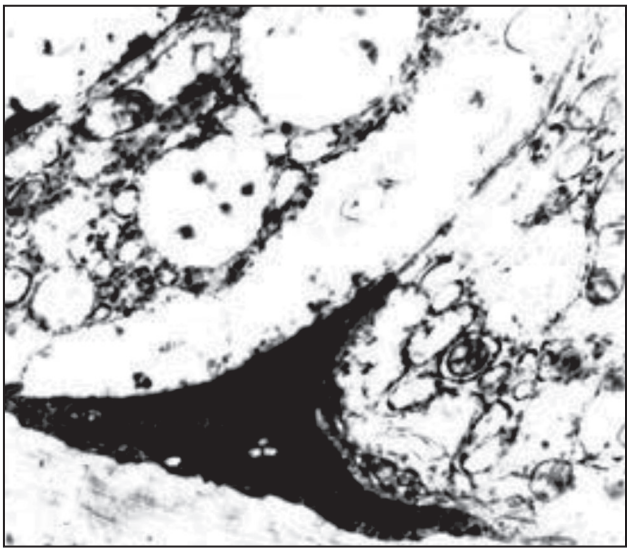

Fig 7. SEM micrograph of Bulbourethral gland. Type B unit. Myoepithelial cells. Note Myofibrils and lipid. 20000 X.

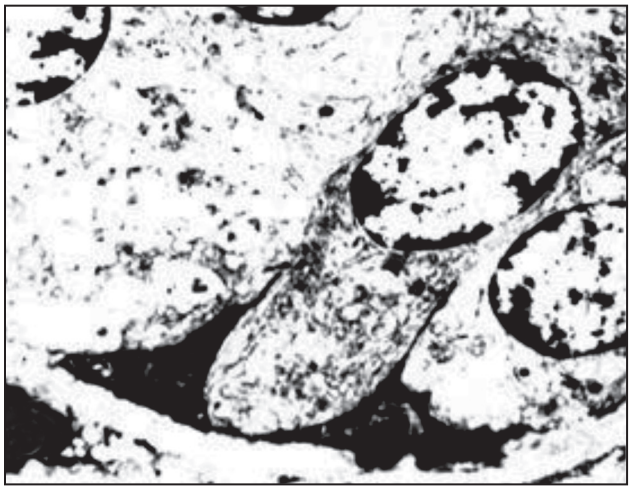

Fig 6. SEM micrograph of Bulbourethral gland. Type B unit. A basal cell (possibly two). Are wedged between the columnar cells and the basement membrane. 43200 X.

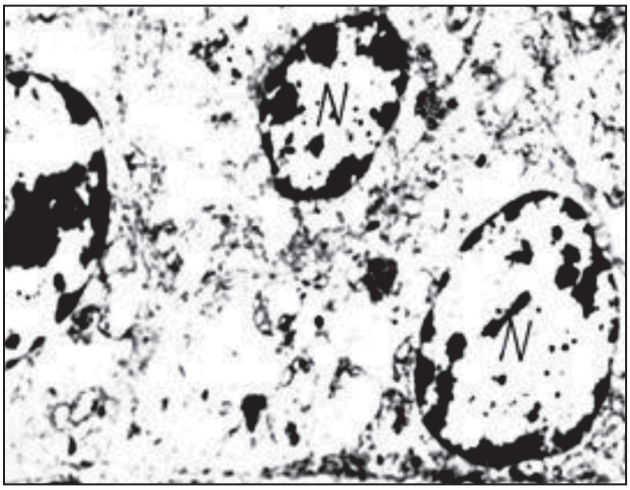

Fig 8. SEM micrograph of Bulbourethral gland. Type B unit showing spherical nucleus (N) disposed at different levels. Note the nucleolus. 44000 X.

of oval, round and elongate bodies. The matrix was traversed by lamellar cristae (Fig 10).

Type C Units

Type C of the secretory units were lined with one layer of cells consisting of both types; A and B (Fig 11, 12).

Basal cells

Basal cells were irregularly pyramidal in shape and they rested directly on the basal lamina. The cell had a large pyramidal nucleus rich in chromatin. The heterochromatin was concentrated around the periphery of the nucleus (Fig 12). The cell had a small Golgi complex, profiles of a few mitochondria, rough endoplasmic reticulum (RER) and small number of vacuoles (Fig 11, 12).

Morphometry

Tables 1, 2 and 3 are showing data and results obtained by using the point-counting technique. The mean volume of the bulbourethral gland, the total points falling on each component, the volume density of each component (Vv), the volume density percentage (Vv%) and the absolute volumes were recorded in these tables. Measurement of the volumes of the bulbourethral glands of 20 camels gave mean values of 13.49 cm^3 and those of weight of bulbourethral glands gave mean values of 14.48 gm. The components of the bulbourethral gland studied

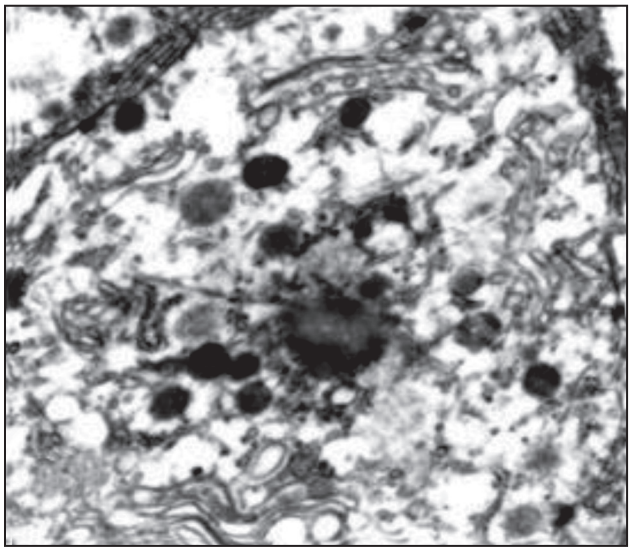

Fig 9. SEM micrograph of Bulbourethral gland. Type B unit showing elements of smooth endoplasmicreticulum including a Golgi complex. 20000 X.

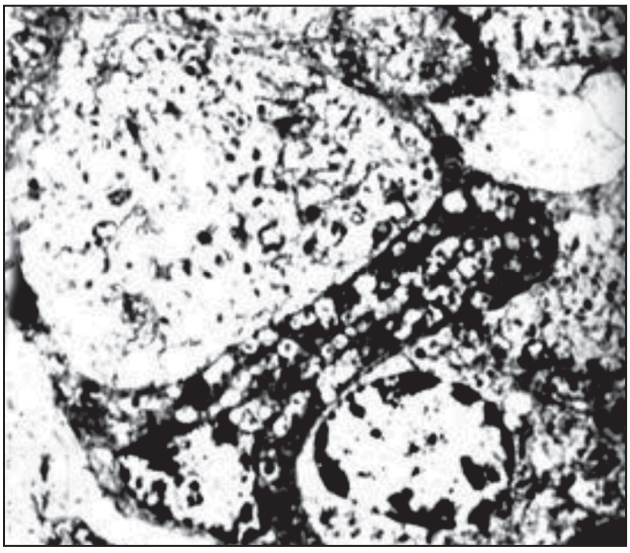

Fig 11. SEM micrograph of Bulbourethral gland. Type C unit showing type A and B in the same unit. 28600 X.

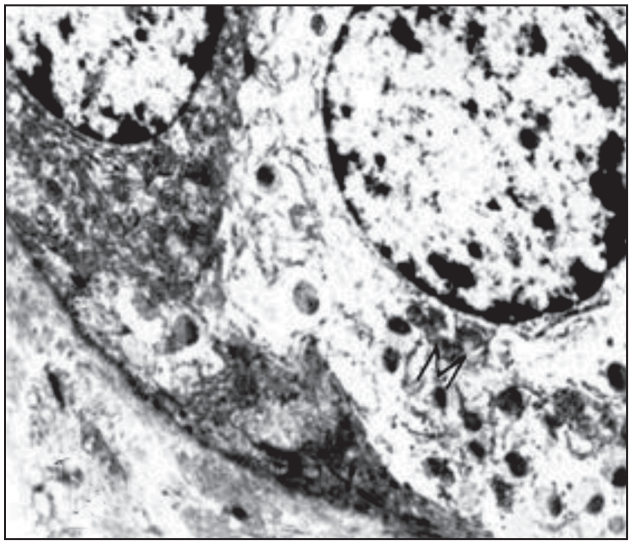

Fig 10. SEM micrograph of Bulbourethral gland. Type B unit showing many mitochondria (M) in the cytoplasm of dark and light cells. 21090 X.

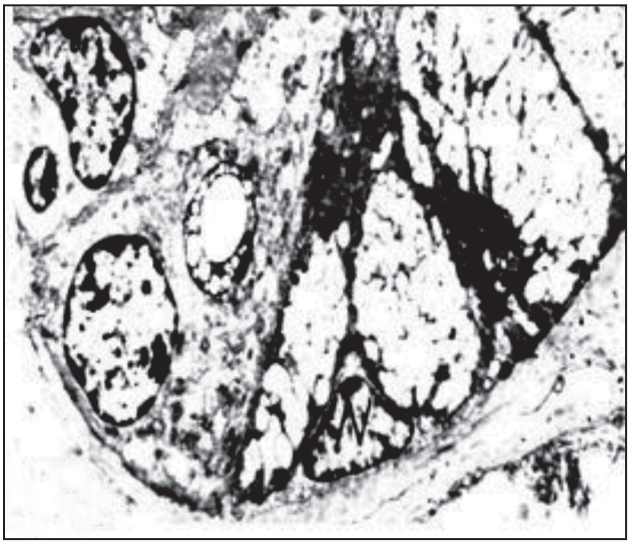

Fig 12. SEM micrograph of Bulbourethral gland. Type C unit showing a pyramidal basal cell with a nucleus (N). 24200 X.

were glandular tissue of the type A, glandular tissue of type B, glandular tissue of type C, connective tissue and muscles, blood vessels and nerves and ducts.

The greater volume of the bulbourethral glands was occupied by connective tissue and the muscle (35.23%) followed by glandular tissue type B (19.76%), glandular tissue type C (18.88) and glandular tissue type A (15.38%). The ducts, blood vessels and nerves occupied 6.39% and 4.36, respectively (Tables 1,2,3). The absolute volume of connective tissue and muscle was 5.08 cm^3 while that of glandular tissue type B, type C and type A was 2.61 cm^3, 2.55 cm^3 and 2.11 cm^3, respectively. The ducts showed an absolute volume of 0.86 cm^3 and that of blood vessels and nerves was 1.57 cm^3 (Tables 1,2,3).

Discussion

This study has shown that secretory units of the gland are lined with one layer of cells which are columnar or pyramidal in shape. These results confirm earlier observations of Ali *et al* (1977) and Mosallam (1981). The present investigation is the only study which hitherto has demonstrated the presence of the basal cells in the bulbourethral glands of the camel. The height of epithelium average is 21 µm, a similar result was reported by Ali *et al* (1977). Their

Table 1. Morphometric analysis of the Bulbourethral glands showing the number of points falling on each component counted from section 1.

No. of F.	G. T.A	G. T. B	G.T.C	C.T+M	B.VS+N	Ducts	Total
1	-	-	-	98	2	-	100
2	11	6	47	16	-	-	100
3	65	-	-	35	-	-	100
4	60	12	13	11	-	22	100
5	-	-	-	98	2	21	100
6	40	5	34	21	-	-	100
7	18	3	66	6	-	23	100
8	22	11	57	8	2	-	100
9	11	9	35	14	-	-	100
10	38	8	15	39	-	-	100
Total	**265**	**54**	**267**	**346**	**6**	**62**	**1000**

Number of field (N. of F). Glandular tissue of bulbourethral gland type A, B and C (G.T.A, G.T.B and G.T.C). Connective tissue and muscles (C.T+M). Blood vessels and nerves (B.VS+N).

Table 2. Morphometric analysis of the Bulbourethral glands showing the number of points counted (p), volume density (Vv), volume density Percentage (Vv%), and absolute volume (v) of the main components from four sections.

No. of F.	G. T.A	G. T. B	G. T. C	C.T+M	B.VS+N	Duct	Total
40	499	716	720	1821	116	128	4000
Vv	0.125	0.179	0.18	0.455	0.029	0.032	
Vv%	12.50	17.90	18.00	45.50	2.90	3.20	
Abs. v	2.44	3.49	3.49	8.87	0.57	0.63	

Number of field (N. of F). Glandular tissue of bulbourethral gland type A, B and C (G.T.A, G.T.B and G.T.C). Connective tissue and muscles (C.T+M). Blood vessels and nerves (B.VS+N).

Table 3. Morphometric analysis of the bulbourethral gland, showing the volume of the glands (v), volume density (Vv), volume density percentage (Vv%) and absolute volume (V) of the main components of 20 camels. Means ± Standard Deviations.

	G. T.A	G. T. B	G. T. C	C.T+M	B.VS+N	Ducts
Total	12304	15808	15103	28188	3486	5111
V	0.1538	0.1976	0.1888	0.3523	0.0436	0.0639
±SD	±0.294	±0.358	±0.0460	±0.0429	±0.0086	±.0132
Vv%	15.38	19.76	18.88	35.23	4.36	6.39
±SD	±2.94	±3.58	±460	±07.62	±0.86	±1.37
Abs.v	2.11	2.61	2.55	5.08	0.59	0.86
±SD	±.74	±0.66	±0.99	±2.34	±0.21	±.28

Number of field (N. of F). Glandular tissue of bulbourethral gland type A, B and C (G.T.A, G.T.B and G.T.C). Connective tissue and muscles (C.T+M). Blood vessels and nerves (B.VS+N).

study has identified three types of secretory units designated as A, B, C; this has been confirmed by the present investigation.

Type A units were lined by tall pyramidal columnar cells as described by Ali *et al* (1977) and Mosallam (1981). The cell possessed an infolded basal plasma membrane and fairly straight lateral membrane with moderate junctional complexes similar to those of the water buffalo (Abou-Elmagd and Wrobel, 1989).

The lining cells possessed a massive Golgi complex. This correlates well with the secretory function of this organelle as evidenced by the large number of the mucous secretory granules found in the cytoplasm.

The cytoplasm of the mucous cells was occupied by abundant electron lucent granules. This is in accord with results reported in the human bulbourethral glands (Riva *et al,* 1990).

Type B units were lined with one layer of cuboidal cells (Ali *et al*, 1977). Similar results were identified in the present investigation. However, the dark cells were apparently more electron dense. In the

current research a massive Golgi complex together with elements of smooth endoplasmic reticulum were randomly scattered in the cytoplasm. Nevertheless, in the water buffalo's bulbourethral glands, the Golgi complex confined to the middle part of the cell (Abou-Elmagd and Wrobel, 1989). The large size of the Golgi complex is indicative of the magnitude of secretory activity of the cells. This is confirmed by the presence of abundant secretory granules of which, some possess fine foci of electron dense material.

The secretory units of type C were lined by one layer of cells consisting of both type A and type B. Similar result was given by Ali *et al* (1977). However, in the water buffalo only type A and type B were reported (Abou-Elmagd and Wrobel, 1989). The basal cells were observed wedged among the bases of the main cells. They were irregularly pyramidal in shape with large pyramidal nucleus that was rich in chromatin. As they were poor in organelles it was concluded that they were not secretory.

Earlier studies made no reference to the presence of myoepithelial cells in the bulbourethral gland of the camel. The present study is the first to identify such cells and showed that they possess characteristic cytoplasmic myofibrils and they were interposed between the basal lamina and the alveolar cells of the secretory units of type B. Such cells were widely reported in exocrine organs of the camel, for instant the epididymis (Tingari, 1989), salivary glands (Khalil, 1989) and mammary glands of human, monkey, shrew, rat and mouse (Tsubura et al 1991), they are considered to help, through their contraction; in the empting mechanism of secretory material.

The morphometric data on the bulbourethral of the camel is virtually lacking. It was scarce for other species. From morphometric results it was clear that the glandular tissue occupied 54.02% of the volume of the gland. It was relatively larger than the volume occupied by the glandular tissue of camel prostate gland (Shaaeldin and Tingari, 2019). Within the glandular tissue type B cells have the largest volume followed by type C and then type A units. A correlation have already established between testicular weight, amount and quantity of testicular interstitial tissue, spermatogenesis and epididymis sperm content and hence testicular function on one hand and season of the year on the other hand (Maiada *et al*, 2013). It would be interesting to carry a similar study to confirm whether there is fluctuation in the amount of glandular tissue of the bulbourethral gland and to correlate the findings with the sexual activity.

Acknowledgements

This research was supported by a grant from Ahfad University for women (Sudan), The Gordon Memorial Trust (UK) and The British Council Sudan.

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