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MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF THE NON- AMPULLATED PART OF THE DUCTUS DEFERENS OF THE CAMEL (CAMELUS DROMEDARIUS)

(With One Table and 16 Figures)

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دراسات مورفولوجية مناعية هستوكيميائية على الجزء الغير انبورى للقناة الاسهرية فى الجمل وحيد السنام

عبدالمهيمن مصطفى صالح

اجريت دراسة مورفولوجية عيانيا ومجهريا بواسطة الميكورسكوب الضوئى والالكترونى الماسح وكذلك دراسة مناعية هستوكيميائية للجزء غير الانبورى للقناة الاسهرية فى الجمال وحيدة السنام السليمه ظاهريا وناضجه جنسيا. ينقسم الجزء الغير انبورى الى ثلاثة مناطق تشريحية منطقة دانية شديدة التعرج، ومنطقة وسطى بسيطية التعرج، ومنطقة بعيدة غير متعرجة. تغطى القناة الاسهرية بظهارة عمودية مطبقة كاذبة ومهدبة وترتكز على صفحة اساسية رقيقة. وتحمل الخلايا الظهارية اهدابا طويلة متفرعة فى المنطقة الدانية بالمقارنة مع اهداب طويلة غير متفرعة فى المنطقة الوسطى وخملات قصيرة فى المنطقة السفلية. اما الغلالة العضلية فهى تتكون من طبقتين: داخلية دائرية وخارجية طولية. تم استخدام التفاعلات المناعية الهستوكيميائية المضادة كل من PGP-9.5 و DBH و ChAT لدراسة التوزيخ العصبى العام والسمبثاوي والجارسمبثاوي للقناة الاسهرية. اظهرت هذه الدراسة ان الاعصاب الداخلية الموجودة فى القناة الاسهرية تكون ليفة عصبية فى النسيج تحت الطلائى واخرى فى النسيج العضلى.

SUMMARY

Gross, light & scanning electron microscopic and immunohistochemical morphology of the non-ampullated part of the ductus deferens of sexually mature dromedary camels were studied with special reference to the changes and modifications occurring throughout its entire length. This part was subdivided into three anatomical regional segments; proximal tortuous (scrotal), middle less tortuous (inguinal) and distal straight (abdominal) segments. It characterized by simple and low folded mucosa. It lined by pseudostratified ciliated columnar epithelium with a thin lamina propria-submusosa. By scanning electron microscope,

stereocilia of epithelial cells were tall and branched in the proximal segment tall, non-branched stereocilia in the middle segment and thick short microvilli in the distal segment. The muscular coat arranged in two layers; the inner layer was circular, while the outer layer was longitudinal. The intramural nerve fibers of the ductus deferens form plexuses in subepithelial tissue and in the muscular coat in addition larger nerve bundles in the serosa. Immunohistochemical reaction to protein gene product–9.5 (PGP-9.5) was used to demonstrate the general innervation pattern. The adrenergic and the cholinergic innervation were demonstrated using by immunohistochemical reactions to DPH and ChAT. These structural variations along the length of the vas deferens suggest that it performs functions other than just as a passageway for spermatozoa.

Key words: *Ductus deferens, camel, immunohistochemistry, scanning electron microscope, mesoductus deferens.*

INTRODUCTION

The ductus deferens is a tubular structure connecting the tail of the ductus epididymis with the pelvic urethra (Popovic *et al.,* 1973). It has been reported that it is a major storage site for sperms (Turner 1995). It probably plays important role in providing sperm with a suitable environment for their maturation, viability and protection during their passage through its terminal portion (Bergerson *et al.*, 1994; Andonian and Hermo, 1999). The anatomical studies on the non-ampullated part of the ductus deferens have been conducted by several authors in different domestic animals. Schummer *et al.* (1979) in the domestic animals and Skidmore (2002) in the camel conducted the gross anatomical observation. Trautman and fiebiger (1957), Goswami *et al.* (1994) in camel, Wrobel and Dellmann (1993) in the domestic animals conducted the microscopic observation. The innervation had been studied histochemsitry and immunohistochemsirty by Duminika (1983) in the dog, Kujat *et al.* (1993) in bulls, Kaleczyc *et al* (1997) in boar and Mirabella *et al.* (2003) in water buffaloe. Studies on the camel ductus deferens are meager and focus mainly on some histological structures. There exist, so far, no reports on the scanning electron microscopy and the innervation of the camel ductus deferens. Therefore, the present study was undertaken to give more information on the morphology of the non-ampullated part of the ductus deferens in the dromedary camel grossly, histologically, immunohistochemically and by the scanning microscopy.

MATERIALS and METHODS

The present study was carried out on the ductus deferens of 30 sexually mature and apparently healthy one humped camels (Camelus dromedarius) collected from Bany Ady slaughter house (Assiut - Egypt). Specimens were collected from the deferent segments of the ductus deferens and processed for light and scanning electron microscopy. Sections, 5-7μm thick, were stained with H&E, Periodic acid Schiff (PAS) and Alcian blue (AB) techniques. Some morphometric aspects were performed using Leica Q 500 MC Image analyzer. For Scanning electron microscopy, specimens were immersed in 5% glutaraldehyde, post-fixed in 1% Osmium tetroxide, dehydrated in alcohol followed by amyl acetate and critical point dryed using liquid Co_2 and mounted on specimen stubs, sputter-coated with gold and examined in a JEOL 5400 LV scanning electron microscope.

Sample from the different segments of the ductus deferens were immunohistochemically treated for visualization of PGP-9.5-, DBH- and ChAT-positive nerves by light microscopy according to methods described fully by Kujat *et al.* (1993) and Saleh (2002).

RESULTS

The non-ampullated part of the ductus deferens of dromedary camel was relatively long and measured about 57.7 cm in length. It was enclosed in a narrow serosal fold, the mesoductus deferens. It was divided into three segments according to its position; scrotal, inguinal and abdominal. The scrotal one ran highly tortuous on dorsomedial aspect of the testis, measured about 25.5 cm in length (Table 1, Fig. 1, 2). The inguinal segment ran less tortuous in the inguinal canal medial to the vascular part of the spermatic cord, formed the longest segment of the ductus deferens (24.7 cm in length) (Table 1, Fig. 1). The abdominal segment, gained the abdominal cavity at the internal inguinal ring, ran dorsocaudally, entered the genital fold and continued as ampulla ductus deferens. It measured 16.5 cm in length and 2.6 mm in diameter (Table 1)

The wall of the non-ampullated part of the ductus deferens consisted of three circumferentially arranged layers around a centeral lumen: tunica mucosa, tunica muscularis and tunica serosa (Fig. 2, 3, 4). The latter was continuous with the mesoductus deferens and contained blood vessels, nerves and variable amount of adipose tissue. The lumen of the ductus deferens was narrow at the initial part, dilated distal-wards and frequently contained masses of sperms.

The tunica mucosa of the ductus deferens of the dromedary camels showed low longitudinal folds, lined with ciliated pseudostratified columnar epithelium. Four types of cells; principle, basal, apical and granular were recognized (Fig. 5).

The principle columnar cells predominated the lining epithelium of the ductus deferens. It characterized by acidohphilic foamy cytoplasm and rounded or oval basally situated nuclei. The basal cells were laid next to the basement membrane and characterized by slightly basophilic cytoplasm and ovoid or round nuclei. The apical cells are few in number and characterized by small deeply stained apical located nuclei. Granular cells were found among the principle columnar cells in the scrotal segment, extending from the basement membrane to the luminal surface. They presented wide apical portion, fine basiophilic granules in cytoplasm and round deeply stained nuclei (Fig. 5).

The scanning electron microscopy revealed the presence of three different pictures in the luminal surface of the non-ampullated part of the ductus deferens. In the scrotal segment, it showed some non-ciliated cauliflower-shaped cells among the dense ciliated epithelium (Fig. 7). The ciliated cells were dominating and studded with dense tall (6.6 μm) branched stereocilia (Fig. 8). The non-ciliated cells were few and presented micro-secrotory granules (Fig. 9). In the inguinal segment, the epithelial surface showed only ciliated cells. The stereocilia were dense non-branched and relatively shorter (4.3 μm) than that of the scrotal segment (Table 1, Fig. 10). In the abdominal segment, the surface of the epithelium showed short (2.5 μm) and thick microvilli (Table 1, Fig. 11).

PAS staining revealed mild to moderate reaction in the epithelial cells. The principle columnar cells contained large number of PAS positive granules in the apical zone and few ones in the basal portion. The secretory blebs and stereocilia showed intense PAS reaction. The basal cells showed weakly PAS reaction in the form of few fine positive granules (Fig. 6). With Alcian blue stain, the lining epithelium of the ductus deferens showed very weakly or no reaction.

The epithelium height reached its maximum value in the scrotal segment and decreased gradually toward the abdominal segment. It measured about 25.9, 20.21and 16.21μm in the scrotal, inguinal and abdominal segments respectively (Table 1).

The lamina propria-submucosa composed of dense connective tissue with collagenous fibers. Some smooth muscle fibers derived from the tunica muscularis were scattered in the outermost part of the lamina propria. Lymphocytes and numerous blood vessels were also

demonstrated (Fig. 4). The thickness of this layer generally increased toward the termination of the abdominal segment. It measured 48.31μm in the scrotal segment and reached about 70.31μm in the abdominal segment (Table 1).

The tunica muscularis consisted of muscular bundles arranged in two layers. The inner layer was circular, while the outer layer was longitudinal (Fig. 2, 3 and 4). It formed the thickest layer of wall of the ductus deferens. It measured about 575.42 μm, 602.6 μm 656.2 μm in the scrotal, inguinal and abdominal segments respectively (Table 1).

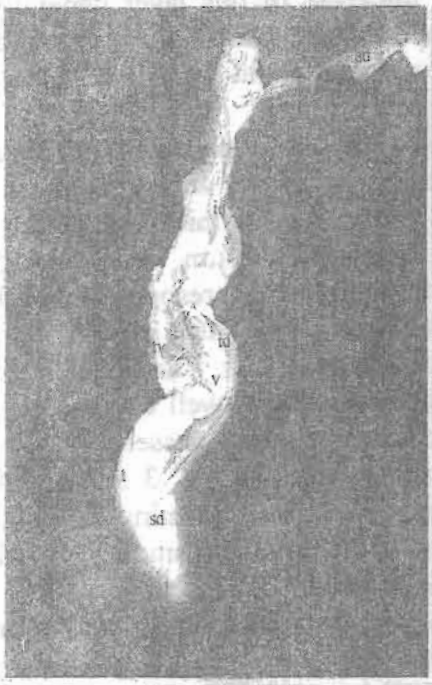
The tunica serosa consisted of an outer mesothelial epithelium covering sub-serosal connective tissue layer. The latter layer contained numerous small-sized arteries and veins as well as venules, arteriolies, smooth muscle bundles and fibers, lymph vessels, nerve fibers and continued with the mesoductus deferens (Fig 2, 3 and 4.). At its outer part, the collagenous fibers appeared denser forming band-like structure containing numerous longitudinally oriented smooth muscle bundles extending to the mesoductus deferens.

The intramural innervation was generally studied by using PGP-9.5- IR. In the ductus deferens, the larger nerve bundles are situated in the tunica periductular tissue and mesoductus deferens and send finer bundles and solitary fibers to the muscular layer, subeepithelial and the vascular plexuses. These fibers formed two plexuses; one was situated in the muscular layer and the other in the subepithelial connective tissue (Fig. 12).

ChAT-IR and DBH-IR were used to differentiate the types of the nerve fibers. All nerve bundles that reach to the ductus deferens were contained a certain number of ChAT-positive fibers. Inside the ductular wall, the ChAT-positive nerves concentrated mainly in the subepithelial tissue and only very few fibers demonstrated in the muscular coat. The vascular wall was devoid from the cholinergic nerve fibers. DBH-IR allowed to visualization of virtually all fibers in the muscular coat and serosal layer and some nerve fibers in the subepithelial tissue and the vascular plexus. ChAT-IR is specific for the demonstration of cholinergic nerves. DBH-IR visualized the fine nerve fibers in the wall of the ductus deferens better than the general nerve marker (Fig. 14, 15, 16).

Table 1: Showing the measurements of the diferent segments of the ductus deferens.

	Testicular	Inguinal	Abdominal
Length in cm	25. 5 ± 2.8	24.7 ± 2.4	16.5 ± 1.9
Epithelium height in μm	25.9 ± 1.3	20.21 ± 0.9	16.21 ± 0.67
Sterocilia height in μm	6.6 ± 0.78	4.3± 0.63	2.5 ± 0.3
Lamina propria thickness in μm	65.31 ± 2.57	85.23 ± 3.42	90.31 ± 3.95
Tunica muscularis thickness in μm	675.42 ± 8.6	702.6 ± 10.3	756.2 ± 9.4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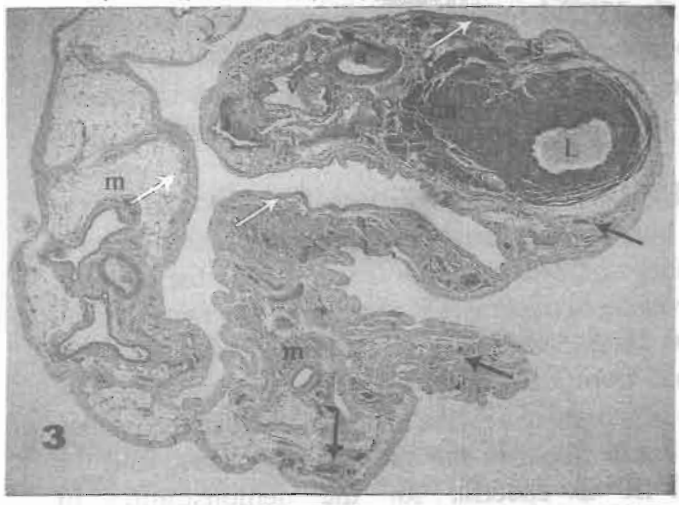
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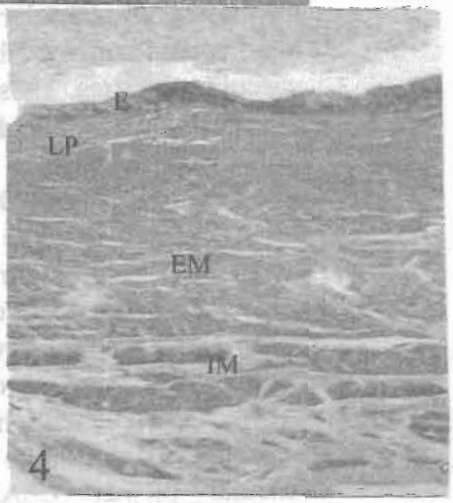
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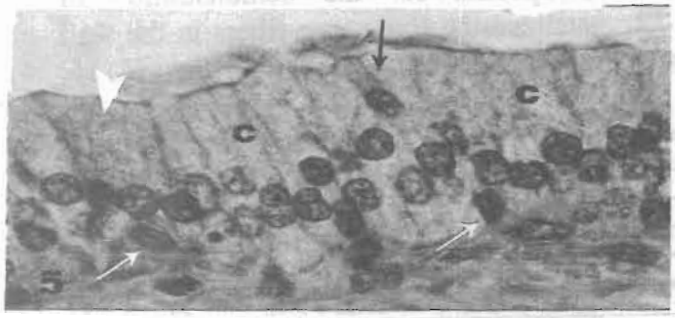
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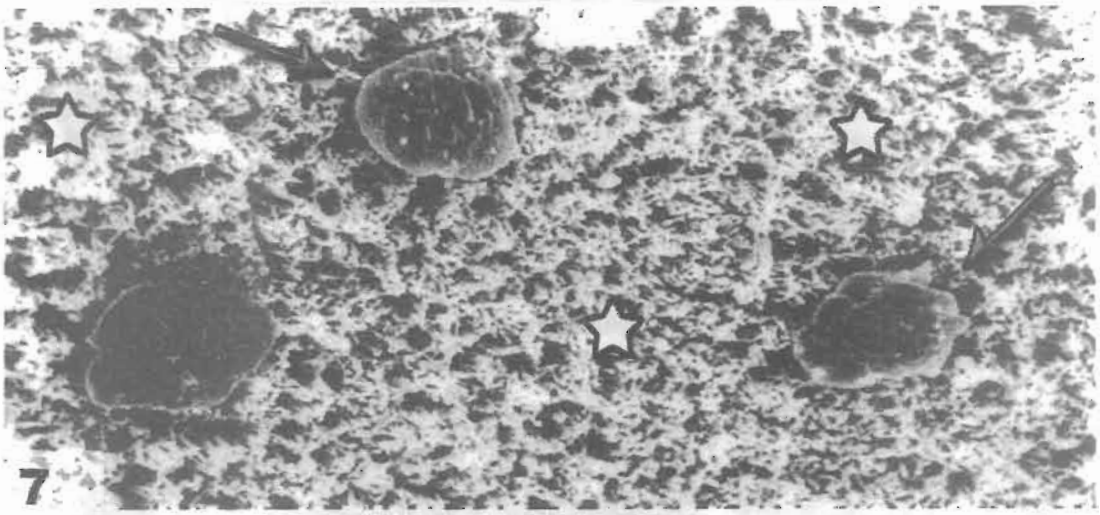

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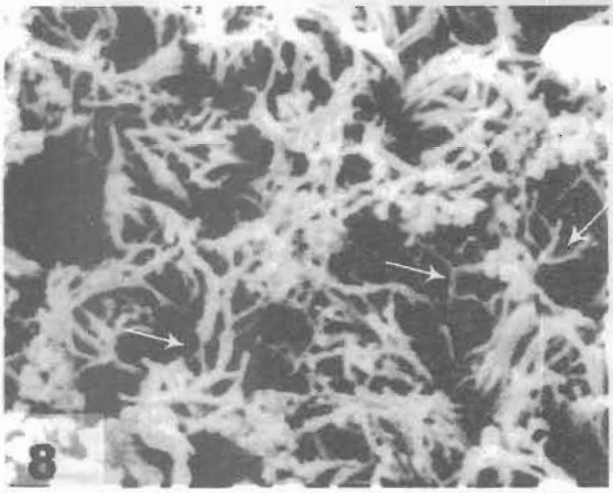

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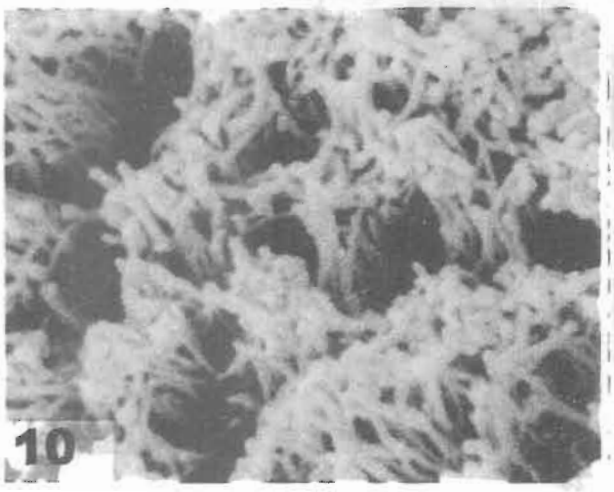

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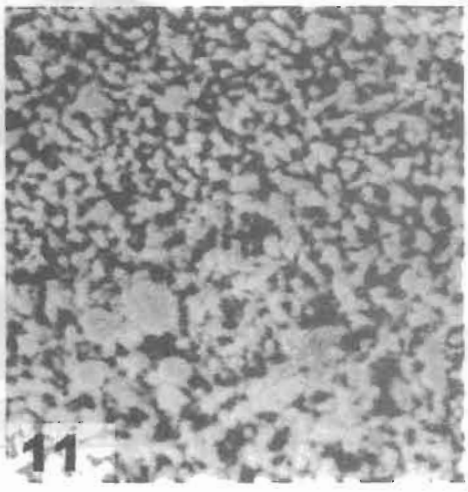

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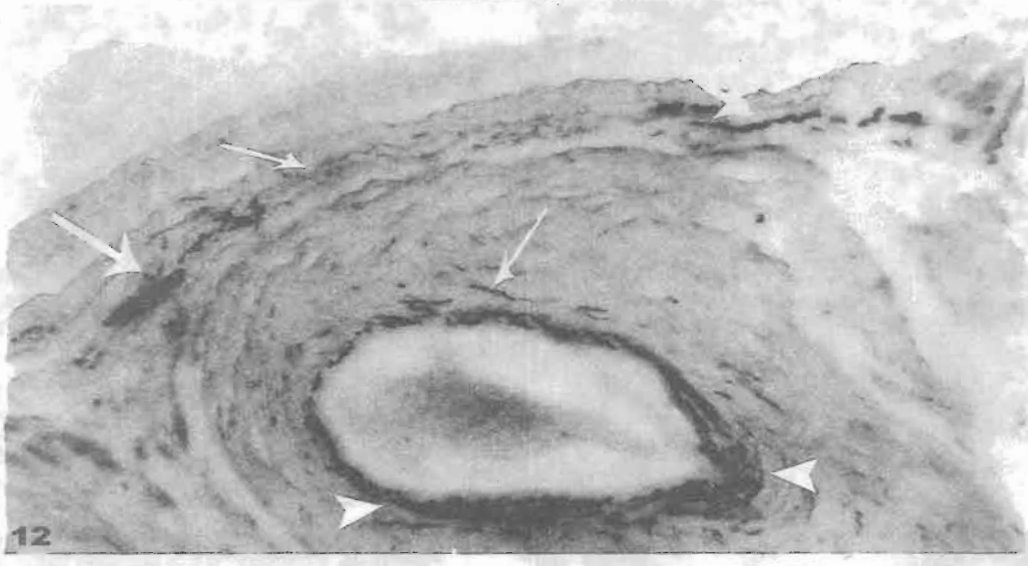
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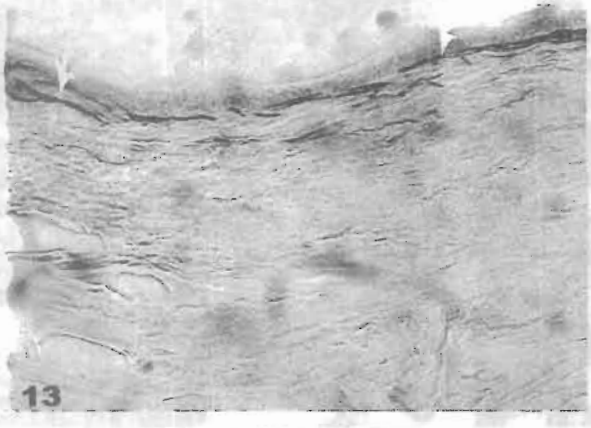
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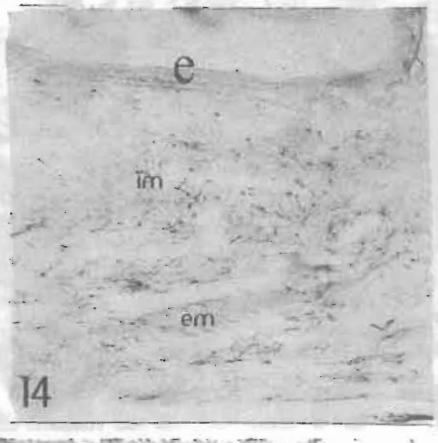

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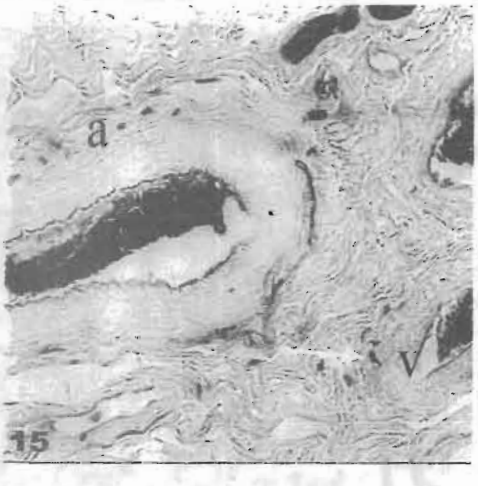

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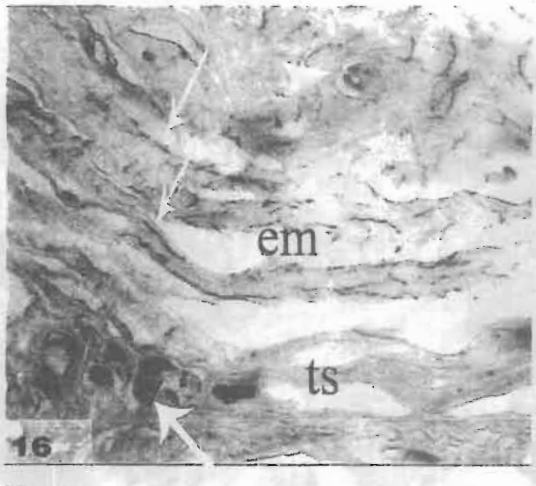

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LEGENDS

Fig. 1: The non ampullated part of the ductus deferens consisted of scrotal (sd) inguinal (id) and abdominal (ad) segments. Testis (t), Vascular part of the spermatic cord (v), Tunica vaginalis (tv).

Fig. 2: The scrotal segment run in highly tortuous manner. The deferent duct (Arrows), Tail of the epididymis (e)

Fig. 3: Due to tortuous manner of the ductus deferens, most of sections were cut obliquely. The lumen (L) packed with spermatozoa surrounding by epithelium (e), lamina propria and muscular coat (tm). The mesoductus deferens is large (m) and expended in the non-attached side of the ductus deferens. The collagenous fibers appeared denser containing numerous smooth muscle bundles in the outer layer of the tunica serosa and mesoductus deferens (Arrow), the inner layer. H&E x10.

Fig. 4: The wall of the non-ampullated part of the ductus deferens consisted of of epithelium (E), Lamina propria-submucosa (Lp), tunica muscularis ccomposed of inner circular (IM) and outer longitudinal (EM) layers of the smooth muscle fibers, and tunica serosa (TS).

Fig. 5: The The lining epithelium of the ductus deferens shows four types of cells: principle (C), basal (Arrow), apical (arrow with double head) and granular (Arrow head). (oil immersion lens), x1000.

Fig 6: The epithelium of the ductus deferens presents PAS-positive granules in the columnar cells (star) and some in the basal cells (arrow). (oil immersion lens), x1000.

Fig. 7: In the scrotal segment, the luminal surface of the presents two types of cells; ciliated (Stars) and non-ciliated cells (Arrows). SEM, x 2000.

Fig. 8: The ciliated cells show dense tall and branched steroocilia. (Arrows) SEM, x 10,000.

Fig. 9: The surface of the nonciliated cells shows micro-secrotory granules.

Fig. 10: In the inguinal segment, the epithelial surface presents only ciliated cells cover with dense tall stereocilia. SEM, x 10,000.

Fig. 11: In the abdominal segment, the epithelial surface covered with microvilli. SEM, x10,000.

Fig. 12: The intramural innervation of the ductus deferens composed of larger nerve bundles (Thick arrows) finer bundles and solitary fibers (Thin arrows)in the periductular tissue, foming dense nerve plexus in the subeepithelial tissue (Arrow heads) and fine one in the muscular layer. PGP 9.5-IR, x100.

Fig. 13: The ChAT-positive nerve fibers (Arrows) concentrated mainly in the subepithelial tissue in addition to few fibers in the muscular coat.

Fig. 14: DBH-IR shows dense nerve plexus of fine nerve fibers in the inner (im) and outer (em) muscular layer, in addition to few fine fibers in the subepithelial tissue (e). DBH-IR, x200.

Fig. 15: The vascular wall of the arteries and veins contains DBH-positive fibers. artery(a), vein(v). DBH-IR, x200.

Fig. 16: The periductlar tissue contains thick DBH-positive fibers (arrow) and the large nerve bundle are strongly DBH-IR. The small artery contains also DBH-positive fibers. DBH-IR, x200.

DISCUSSION

In the present study, the non-ampullated part of the ductus deferens of the camel is relatively long (66.2 cm in length average). It divided into three different segments; very tortuous scrotal, slightly tortuous inguinal and straight abdominal. Skidmore (2000) in the same animal reported that the ductus deferens ran tortuous at its initial part and measured 45-50cm in length. In other animals, the ductus deferens ran flexuous in the initial part and then straight in its most part and measured 52.5 - 82, 30 - 32 and 17 - 18 cm in buffaloe, donkey and dog respectively (Pal and Bharadwaj 1985; Salem 2003). In rat, buffaloes and donkey only two different structural segments were mentioned; extra-abdominal and intraabdominal (Hamilton and Cooper, 1978; Pal and Bharadwaj, 1985 and Salem, 2003).

The lumen of the ductus deferens was wider in the scrotal and inguinal segment in comparison with the abdominal one; it frequently contained masses of sperms. These results are disagreed with the results of Stallcup and Griffon (1969) in bull, and Goswami *et al.* (1994), in camel. The ducts deferens was lined by pseudostratified columnar epithelium and studded with stereocilia as confirmed by scanning electron microscopy. These results are similar to that mentioned by Goswami *et al.* (1994) in camel, Wrobel and Dellman (1993) in the domestic animals, Schimming (2001) in tufted capchin monkey and

Salem (2003) in donkey. In agreement with Pal and Bharadwaj (1985) in buffaloes and Salem (2003) in donkey, the epithelial lining of extra-abdominal part (scrotal and inguinal) of ducts deferens was higher than that of the abdominal one. These results disagree with that observed by and Goswami *et al.* (1994) in the same animal, Hoffer and Greenberg (1978) in guinea pigs, Kennedy and Heidger (1979) in rats

The present study demonstrated that the lining epithelium of the camel duct deferens consisted of four types of the cells; principle, basal, apical, dark stained cells. While Goswami *et al.* (1994) in the same animal, Pal and Bharadwaj (1985) in buffaloes and Salem (2003) in donkey, mentioned that the lining epithelium composed mainly of principle and basal cells.

The principle cells were the major cell type; they possessed rounded or ovoid basally situated nuclei. By scanning electron microscope, the surface of these cells showed dense tall-branched stereocilia in the scrotal segment, dense less tall non-branched stereocilia in the inguninal segment and thick short microvilli in abdominal segment. The stereocilia were microvilli projections of the cell membrane increased greatly the surface area of the duct cells (Niemi 1965). The cytologic characteristics of principle cells, as stereocilia, apical blebblings, primary and secondary lysosomes, seem to indicate that these cells are mainly responsible for the absorptive function (Paniagua *et al.,* 1981). These cells eliminated water from the lumen of the duct during and after ejaculation into the underlying vascular channel in the lamina propria in order to concentrate the sperms (Liebish, 1990; Andonian and Hermo, 1999).

The basal cells were oval or spherical with slightly basophilic cytoplasm and round nuclei. They were probably undifferentiated cells that are capable of differentiation into columnar cells (Paniagua *et al.,* 1981; Nistal *et al.* 1992). It could be suggested that the principal, apical, and basal cells seem to be related to a cell cycle, in which the basal cells are the stem cells.

The apical cells were also described by Kennedy and Heidger (1979) in rat, Paniagua *et al.* (1981) & Nistal *et al.* (1992) in man and Schimming (2001) in tufted capuchin monkey. These cells might represent old or "exhausted" columnar secretory cells that have undergone mitochondrial hyperplasia and loss of organelles involved in glycoprotein synthesis (Nistal *et al.* 1992). It might be involved in the acidification of the seminal plasma or transport of electrolytes, hydrogen ions and water across the mucosa (Schimming, 2001).

The granular cells which found only in the scrotal segment, had wide apical portion, basiophilic granular cytoplasm and round deeply stained nuclei. By scanning electron microscope, appeared cauliflower in shape and presented secrotory granules and short microvili on their surface. They were also reported in others mammals as rat (Rodríguez & Bustos-Obregón, 1993), in the tufted capuchin monkey (Schimming, 2001). These cells appeared to be involved in microapocrine secretion, in addition they aid in phagocytosis of the sperm remnants or residual bodies (Hamilton and Cooper, 1978). Some authors considered these cells as: holocrine secretory cells (Martan *et al.*, 1964), dead or dying columnar cells (Hoffer, 1976 and Paniagua *et al.*, 1981).

The lining epithelium of the camel ductus deferens contained PAS-positive granules in the columnar and basal cell similar to that mentioned by Stallcup and Roussel (1968) in the bull. While, in buffaloes, the PAS-positive granules demonstrated only in the columnar cells (Pal and Bharadwaj 1985). With Alcian blue stain, the lining epithelium of the ductus deferens showed very weakly or no reaction. This indicated that the epithelium of the camel ductus deferens contained mainly neutral mucopolysaccharids.

The ductus deferens has been classically described as exhibiting a folded mucosa with portions of the epithelium and underlying lamina propria projection into the lumen Goswami *et al.* (1994) in camel, Wrobel and Dellmann (1993) in the domestic animals, and Salem (2003) in donkey. In the monkey the mucosal folds appeared in the middle and distal segments of duct. Kennedy and Heidger (1979) observed a folded mucosa only in the terminal segment of vas deferens of the rat. In the camel, the ductus defrens showed low or absence mucosal invaginations but not have uniform lumen.

The tunica muscularis of the camel ductus deferens consisted of many muscular bundles arranged into inner thick circular and outer thin longitudinal layers similar to that stated by Goswami *et al.* (1994). In the donkey, it consisted of inner interwoven and outer longitudinal layers of smooth muscle fibers (Salem, 2003). While in stallions, bulls, boars and carnivores, it consisted of intermingled circular, longitudinal and oblique smooth muscle layers (Trautman and Fiebiger 1957; Wrobel and Dellmann, 1993). In guina pigs and buffaloes, it consisted of inner and outer longitudinal and middle circular layers of smooth muscle fibers (Hoffer and Greenberg, 1978; Pal and baradwaj 1985). The muscular coat of the camel duct deferens increased in thickness distalwards. The increased thickness of the muscular coat in the abdominal segment, may

assisted in maintaining the speed of ejaculated sperms within this part as nearly as that of the initial part (Salem, 2003).

The serosal layer of the ductus deferens of the camel is characterized by presence of smooth muscle bundles distributed in its different parts. In its outer part, the collagenous fibers became denser and formed a band-like structure just under the mesothelium and containing some smooth muscle bundles. The muscle bundles might be derived from the outer layer of the muscular coat. Our results agree with that of other domestic animals reported by Trautman and Fiebiger (1957) Schummer *et al.* (1979) Pal and Bharadwaj (1985). In donkey, the band-like structure in the outer layer of the subserosal layer contained continuous longitudinally oriented layer of the smooth muscle bundles decreased toward the mesoductus deferens but the other part the serosa not contained any muscular bundles (Salem, 2003). The presence of longitudinally oriented smooth muscle bundles within the serosa of the ductus deferens, probably enable it to accommodate itself with the shorting and lengthing action, exerted by contraction of muscular coat during ejaculation. The unique (band-like) arrangement of dense collagenous fibers in the outer layer of the subserosa may indicated that, this layer lay under tension and consequently need more support and great resistance to pulling force (Salem 2003).

The intramural nerve fibers were demonstrated immunohistochemically by using well-established general nerve markers PGP.5-IR (Thompson *et al.* 1983; Wang *et al.* 1990). It showed the distribution of the large and fine fibers in the wall of the ductus deferens and the components of the vascular plexuses. The intramural innervation of the camel ductus deferens composed of larger nerve bundles in the serosal and the periductalar tissue, in addition finer bundles and solitary fibers in the ductular wall formed thick subepithelial and fine intramuscular nerve plexuses. The specific innervations were demonstrated by using adrenergic nerve markers (DBH-IR) and cholinergic nerve marker (ChAT-IR) (kujat *et al.,* 1993; Saleh, 2002). DBH-IR allowed to visualization of virtually all fibers in the muscular coat and serosal layer and many nerve fibers in the subepithelial tissue in addition to the vascular plexus and therefore represented postjunctional sympathetic fibers. As in the boar (Kaleczyc *et al.,* 1993), the DBH-positive fibers were dense in both the inner and outer muscular layer. Adiverging innervation pattern was observed in the dog and bull ductus deferens where the adrenergic innervation was occurred only in the inner muscular layer, while the other muscle layer contained few fibers

(Sjoestrand, 1965, and Kujat *et al.*, 1993). In our result DBH-IR cleared the fine nerve fibers in the wall of the ductus deferens better than the general nerve marker. Similar result has been reported by Saleh (2002) in the innervation of the camel testis and epididymis.

The cholinergic innervation of the camel ductus detferens was demonstrated with ChAT-IR. All nerve bundles that reach to the ductus deferens were contained a certain number of cholinergic fibers. Inside the ductular wall, the cholinergic fibers concentrated mainly in the subepithelial tissue and showed very few fibers in the muscular coat. The vascular wall was devoid from the cholinergic nerve fibers. In the bull vas deferens, the cholinergic fibers formed well develop plexus in the subepithelial tissue and inner muscular wall and few fibers found in the outer muscular layer (Kujat *et al.* 1993). The adrenergic innervation has important role in the spermatozoa transport during emission of semen from the epididymis through the ductus deferens (Billups *et al.* 1990 and Kempinas *et al.* 1998). Restriction of the spermatozoa transport in the vas deferens has been observed after adminstration of sympathetic nerve blocking agents (Leidl, 1965). The adrenergic nerves appeared to stimulate the movement of the ductus deferens, while the cholinergic nerves seem to inhibit the movement of it (Shirai *et al.* 1973). In this study ChAT-IR and DBH-IR were not able to visual all the nerve that detected by general nerve marker. The non-demonstrated fibers may be peptidergic nerves and need further studies to clear the different types of the nerve fibers in the wall of the ductus deferens.

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