Histological and Histochemical Studies of the Efferent Ductules of Male One Humped Camel (*Camelus Dromedarius*)

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Abstract: A total of 9 adult apparently healthy male camels were used to study the histology and histochemistry of the efferent ductules during the winter season (rutting season). The efferent ductules were considered as a part of the excurrent duct system of the testis. They were convolutions of tubules that connect the rete testis to the ductus epididymidis. The tunica albuginea covering the ascending first region of the epididymal head showed many valved veins. This first region contained the extratesticular part of the rete testis and convolutions of efferent ductules, while the second region revealed convolutions of efferent ductules as well as the tubules of the initial segment of the epididymal duct. The extratesticular rete testis was lined by cuboidal epithelium. There was abrupt change in the epithelium lining the rete testis and that of efferent ductules. The efferent ductules were lined by epithelial membrane of three types of cells; columnar (ciliated and non ciliated), basal and migrating cells. The epithelium of the initial part of efferent ductules was lined with numerous columnar non ciliated cells showing numerous cytoplasmic vacuoles and fine granules. They showed signs of apocrine secretion. Along the course of the efferent ductules the columnar ciliated cells increased towards the epididymal duct. Few tall and slender dark cells appeared in the epithelium near the junction with the epididymal duct. The efferent ductules of camel were surrounded by fine peritubular smooth muscle layers, which increased in frequency toward the epididymal duct. Alkaline phosphatase enzyme reactivity was observed throughout the subepithelial connective tissue and blood vessels. Strong granular activity of acid phosphatase enzyme was demonstrated in the whole epithelium.

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Key words: Camel, efferent ductules

1. Introduction

It has been known that dromedary camel has economic significances; it produces, milk, meat, hair, some wool and hides. It is used also in riding, racing, agriculture, short-distance transport and tourists **(Khan, et al., 2003).** Camel was described as a seasonal breeder **(Al Eknah, 2000** and **Marai,et al., 2009)** or it might maintain their reproductive capacity throughout the year **(Zayed, et al., 1995).** The rutting season in Egypt had been reported to occur at December to March **(Ebada, 1994).** The excurrent duct system of the testis include the efferent ductules the ductus epididymidis and the ductus deferens **(Robert, 2010)**. The efferent ductules plays an important role in sperm maturation, absorption and secretion **(Goyal and Hrudka, 1981).**

Like of other economically valuable animals, studying the morphology of the camel reproductive organs was essential to improving and exploiting their reproductive activity. Micromorphology and histochemistry of the efferent ductules had been studied in most mammals **(Gray et al, 1983, Goyal and Williams, 1988** and **Hess and Bassily, 1988).** Detailed description of the structure of the efferent ductules and their histochemical characteristics in the dromedary camel had been quite few **(Ebada, 1994** and **Abd El-maksoud, 2010).** In view of this, the aim of this study is to elucidate more light and some details on the normal histological and histochemical features of the efferent ductules of the male one-humped camel, as one of the excurrent duct system of the testis.

2. Material and Methods:

The present work was conducted on 9 adult male apparently healthy animals. Samples from the head of the epididymis were taken during winter season **(December, January and February)** immediately after slaughter at the central abattoir in Cairo, Egypt.

The first region was taken as a longitudinal section of the ascending first region of the head. The second region was taken as a cross section of the initial segment of the epididymal duct (Fig. 1). The obtained segments were fixed in 10% buffered neutral formalin and Bouin's fluid for confirmation of

the results. Fixed specimens were dehydrated, cleared and embedded in paraffin wax. Serial and step serial sections of 5-6 micrometers thick were obtained and stained with Harris Hematoxylin and Eosin, Weigert's elastic tissue stain, Gomori's reticulin method, Periodic acid Schiff (PAS) technique and Alcian blue pH 2.5 **(Drury and Wallington, 1987).** Crossmon′s trichrome stain **(Crossmon, 1937).** Frozen sections were prepared and stained by azo-dye coupling methods for demonstration of acid phosphatase and alkaline phosphatase enzymes **(Pearse, 1972).**

For epon-araldite embedding, small tissue blocks from the epididymal head fixed in paraformaldehyde-gluteraldehyde solution in phosphate buffer **(Karnovsky, 1965).** Specimens were post fixed in 1% osmium tetraoxide for one hour, washed in 0.1M phosphate buffer (7.3pH), then dehydrated in graded ethanol and embedded in epon-araldite mixture **(Mollenhauer, 1964)**. Semithin sections (1 µm) were cut, stained with Toluidine blue **(Richardson, et al., 1960)** and examined with light microscope.

3. Results

As shown in Fig. (2); the epididymis of camel was surrounded by a thick tunica albuginea covered by the visceral layer of the tunica vaginalis. The tunica albuginea of the ascending region of the epididymal head could be demarcated into an outer dense fibrous layer and inner loose fibrous that showed many blood vessels. In addition smooth muscle fibers could be demonstrated only in the wall of blood vessels. The albuginea covering the ascending first region of the head showed many valved veins (Fig. 3). This ascending first region enclosed the extratesticular part of the rete testis and loops of efferent ductules.

Fig. (4) revealed that the intralobular fibrous connective tissue septa extended from the deeper part of the albuginea dividing the efferent ductules and the epididymal duct into lobules of variable sizes. The intralobular stroma that houses the efferent ductules as well as the loops of epididymal duct was formed of loose connective tissue (Fig. 5). It showed fine collagenous fibers, some connective tissue cells as well as blood capillaries embedded in abundant amorphous ground substance and the intralobular cells were lymphocytes, fibroblasts and macrophages. The ascending first region of the epididymal head enclosed the extratesticular part of the rete testis, and loops of efferent ductules (Fig. 6). The second part revealed convolutions of efferent ductules and tubules of the epididymal duct.

As shown in Fig. (7); the extratesticular rete testis was lined by cuboidal to high cuboidal epithelial cells. The epithelium was surrounded by a thin layer of collagen and reticular fibers. At the junction between the extratesticular rete testis and efferent ductules, the transition was abrupt, the simple cuboidal cells changed into columnar cells (Fig. 8).

As shown in Fig. (9); the lumen of the efferent ductules was usually empty, but sometimes contained spermatozoa particularly near their connection with the epididymal duct. The efferent ductules of camel were lined by epithelial membrane of different cells types. Three types of cells could be encountered; columnar (ciliated and non ciliated), basal and migrating cells. The epithelium of the efferent ductules rested on a well-defined PAS positive basement membrane. The present study revealed that the form of the epithelial membrane varied along the course of the efferent ductules. The initial part of the efferent ductules of camel was lined by alternating groups of high columnar and low columnar cells. Because of this characteristic appearance, the epithelium was looking as like a festoon (Fig. 10). Towards the epididymal duct the epithelial cells of the efferent ductules were nearly of the same height. Also, the terminal portion of the efferent ductules possessed wider lumen than the other portion and their lumen was filled with spermatozoa.

Fig. (11) showed that the epithelium of the efferent ductules in the initial part was lined with numerous columnar non ciliated cells which possessed large oval nuclei occupied a wide nuclear zone, but most of them situated in basal third of the cell. These cells had a light finely granular acidophilic cytoplasm which contained variable number of empty cytoplasmic vacuoles mostly in the supranuclear region. Most columnar non ciliated cells showed bleb-like protrusions which differed in form. The blebs might be cylindrical having the same breadth of the cells from which they arose or pedunculated with a distinct neck. The apical cytoplasmic blebs were protruding into the lumen (Fig. 12). As shown Fig. (13); globules of PAS positive diastase resistant material were encountered in the infranuclear and supranuclear cytoplasm of these cells. Besides, the apical region of the ductular epithelium was PAS positive.

Fig (14) revealed the luminal portion of the ductular epithelium was moderately alcianophilic. As revealed in Fig. (15); the appearance of columnar ciliated cells was increasing towards the epididymal duct. They were long columnar cells with faintly stained cytoplasm and tufts of long cilia protruding into the lumen. The nuclei were ovoid shaped, heterochromatic and located at more apical position within the epithelium. Also near the epididymal duct, another cell type appeared within the epithelium of the efferent ductules (dark cells). They were few,

narrow, tall and slender shaped cells. The heterochromatic nuclei arranged with the long axis of the cells. The cells extended from the basement membrane to the lumen.

Fig. (16) revealed that the columnar ciliated cells showed cytoplasmic vacuoles that increased in intensity towards the epididymal duct. The luminal border of these cells was PAS positive. The cells contained coarse infranuclear PAS positive granules, besides fine ones in the supranuclear region.

Few basal cells were encountered in the epithelium lining the efferent ductules. The basal cells were oval to irregular with oval nuclei might be oval in shape and oriented with their longest diameter parallel to the basal lamina. Their cytoplasm might show variable amount of PAS positive granules.

Some cells were noticed to penetrate the basement membrane of the efferent ductules and migrated toward the lumen. Such migrating cells were found at different levels within the ductular epithelium till they left the luminal border to the lumen. The cytoplasm varied from being clear, basophilic or showed fine PAS positive granules.

Delicate peritubular collagen fibers and smooth muscle fibers were noticed around the efferent ductules that increased in frequency towards the epididymal duct. As showed in Fig. (17); a coarse reticular net was found around the efferent ductules.

Fig. (18) showed that alkaline phosphatase enzyme reactivity was observed throughout the subepithelial connective tissue and blood vessels. Weak reactions were also detected in the apical region of the lining epithelia. As revealed in Fig. (19); high activity due to acid phosphatase enzyme was demonstrated in the form of cytoplasmic granules in the whole epithelial lining of the efferent ductules.

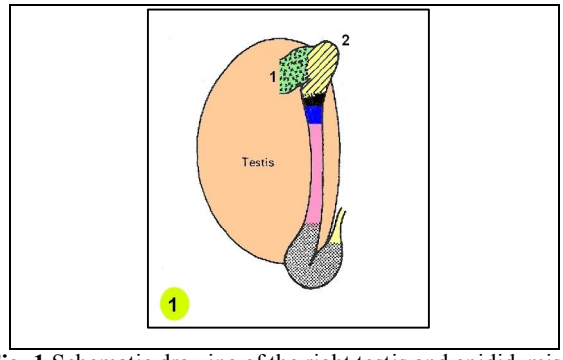

Fig. 1 Schematic drawing of the right testis and epididymis of camel. The head and body have been dissected free from the upper pole of the testis and stretched out to show the regions detectable.

1.Ascending first region of the head.
2. Initial segment of epididymal duct.

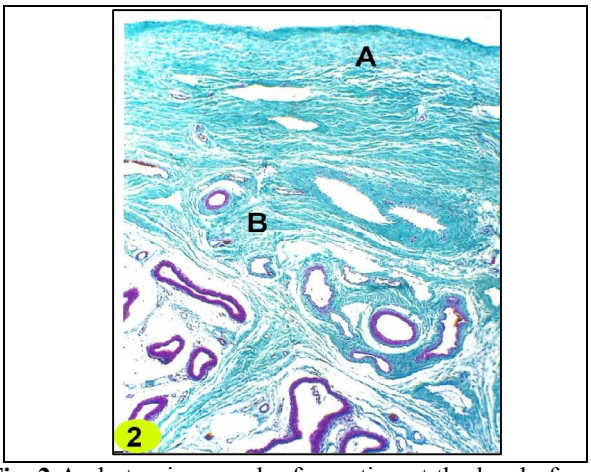

Fig. 2 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing.
The outer albuginea (A)
The inner albuginea (B)
Crossmon′s trichrome stain; x 40

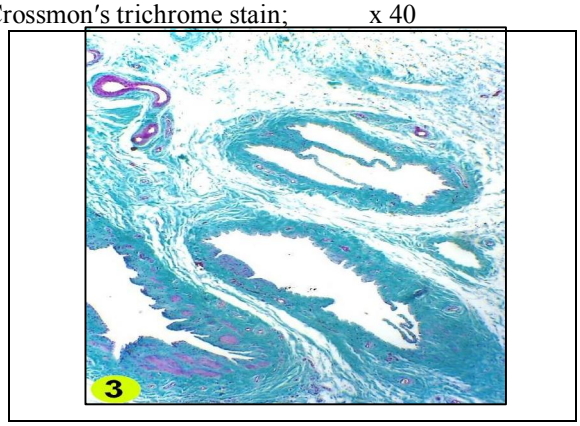

Fig. 3 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing deeper albuginea with muscular and valved veins Crossmon′s trichrome stain; x 100

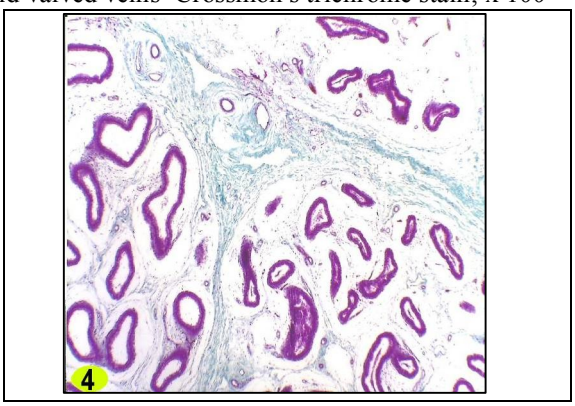

Fig. 4 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing lobules of efferent ductules. Crossmon′s trichrome stain; x 40

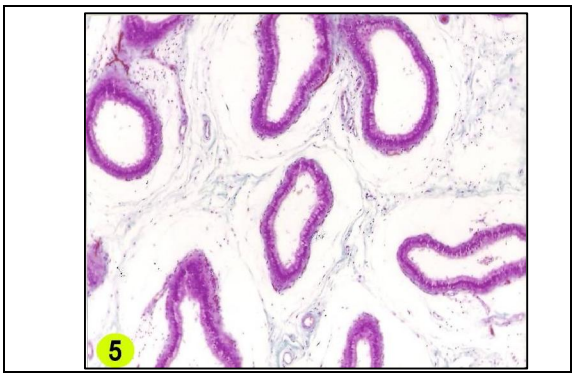

Fig. 5 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing the loose intralobular fibrous connective tissue. Crossmon′s trichrome stain;x 100

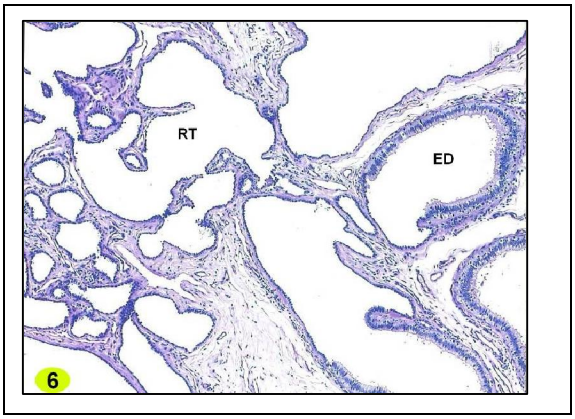

Fig. 6 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing. Extratesticular rete testis (RT) Initial part of efferent ductules (ED)
H & E stain; x 40

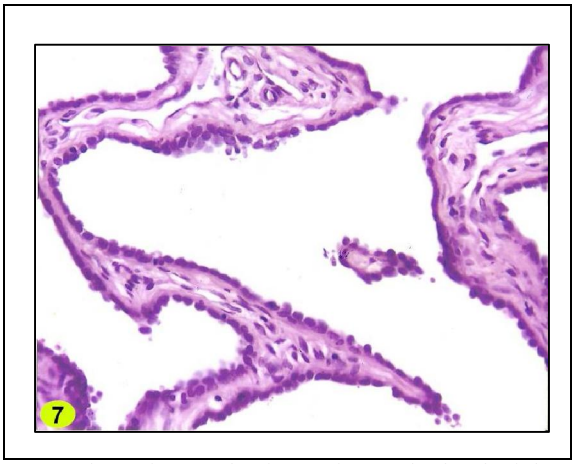

Fig. 7 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing extratesticular rete testis. H & E stain; x 100

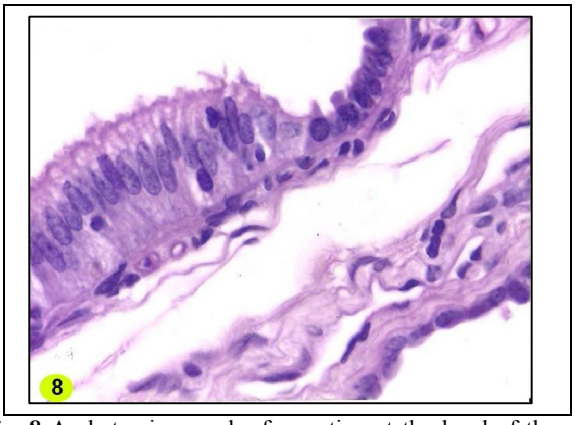

Fig. 8 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing the junction between the extratesticular rete testis and efferent ductules. H & E stain; x 100

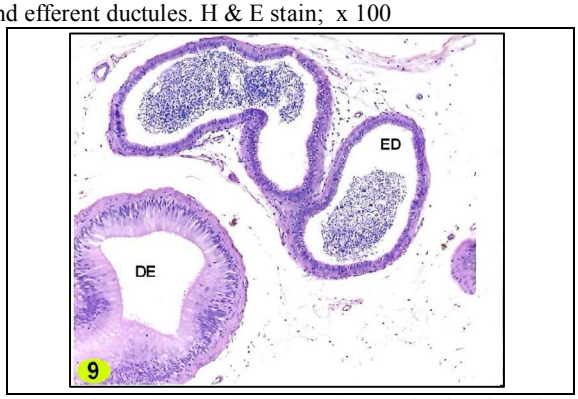

Fig. 9 A photomicrograph of a section at the level of the initial segment of the epididymal duct showing the efferent ductules filled with sperms near the epididymal duct. Efferent ductules (ED) Ductules epididymis (DE) H & E stain; x 100

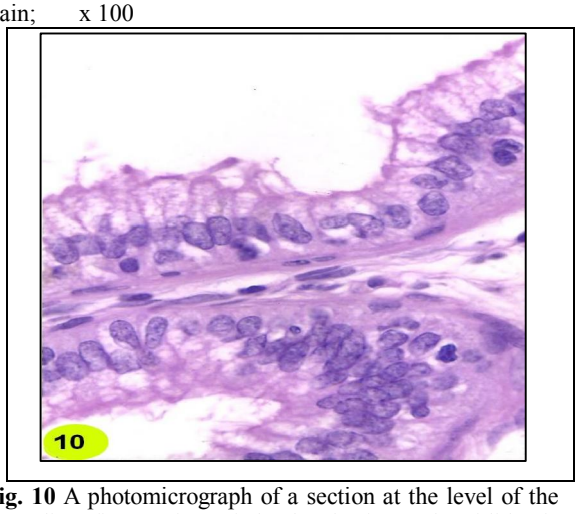

Fig. 10 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing high, low columnar non ciliated cells and basal cells. Notice the few peritubular smooth muscle fibers.
H & E stain; x 1000

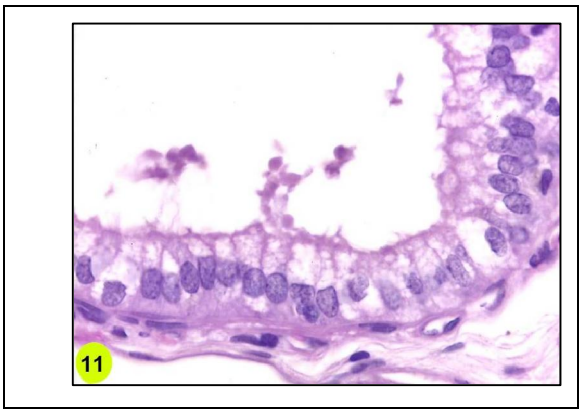

Fig. 11 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing the columnar non ciliated cells with bleb- like protrusions indicative of apocrine secretion. H & E stain; x 1000

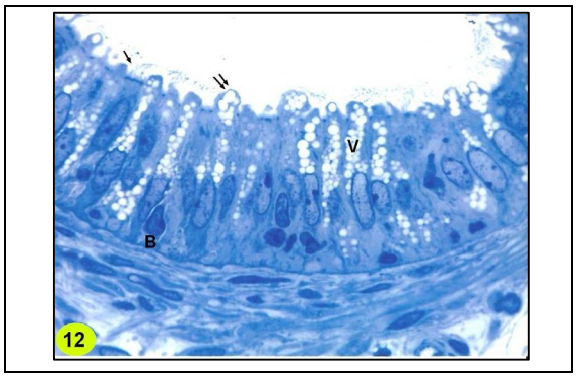

Fig. 12 Semithin section in the efferent ductules showing that the epithelium is composed of two main cell types; ciliated cells (arrow) and non ciliated cells (double arrow head). The latter contain cytoplasmic infranuclear and numerous supranuclear vacuoles (V). The apical secretory blebs are seen protruding from the non ciliated cell surface into the lumen in the form of vesicles. Basal cells (B) are also demonstrated. Notice that the epithelium rests on thin basement membrane which is surrounded by 3 – 4 layers of circularly arranged smooth muscle fibers.
Toulidine blue stain; x 1000

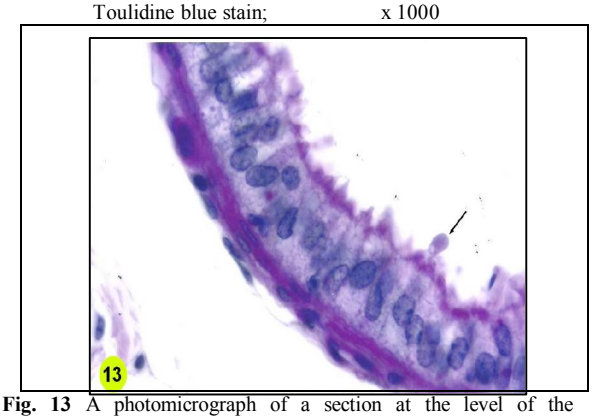

Fig. 13 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing the columnar non ciliated cells with infranuclear and supranuclear PAS positive granules.
PAS positive luminal margin.
PAS positive blebs – like protrusions (arrow).
PAS stain; x 1000

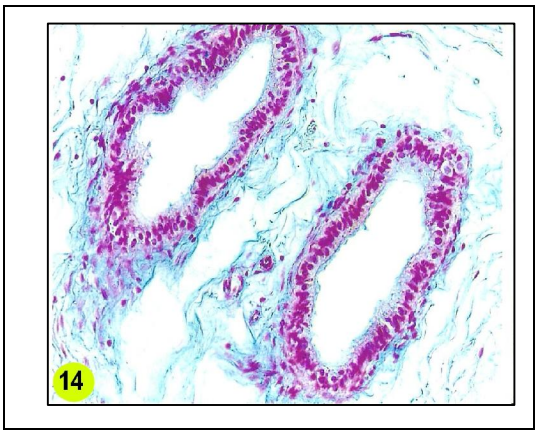

Fig. 14 A photomicrograph of a section at the level of the ascending first region of the head of camel epididymis showing alcianophilic luminal border of the epithelial lining. Alcian blue stain PH 2.5; x 100

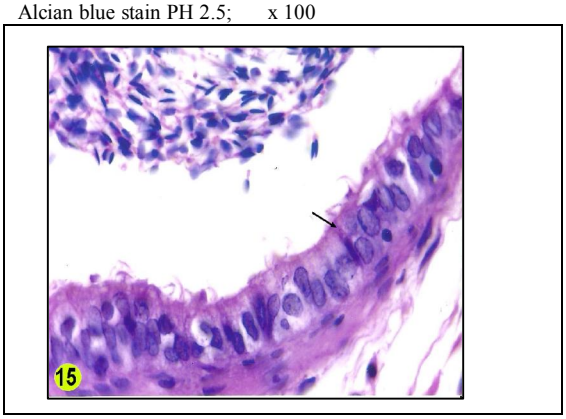

Fig. 15 A photomicrograph of a section at the level of the initial segment of the epididymal duct showing dark cells (arrow), sperms in lumen and peritubular smooth muscle cells. H & E stain; x 400

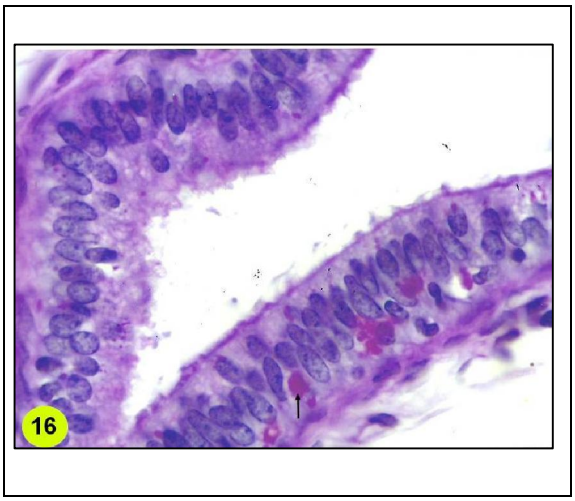

Fig. 16 A photomicrograph of a section at the level of the second region of the head of camel epididymis showing infranuclear coarse PAS positive globules (arrow), PAS positive fine supranuclear granules and PAS positive luminal border.
PAS stain; x 1000

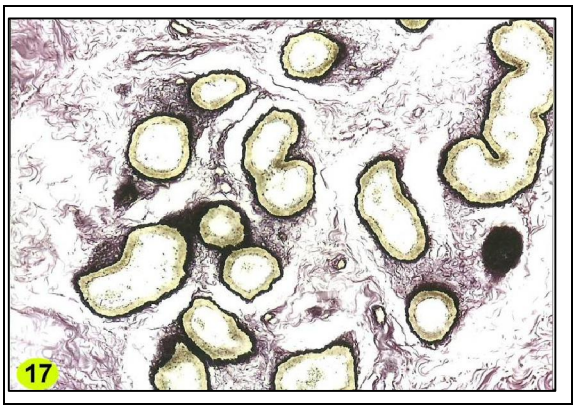

Fig. 17 A photomicrograph of a section at the level of the first ascending region of the head of camel epididymis showing peritubular reticular net.
Gomori's reticulin method; x100

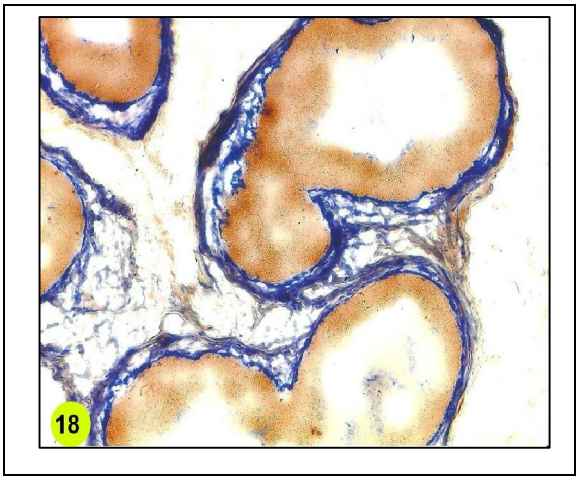

Fig. 18 A photomicrograph of a section at the level of the first ascending region of the head of camel epididymis showing alkaline phosphatase enzyme reactivity in the subepithelial connective tissue and in the apical region of the epithelial lining. Azo-dye coupling method. x400

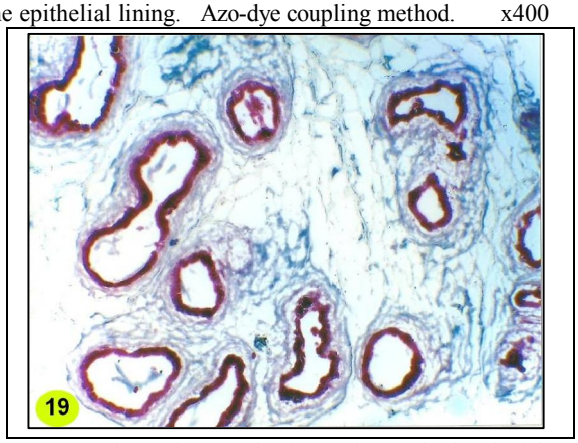

Fig. 19 A photomicrograph of a section at the level of the first ascending region of the head of camel epididymis showing acid phosphatase enzyme reactivity in the epithelial lining. Azo-dye coupling method. x 100

4. Discussion:

The efferent ductules of male camel were formed of convolutions of tubules connecting the rete testis with the epididymal duct. This simulate the result of **(Wrobel, 1998).**

The albuginea covering the ascending, first segment of the epididymal head showed many valved veins as well as loops of efferent ductules. The presence of valves in the interstitial epididymal veins might be of functional importance in regulating blood circulation in an ascending direction **(El-Rafey, 1985).**

The extratesticular rete testis of camel was lined by cuboidal to high cuboidal epithelial cells. The junction between the extratesticular rete testis and efferent ductules was an abrupt. These findings were in agreement with the findings of **Ebada (1994)** in camel and **Goyal,et al., (1992)** in goat**.**

Our results pointed out that the non ciliated cell always showed variable number of vacuoles and granules. These vacuoles and granules had been reported to be released in the lumen along with their contents through apical protrusions; the same results were obtained by **Goyal and Hrudka (1981).** Variations in histological appearance in different regions of efferent ductules had been reported in various species; in bull **(Alkafafy, 2005),** goat **(Goyal,et al., 1992)** and horse **(Aureli et al., 1984).** All these reports tend to divide the lining epithelial cells into two main types, ciliated and non ciliated, of which the non ciliated cells were three subtypes, cells with vacuoles, cells with granules and cells with neither vacuoles nor granules. In agreement with **Abd El-maksoud (2010)** in camel, we assume that, these differences were different stages of cellular functional status. Similarly, **Wrobel (1972)** interpreted the variation between non ciliated cells as two phases of absorptive process. The later author mentioned that, the vacuoles were a result of fluid absorption which condense into granules and were then eventually released into the lumen through apical protrusions.

The lumen of the efferent ductules in camel appeared free of sperms except few sperm collections toward the connection with the initial segment of the epididymal duct. The presence of these sperms was logic according to **Talo (1981)** and **Abd El-maksoud (2010)**. **Talo (1981)** postulated that, the presence of sperms in the terminal segment of efferent ductules was logic because many efferent ductules join the initial segment of the epididymal duct along short distance that might hinder the passage of sperms in these regions.

In the current study, the non ciliated cells showed bleb-like protrusions which differed in from. The blebs might be cylindrical having the same

breadth of the cells from which they arose or pedunculated with a distinct neck. The blebs showed variable number of PAS positive globules as well as vacuoles. The secretory activity of the non ciliated cells was denoted by the presence of PAS positive granules and globules in the infranuclear as well as the supranuclear cytoplasm. The blebs at the luminal border of the ductular epithelium could be taken as indication for the apocrine secretory activity of the non ciliated cells of the efferent ductules of camel. The blebs in the epididymis were described as manifestation of apocrine secretion **(Hess and Bassily, 1988).**

The acid mucopolysaccharides were mainly located in the apical region of epithelial cells of efferent ductules of the camel. Similar findings were encountered in some species **(Goyal and Dhingra, 1975a** and **López et al., 1989).** This observation suggested that the epithelial cells might be secretory in function as indicated in rabbit **(Nicander, 1957).** The efferent ductules had been reported to perform functions of absorption **(Pineda, 1989)** and secretion **(Goyal and Hrudka, 1981). Goyal and Dhingra (1975b)** in buffalo denoted the presence of vacuoles in the apical cytoplasm of the ductular epithelium as suggestive of their absorptive function.

In the present work, few tall, slender dark cells appeared in the efferent ductules near the epididymal duct. **Tingari (1989)** in camel considered these dark cells as intermediate stages prior to death of principal columnar cells.

Peritubular smooth muscle fibers were noticed around the efferent ductules of camel which increased in frequency towards the epididymal duct. Our findings were in accord with those of **Abd El-maksoud (2010)** in camel**, Ebada (2000)** in buffalo and **Goyal et al., (1992)** in goat and disagree with **Goyal and Dhingra (1975b)** in buffalo. The later author could not notice any muscle fibers around the efferent ductules.

Similar to the findings of **Tingari and Moniem (1979)** in camel, a positive reaction for alkaline phosphatase was observed only in the subepithelial connective tissue, blood vessels and stereocilia. The stereocilia had been reported to be positive in camel **(Al-Shaikly et al., 1981)** and bull **Goyal and Vig, 1984)** and slightly positive in stallion **(López et al., 1989).** The alkaline phosphatase activity had been associated with the active transport **(López et al., 1989)** and with secretion of metabolites participating in the maturation of spermatozoa **(Al-Shaikhly,et al., , 1981** and **Beu et al., 2007).**

The activity of acid phosphates was observed to be high in the efferent ductules of camel. The activity had been associated with lysosomes present in the mammalian epididymis **(Goyal, 1985, López et al., 1989** and **Delhon and von Lawzewitsch, 1994).**

5. Conclusion:

This study revealed that micromorphological and histochemical features of the efferent ductules of the camel provide evidence for the presence of three different types of epithelial cells, columnar (ciliated and non ciliated), basal cells and migrating cells. In addition to these common cells, there was evidence for the presence of dark cells near the junction with the epididymal duct. Indication of apocrine secretion was demonstrated in camel efferent ductules by the presence of vacuoles, PAS positive granules as well as the prominent blebs at the apical cytoplasm. The presence of cilia in the efferent ductules might participate in rapid transportation of spermatozoa.

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