DOI : 10.5958/2277-8934.2023.00016.4 *Vol 30 No 1, p 119-124*

ULTRASTRUCTURAL AND MORPHOMETRIC STUDIES ON THE PELVIC URETHRAL GLAND OF THE ONE-HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

The urethral glands from 40 dromedary camels were investigated using histological, morphometric and electron microscopic techniques. The secretory units of the gland were characterised by columnar or pyramidal cells rich in rough endoplasmic reticulum, Golgi complex and various forms of mitochondria. Basal cells were observed located between the cells of the secretory units. Morphometric data revealed that the connective tissue occupied most of the gland (81.66%) while the glandular tissue was reduced to only 8.94%. Morphometric data was indicative of the relatively minor contribution of the urethral gland to the seminal plasma.

Key words: Camel, dromedary, morphometry, ultrastructure, urethral gland

The male urogenital tract was confirmed to contain urethral glands in many rodents (Hall, 1936; Hebel and Stromber, 1986). The pelvic urethral glands have been studied in the male cat (Cullen *et al,* 1983), dog and stallion (Trautman and Fiebiger, 1952; Bharadwaj and Calhoun, 1959) and man (Sirigu *et al,* 1991).

Various studies described the histology or histochemistry of the camel urethral glands (El Jack, 1970; El-Wishy *et al,* 1972; Ali, 1975; Ali *et al,* 1976, 1978; Mosallam, 1981; Badawy and Yousef, 1982; Degan and Lee, 1982; Marroni *et al,* 1982; Mobarak *et al,* 1990; Hafez and Hafez, 2001; Yousefi *et al,* 2010; Masood *et al,* 2022).

The urethral glands are located just behind the body of the prostate and extend to the level of the urethral bulb before opening into the urethral lumen *via* numerous ducts. These glands and the pelvic urethra are responsible for the contraction of the muscle and expulsion of glandular secretion (Ali *et al*, 1978). The seminal colliculus is fibroglandular in the males of camel, ox, buffalo and pig are found on the medial aspect of the dorsal wall of the urethra, little caudal to the internal opening of the urethra from the bladder (Shehata, 1980).

The ultrastructure and morphometry of urethral glands of camels are least studied. The present study was, therefore, undertaken to reveal the ultrastructure and morphometry of this gland.

Materials and Methods

The pelvic urethral glands were collected from 40 adult one-humped camels (*Camelus dromedarius*) immediately after slaughter at Tambul Slaughterhouse, Sudan. All collected samples were apparently normal without any pathological lesions.

Light Microscopy

Samples from 11 animals were used for the preparation of histological sections. Tissue pieces from the urethral 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

Materials 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. These 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. These were then

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washed in 2.14% sodium cacodylate buffer pH 7.4 twice for 30-60 minutes. Dehydration was carried 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 (Scherie, 1970).

Each gland was cut into 4 slices about 5 mm thick. Since the slices were more or less identical it was necessary to analyse all of them and therefore, 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 and Discussion

Light Microscopy

The secretory units were lined with one layer of pyramidal or columnar cells about 17 µm in height. Their nuclei were spherical in shape and usually occupied a basal position. Some units presented basal cells wedged between the lining cells and the basement membrane.

The camel pelvic urethra was described by El Jack (1970) and Degan and Lee (1982). Camel urethra was different from other species, as it was glandular along its entire length. Such an observation was reported by Ali *et al* (1978) and confirmed in the present study.

Current findings on pelvic urethral gland of the camel confirmed those of Ali (1975), Ali *et al* (1978) and Mosallam (1981). The secretory units were lined with a single layer of pyramidal or columnar cells (13µm) with spherical basal nuclei. Basal cells were observed in some secretory units, an observation which were not reported elsewhere.

Electron Microscopy

Columnar Cells

The luminal border of the columnar cells carried microvilli which were long, numerous and pleomorphic; some were short (Fig 1). Pinocytotic vesicles were observed between the bases of the microvilli. The lateral plasma membrane showed extensive junctional complexes towards the luminal tips (Fig 3). There was also an extensive infolding of the basal part of the lateral plasma membranes whereas the basal plasma membrane was moderately folded. Nuclei of the columnar cells were spherical in shape and frequently occupied basal positions (Fig 1) and rich in chromatin. Heterochromatin, is the form of irregular, dense granular masses disposed around the periphery of the nucleoplasm, while euchromatin occupied the central areas of the nuclei (Figs 1-4). Columnar cells contained sizeable Golgi complex, usually seen in the supranuclear cytoplasm, consisting of several arrays of cisternae and vesicles of various sizes (Fig 2). Numerous mitochondria were scattered throughout the cytoplasm in a form of oval, rounded and elongated bodies (Fig 3). Rough endoplasmic reticulum (RER) consisting of branching and anastomosing tubules were distributed throughout the cytoplasm. Some profiles were observed in the form of whorled lamellae (Fig 4). Large electron-lucent bodies, numerous vacuoles and secretory granules were often seen in the cytoplasm (Fig 5).

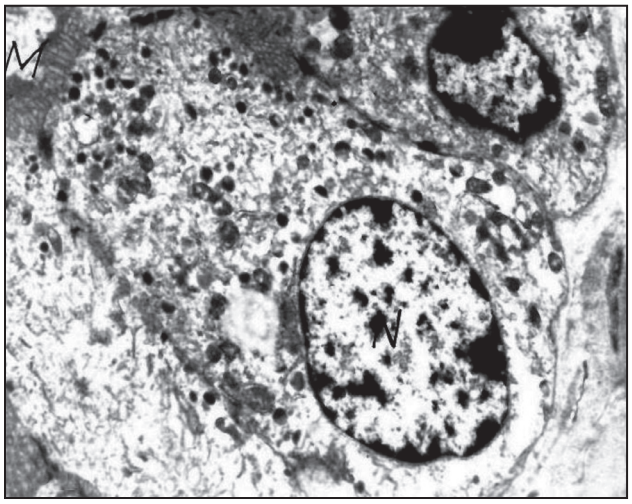

Fig 1. TEM micrograph of the urethral gland. The columnar cell carries microvilli (M) with spherical nucleus (N) in the basal cytoplasm. X30800.

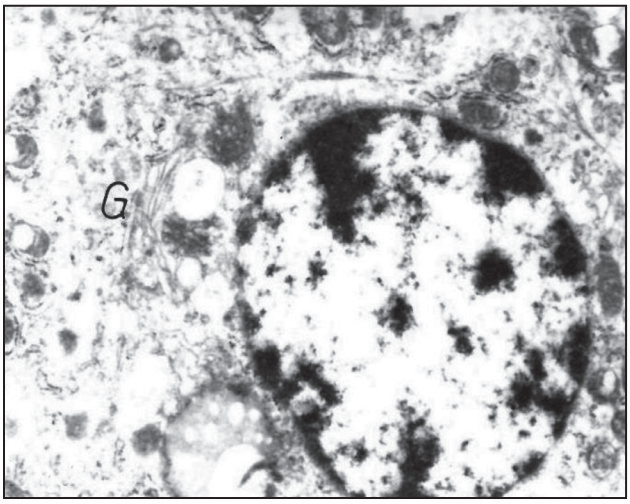

Fig 2. TEM micrograph of the urethral gland. The cell showing a sizeable Golgi complex (G). X32700.

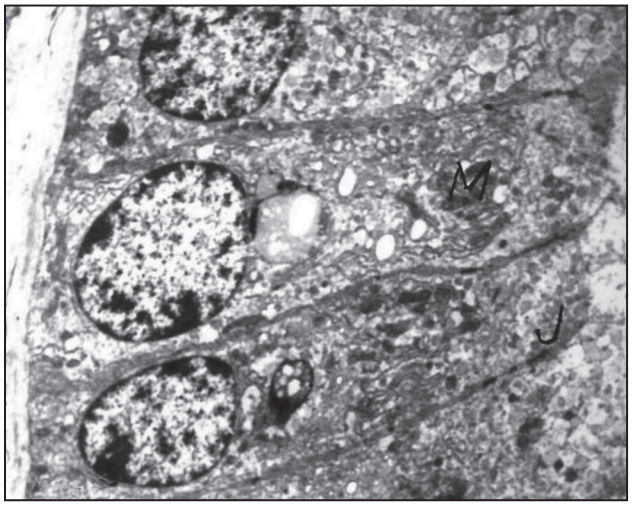

Fig 3. TEM micrograph of the urethral gland. Two cells showing many mitochondria. Note the junctional complex (J) towards the luminal tips. X19500.

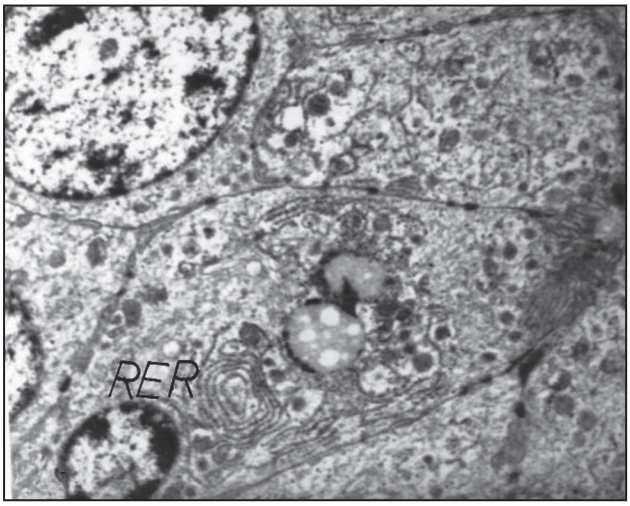

Fig 4. TEM micrograph of the urethral gland. A cell showing Rough Endoplasmic Reticulum, some profiles observed in the form of whorled lamellae (RER). X44000.

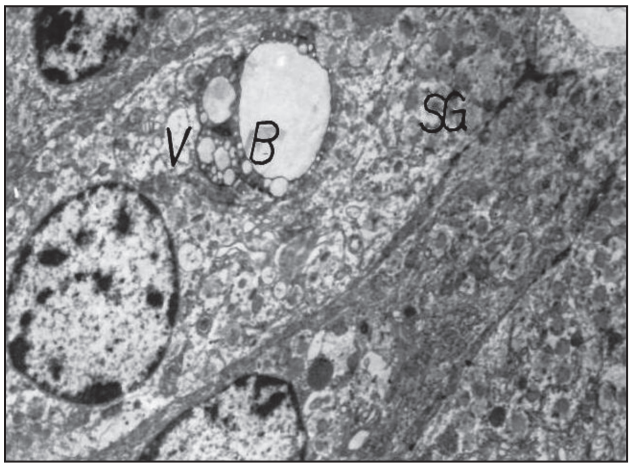

Fig 5. TEM micrograph of the urethral gland. Large electron lucent bodies (B) are observed, secretory granules (SG),and vacuoles (V). X21900.

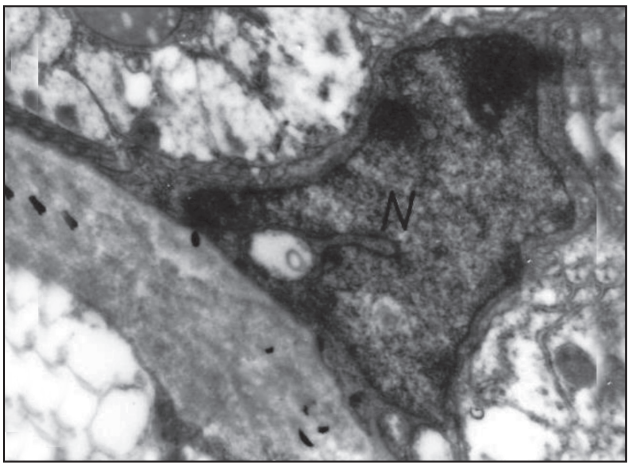

Fig 6. TEM micrograph of the urethral glands. The basal cell is irregularly pyramidal in shape with dense nucleus occupies most of the cell. X23000.

Basal Cells

The basal cells were irregularly pyramidal in shape and rested directly on the basal lamina. Their cell membrane were somewhat irregular. These cell possessed large dense irregularly pyramidal nuclei that filled most of the cell and had heterochromatin concentrated peripherally (Fig 6). These cells had small Golgi complex, few mitochondria, rounded or elongated and some profiles of rough endoplasmic reticulum (RER). The cytoplasm contained also a small number of vacuolar spaces.

As revealed by electron microscope the luminal surface of the lining cell was provided with tiny microvilli together with interspaced pinocytosis vesicles. The lateral plasma membrane was extensively folded in it was basal part and showed large junctional complex towards the luminal tip. The basal plasma membrane was only moderately folded.

The nucleus was spherical and basally located, similar results was reported by Ali *et al* (1978) and Mosallam (1981). The heterochromatin was located at the periphery. A large Golgi complex indicative of secretory activity, appeared in the supranuclear cytoplasm. There was an abundance of mitochondria which was either oval, round or elongated with lamellar cristae. The cytoplasm also contained elements of rough endoplasmic reticulum (RER). Some were observed in the form of whorled lamellae as in prostate gland.

The cells were characterised by the presence of a large electron-lucent bodies located above the nucleus and a large number of secretory granules were concentrated in the luminal cytoplasm.

Morphometry

Tables 1, 2 and 3 present data and results obtained by using the point-counting technique. The mean volume of the urethral 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 urethral glands of the 20 camels gave mean values of 64.7 cm^3; and those of weight of the urethral glands gave mean values of 69.97 gm.

The components of the urethral gland studied were glandular tissue, connective tissue and muscles, blood vessels and nerves and ducts. The greater volume of the urethral glands was occupied by connective tissue and muscle (81.66%) followed by glandular tissue 8.94%). The blood vessels and nerves occupied 6.04% and ducts occupied the least volume 3.36% (Tables 1-3). The absolute volume of connective tissue and muscle was 53.20 cm^3 while that of glandular tissue was 5.10 cm^3. The ducts showed an absolute volume of 2.01 cm^3 and that of blood vessels and nerves was 3.50 cm^3 (Tables 1-3).

Table 1. Morphometric analysis of the urethral gland showing the number of points counted from one section.

No. of fields	G. T. P	C.T+M	B.VS+N	Ducts	Total
1	-	85	15	-	100
2	65	25	03	07	100
3	-	94	06	-	100
4	75	12	06	07	100
5	81	09	03	07	100
6	-	95	05	-	100
7	67	16	04	13	100
8	87	12	01	-	100
9	-	100	-	-	100
10	35	40	05	20	100
11	-	90	10	-	100
12	25	48	05	22	100
13	17	72	08	03	100
14	-	89	11	-	100
15	07	86	03	04	100
16	-	96	04	-	100
17	-	97	03	-	100
18	-	100	-	-	100
19	-	96	04	-	100
20	-	88	12	-	100
21	-	94	06	-	100
22	-	94	02	-	100
23	-	100	-	-	100
24	-	95	05	-	100
25	-	94	06	-	100
26	-	100	-	-	100
27	-	97	03	-	100
28	-	100	-	-	100
29	-	98	02	-	100
30	-	90	10	-	100
Total	459	2316	142	83	3000

Glandular tissue of urethral gland (G.T.P), Connective tissue and muscles (C.T+M), Blood vessels and nerves (B.Vs+N).

Search in the literature has shown that quantitative data on the urethral gland was virtually lacking. In the camel the mean values of the volume was 64.7 cm^3, and that of weight was 69.97 gm. The connective tissue and muscle occupied about 81.66%

of the gland, followed by glandular tissue 8.94%, blood vessels and nerves 6.04% and lastly the duct 3.36%.

Table 2. Morphometric analysis of urethral gland 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 fields	G. T. P	C.T+M	B.VS+N	Ducts	Total
120	1070	9775	736	419	1200
Vv	0.089	0815	0.061	0.035	
Vv%	8.90	81.50	6.10	3.50	
Abs. v	5.10	46.70	3.50	2.01	

Glandular tissue of urethral gland (G.T.P), Connective tissue and muscle (C.T+M), Blood vessels and nerves (B.Vs+N).

Table 3. Morphometric analysis of urethral glands showing the volume of gland (V) in cm^3, volume density (Vv), volume density percentage (Vv%), and absolute volume (V), of the main components of 20 camels.

No. of fields	G. T. P	C.T+M	B.VS+N	Ducts
Total No.	21459	195984	14488	8069
Vv	0.0894	0.8166	0.0604	0.0336
±SD	±.0.0165	±0.0111	±0.1032	±0.0071
Vv%	8.94	81.66	6.04	3.36
±SD	±1.65	±1.12	±1.02	±0.71
Abs. v	5.79	53.20	3.92	2.22
±SD	±1.37	±9.59	±0.97	±0.70

Glandular tissue of urethral gland (G.T.P), Connective tissue and muscle (C.T+M), Blood vessels and nerves (B.Vs+N).

The absolute volume of connective tissue and muscle was 53.20 cm^3, glandular tissue 5.79 cm^3, blood vessels and nerves was 3.93 cm^3 and duct was 2.22 cm^3.

The analysis of the quantitative data of the pelvic urethra showed that the glandular tissue occupied about 8.94% being much lower than those of prostate gland (52.91%) and bulbourethral glands (54.02%). The connective tissue and muscle occupied 81.66%, a ratio which exceeds other glands of the dromedary camel (Shaaeldin and Tingari, 2019; Shaaeldin *et al,* 2020). Blood vessels and nerves amounted to 6.4%, this ratio was lower than that of prostate gland and exceeded that of the bulbourethral glands. The quantitative data of the pelvic urethra, especially that of the glandular tissue was indicative of relatively minor contribution of the pelvic urethral glands to the seminal plasma.

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