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Original Research

Histological and Histochemical Studies on the Ampulla of the Deferent Duct of Donkey (*Equus asinus*)

Alaa S. Abou-Elhamd*, Ahmed O. Salem, Aziza A. Selim

Department of Anatomy and Histology, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt.

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Abstract

The present study was performed on the ampulla of the deferent duct of 24 sexually mature apparently healthy male donkeys (5 to 7 years) distributed over the four seasons of the year. The ampullary glands were branched tubulo-alveolar in type which they were opened in a central lumen. The luminal and the glandular epithelium were formed of principal and basal cells. Singly scattered rod- shaped cells were observed among the principal cells. PAS positive reactivity was observed in the surface and the glandular epithelium of the ampulla ductus deferentis. Only the luminal contents and the secretory materials attached to the apical border of the principal cells, showed positive reactivity for alcian blue. The present study presents the first description of the ampulla ductus deferentis of donkey. In addition to, it acts as a reservoir for sperms it had a secretory function for neutral and acid mucopolysaccharid.

Keywords: Ampulla ductus deferentis; donkey; mucopolysaccharid

Introduction

It is well known that the efficient reproduction in male animals is dependant to a great extent on the effective functions of the genital glands. The accessory genital glands are a series of glands situated between the vas deferens and the root of the penis. They include the ampullary, vesicular, prostate, bulbourethral and urethral glands (Banks, 1993; Davies Morel, 2003). Collectively, these glands are responsible for the secretion of the seminal plasma, which forms the major fluid fraction of semen. Seminal plasma provides the substrate for conveying the sperm to the female genital tract and ensuring final maturation.

Surveying the available literature revealed that the accessory genital glands were extensively investigated in men and other mammals (Tsukise and Yamada, 1981; Amselgruber and Feder, 1986; Abou-Elmagd and Wrobel, 1989; Sirigu *et al*., 1993; Cormack, 1997; Marei et al., 2004). From the vast volume of literature on these glands, little attention has been paid to those of equidae (Ellery, 1971; Parillo and Verini Supplizi, 2008; Parillo *et al.*, 2010). Therefore the present investigation was performed to describe the histological and histochemical features of the ampulla ductus deferentis of donkey. This is to provide a basic knowledge on it for both theoretical and reproductive reasons as well as to compare it with the accessory genital glands of the other mammals.

Materials and methods

The present study was performed on 24 sexually mature apparently healthy male donkeys (Jacks) that were collected during four seasons of the year, six for each. The age of the animals ranged from 5 to 7 years.

The animals were anesthetized and then thoroughly bled to death from the common carotid artery. Jacks were dissected and their accessory genital glands were perfused in situ through the right and left internal pudendal arteries with the appreciate fixatives. They included neutral buffered formaldehyde, Bouin´s fluid (for routine histolog-

*Corresponding author: Alaa S. Abou-Elhamd

E-mail address: alaa88@yahoo.com

ical examinations), Carnoy´s fixative (for carbohyderates histochemistry).

The specimens collected from the ampulla of the deferent duct were fixed in the aforementioned fixatives and processed for paraffin embedding. 5-7 µm thick paraffin sections were obtained and stained with the following stains: Harris haematoxylin and eosin (Harris, 1900), Crossmon´s trichrome (Crossmon, 1937), Verhoeff´s methods (Verhoeff, 1908), Gomori´s reticulin (Gomori, 1937), Periodic acid-schiff, PAS (Mc, 1946), Best´s carmine method for detection of glycogen content (Best, 1906). Alcian blue technique, pH 2.5 (Steedman, 1950) and Combined Alcian blue-PAS technique (Mowry, 1956).

Results

The accessory genital glands of the donkey consisted of a series of four glands grouped around the pelvic urethra (Figs. 1A- D). These included the paired ampullae of the deferent duct, the paired seminal vesicles, the prostate and the paired bulbourethral glands, in addition to the urethral glands. The latter were embedded in the tunica propria/ submucosa of the male pelvic urethra.

The ampullae of the paired deferent duct (Ampulla ductus deferentis) of the donkey were finger- or sausage-shaped. They constituted a glandular enlargement of the terminal part of each duct. They were enclosed within the genital fold except their caudal third, which was situated retroperitoneally. The ampullae were directed caudalwords at the ventral aspect of the seminal vesicles and the prostate, and dorsal to the neck of the urinary bladder. Remnants of the paramesonephric (Müllerian) ducts were demonstrated within the genital fold in-between the two ampullae. The terminal portions of the deferent ducts formed with the corresponding seminal vesicle ducts, the common right and left ejaculatory ducts. Each one opened by an ejaculatory orifice at the Colliculus seminalis (Figs 1A-1D).

Fig. 1(A-D). Photograph showing the accessory genital glands of the donkey during spring (dorsal view, 1A; Ventral view, 1B- 1C). Ductus deferens (arrowhead), Ampulla of the deferent duct (Am), seminal vesicles (S), prostate gland (P) and its isthmus (I), band of urethralis muscle (arrow) covers the caudal pole of the prostate (P), bulbourethral glands (Bu), genital fold (asterisk), vestige Mullerian ducts (arrow), urinary bladder (Ub), pelvic urethra (Pu). 1D, High magnification photograph at the ventral aspect of an incised pelvic urethra of the donkey during spring showing opening of the ejaculatory ducts (double arrows), prostate gland openings (arrows), bulbourethral gland openings (arrowhead), urethral gland duct openings (double arrowheads).

Histological observations:

The wall of the ampulla of the deferent duct was formed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa or adventitia (Fig. 2). The ampulla of the deferent duct of the donkey had a folded mucosa, surrounding an irregular oval or rounded central lumen. These folds were interrupted with shallow depressions, in which the ampullary glands opened (Fig. 3). The latter mostly contained spermatozoa and often homogenous acidophilic materials (Figs. 4A& 4B).

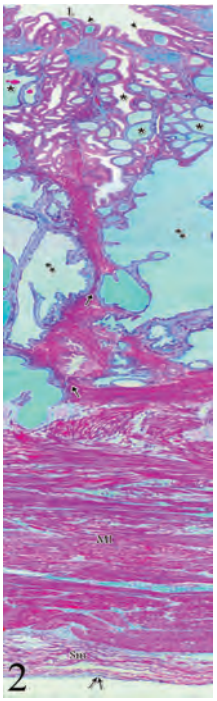


Fig. 2. Photomicrograph of the wall of the ampulla of the deferent duct of the donkey during spring showing the arrangement of its layers. Lamina epithelialis (arrowhead), tunica propria/ submucosa containing secretory end-pieces with narrow lumina (asterisk) near the central lumen (L) and with wide lumina (double asterisk) at the periphery, muscular layer (Ml), mesothelium (double arrow), submesothelium (Sm). Muscular band extends from the inner circular layer till the base of the mucosal folds (thick arrow). Collagenous fibers (green colour) supporting the ampullary glands, smooth muscle bundles of the tunica muscularis and submesothelial connective tissue. Crossmon´s trichrome stain. (X50).

The lamina epithelialis was formed of two types of cells; principal columnar and basal cells. The principal columnar cells possessed mostly brush borders and nearly oval or spherical vesicular nuclei with distinct nucleoli. The cytoplasm was acidophilic, and occasionally contained vacuoles of variable sizes (Fig. 4A). Bleb-like apical protrusions containing acidophilic substances were often seen at the apical border of some principal cells (Fig. 4B). The basal cells (Fig. 4A) were small and did not form a continuous layer. They appeared nearly rounded-, flattened- or wedged-shape with spherical, flat or irregular-shaped nuclei. The cytoplasm of basal cells were mostly appeared vacuolated and in others, it was granular acidophilic. The vacuoles within some basal cells displaced the nucleus to one side of the cell (Fig. 4B).

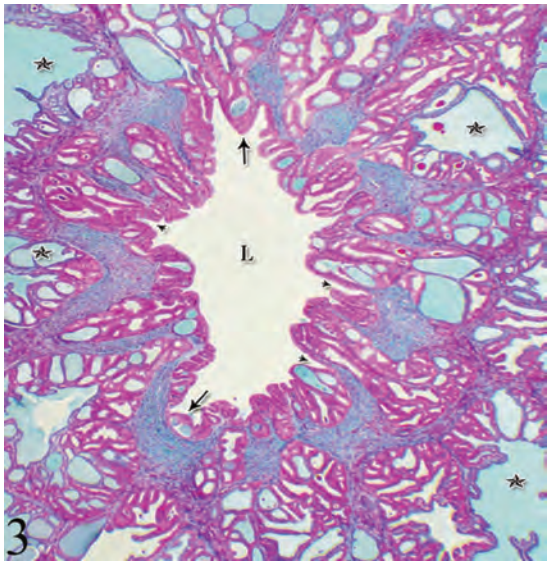


Fig. 3. Photomicrograph showing the irregular oval central lumen of the ampulla of the deferent duct of donkey (L). It has a folded mucosa (arrow). This folds was separated by shallow depressions (arrowhead). Secretory portions of the ampullary glands (star). Crossmon´s trichrome stain. (X25).

Singly scattered small rod-shaped cells were observed among the principal ones. These cells were characterized by deeply stained acidophilic cytoplasm and compressed oval- or elongated- deeply stained nuclei (Fig. 4A).

The tunica propria/ submucosa were occupied completely with the ampullary glands. It was formed of dense connective tissue layer containing collagenous, reticular and elastic fibers (Figs. 2, 3& 5A-5C). The elastic fibers constituted a subepithelial network, like a girdle surrounding the central lumen (Fig. 5C). This girdle-like was interrupted by the ampullary gland openings. Bundles of smooth muscle fibers from the inner portion of the inner circular layer of the tunica muscularis traversed, like partitions, the whole thickness of the lamina propria-submucosa in a radial manner till the base of the mucosal folds. It was also observed that these bundles along their course give lateral branches between the secretory portions, in the form of thin muscular layers or evenly singly scattered smooth muscle fibers. Blood capillaries and fibroblasts were also seen (Fig. 2).

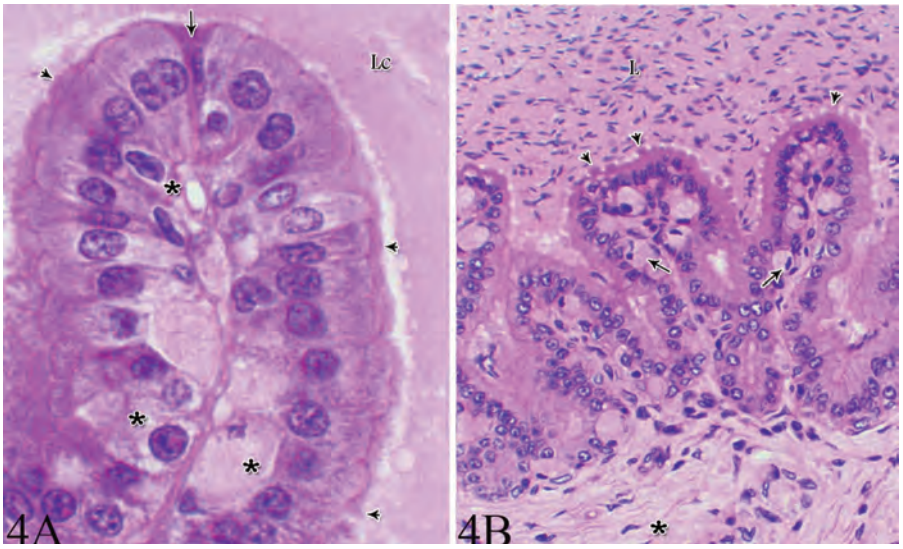
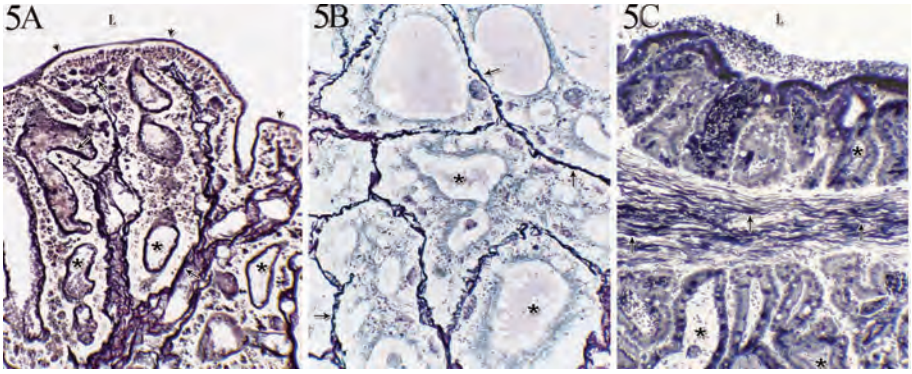


Fig.4.A) Photomicrograph of the lamina epithelialis of the ampulla of the deferent duct of the donkey during spring showing the principal columnar cells with brush border (arrowhead), basal cells (asterisk), rod-shaped cell (arrow) and luminal contents (Lc). Haematoxylin & eosin stain. (X1000). 4B) Photomicrograph showing the bleb-like apical protrusions (arrowhead) of the principal cells of the lamina epithelialis of the ampulla of the deferent duct of the donkey during spring. Vacuolated basal cells (arrow), lumen containing spermatozoa and secretory materials (L), lamina propria-submucosa (asterisk) containing fibroblasts and fibrocytes. Haematoxylin & eosin stain (X400).



Figs. 5. 5A) Photomicrograph showing the reticular fibers network (arrow) supporting the lamina epithelialis (arrowhead) and the secretory end-pieces (asterisk) near the central lumen (L) of the ampulla of the deferent duct of the donkey during winter. Gomori´s stain. (X400). 5B) Photomicrograph showing the reticular fibers network (arrow) around the peripherally located secretory end-pieces of the ampullary glands (asterisk) during spring. Gomori´s stain (X400). 5C) Photomicrograph showing a part of the subepithelial elastic fibers network (arrow) around the central lumen (L) of the ampulla of the deferent duct of the donkey during summer. Secretory end-pieces (asterisk). Verhoff´s stain. (X 200).

The ampullary glands were branched tubuloalveolar variety, which opened directly into the central lumen of the ampulla of the deferent duct (Fig. 6). The luminal diameter of the secretory portions always increased towards the periphery, than those near the central lumen (Figs. 2 & 6).

The glandular epithelium of the ampullary glands was formed of principal and basal cells (Fig. 7A-7B). The principal cells were columnar in shape, and mostly had brush borders. They possessed acidophilic cytoplasm and spherical nuclei with one or two prominent nucleoli and fine dispersed chromatin. Some principal cells contained vacuoles. These cells appeared few or rarely seen during spring and increased gradually during summer, autumn and appeared more numerous during winter. Few rod-shaped cells were also observed between the principal glandular cells. These cells had the same characteristics to those of lamina epithelialis (Fig. 7B).

Some types of the principal cells showed bleb-like apical projections containing deeply stained acidophilic substance (Fig. 7C). Few secretory end-pieces were also lined with low cuboidal principal cells with irregular oval or spherical nuclei. Their deeply stained acidophilic cytoplasm revealed neither granules nor blebs. The lumina of these secretory tubules were completely filled with acidophilic secretory materials (Fig. 7D).

The basal cells simulated nearly that of the lam-

ina epithelialis. They formed a discontinuous layer. Their acidophilic cytoplasm contained vacuoles of variable sizes. The vacuolated cells were numerous during winter and decreased during summer and autumn. During spring, they reached their minimal amount.

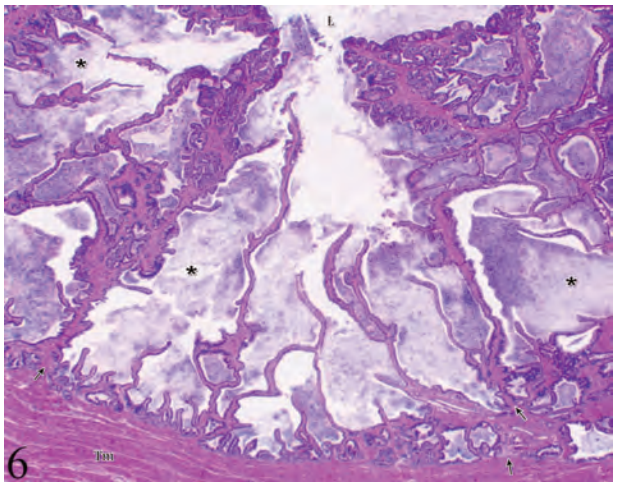


Fig. 6. Photomicrograph of the ampullary glands in the lamina propria-submucosa during winter open directly into the central lumen (L) and they possess diverticulae-like appearance containing spermatozoa and secretory materials (asterisk). Part of the tunica muscularis (Tm) from which bundles of smooth muscle fibers extend inbetween the secretory endpieces of the ampullary glands (arrow). Haematoxylin & eosin stain. (X25).

The lumina of the secretory portions contained variable amounts of secretory substance, concretes, spermatozoa and detached blebs.

The tunica muscularis of the ampulla of the deferent duct of the donkey was composed of smooth muscle fibers arranged into inner circular and outer longitudinal layers. Some oblique bundles were observed within the inner circular layer (Fig. 8A). The muscular layers were supported with collagenous, elastic and reticular fibers. The collagenous (Fig. 8A) and the few elastic fibers (Fig. 8B) were demonstrated surrounding the muscular bundles, while reticular fibers network was observed enclosing each muscle fiber separately (Fig. 8C).

The tunica serosa covered most of the ampulla of the deferent duct, which enclosed within the genital fold, while the reteroperitoneal part was invested with tunica adventitia. The tunica serosa was formed of flat mesothelial cells and a submesothelial connective tissue layer (Figs. 2 & 8A). The latter or the adventitia was composed mainly of collagenous fibers. Network of elastic fibers was seen in relation to the outer muscular layer. Fibroblasts, blood vessels of variable calibers as well as myelinated nerve bundles were also recorded within this layer.

Histochemical observations

Carbohydrate histochemistry

A- Neutral mucopolysaccharides:

The principal cells of the lamina epithelialis and the glandular epithelium of the ampulla of the deferent duct showed strong PAS positive reactions (Fig. 9A). These reactions were represented by diffuse homogenous positive substance occupying mostly the whole cell. Some cells revealed few PAS positive granules in their basal cytoplasm.

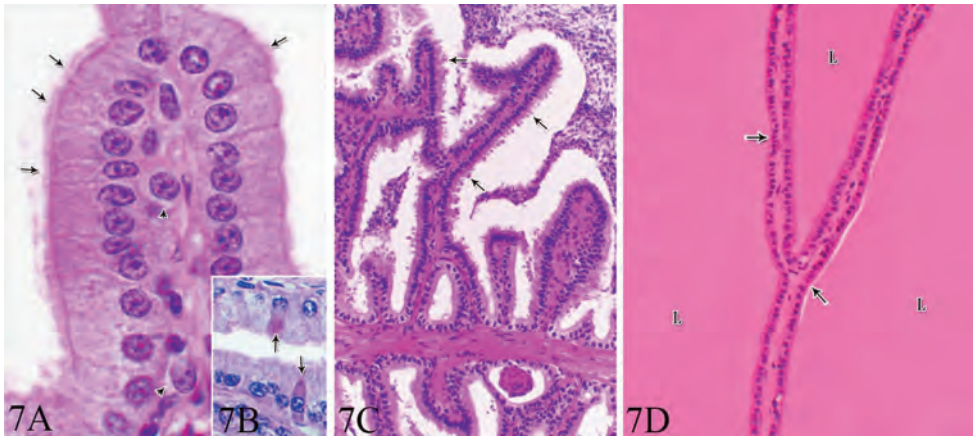


Fig.7. Photomicrograph of the lining epithelium of the ampullary gland (7A) during spring showing its principal cells with brush border (arrow) and spherical nuclei; vacuolated basal cells (arrowhead). Rod-shaped cells with darkly stained acidophilic cytoplasm (arrow) were occasionally observed inbetween the principal cells lining the ampullary glands (7B). Haematoxylin & eosin stain. X Obj. 1000. 7C) The principal cells had Bleb-like apical projections (arrows). Haematoxylin & eosin stain. (X200). Inest: (X400). 7D) During spring some of the ampullary glands were lined with low cuboidal or flattened principal cells with irregular oval or spherical nuclei (arrow). Lumen (L) filled with acidophilic secretory material. Haematoxylin & eosin stain. (X200).

Others were moderately or even negatively reacted. The basal cells of the luminal and the glandular epithelium were reacted strongly positive for PAS in the form of large globular mass of variable shapes occupying mostly the whole cell cytoplasm or appeared as granular deposits. In some basal cells, this PAS positive globular mass was observed compressing or displacing its nucleus to one side of the cell. In few basal cells, the nuclei were seen within the PAS positive mass (Fig. 9A& 9B). The bleb-like apical protrusions of the principal cells were strongly PAS positive (Fig. 9C).

The luminal content of the glandular portions was PAS positive, while the concretes were more strongly reacted (Figs. 9A-C).

With Best´s carmine technique, all the forming layers of the ampulla of the deferent duct of the donkey were negatively reacted during the studied seasons (Data not shown).

B- Acid mucopolysaccharides:

All layers of the ampulla of the deferent duct of the donkey were negatively reacted with alcian blue staining except the luminal contents and the secretory materials attached to the apical border of the principal cells, which were moderately reacted (Figs 10, inset).

After staining sections of the ampulla of the deferent duct with alcian blue-PAS combination, no differences could be recorded in surface and glandular epithelium (Fig. 11A) concerning both the

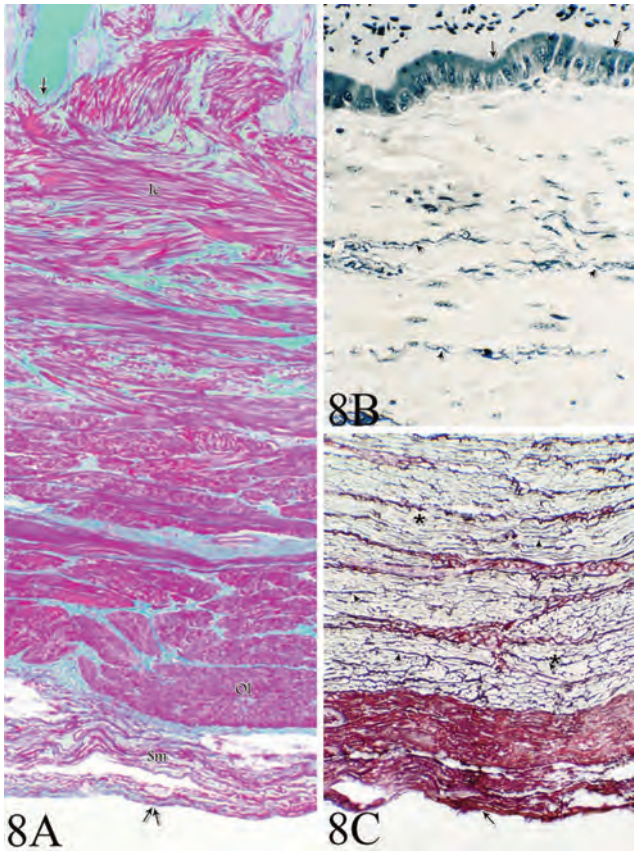


Fig. 8. A) The tunica muscularis of the ampulla of the deferent duct of the donkey formed of inner circular (Ic), outer longitudinal (Ol) layers. Collagen fibers inbetween the muscle layers (green colour), glandular epithelium (arrow), submesothelium (Sm), mesothelium (double arrow). Crossmon´s trichrome stain. (X50). 8B) Photomicrograph showed the distribution of the elastic fibers (arrowhead) inbetween the smooth muscle fiber bundles of the tunica muscularis of the ampulla of the deferent duct of the donkey during spring. Verhoff´s stain. X Obj. 40 & Oc. 10.8C) The tunica muscularis of the ampulla of the deferent duct of the donkey was supported by reticular fibers network (arrowhead). Collagen fibers (brown colour) inbetween the muscular bundles (astrisk) and in the tunica adventitia (arrow). Gomori´s stain. (X200).

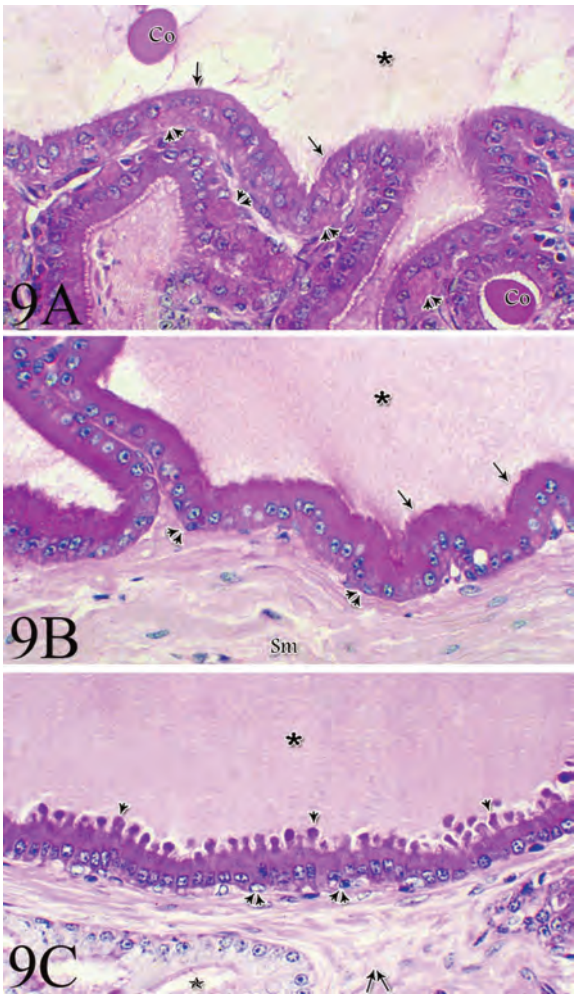


Fig. 9. Photomicrographs showing strong diffuse PAS reactivity in the surface (18A) and glandular epithelium (9B-9C) of the ampulla of the deferent duct of the donkey during spring. Strong diffuse PAS reactivity within the principal cells with the brush border (arrow) or with bleb-like apical protrusions (arrowhead); basal cells (double arrowhead), negatively reacted secretory end-pieces (star), secretory materials (asterisk), concretes (Co), weakly reacted connective tissue stroma (double arrow) and smooth muscle fibers (Sm). PAS / haematoxylin stain. (X400).

pattern of distribution and the intensity of reaction beyond that mentioned for PAS stain during the studied seasons except for the luminal content, which showed a mixed staining reactivity. However, in some secretory portions the luminal contents were either alcian blue or PAS positively reacted (Fig. 11B).

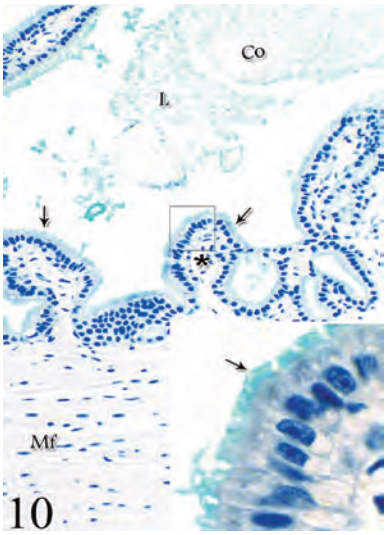


Fig. 10. Photomicrographs of the glandular epithelium (arrow) of the ampulla of the deferent duct of the donkey during spring. High magnification of the marked area (inset). The alcian blue positive reaction was observed in the luminal contents (L) and the secretory materials attached to the brush border of the principal cells (arrows). Concretes (Co), connective tissue fibers (asterisk) as well as the muscle ones (Mf) were negatively reacted. Alcian blue / haematoxylin stain. X200. (Inset):1000).

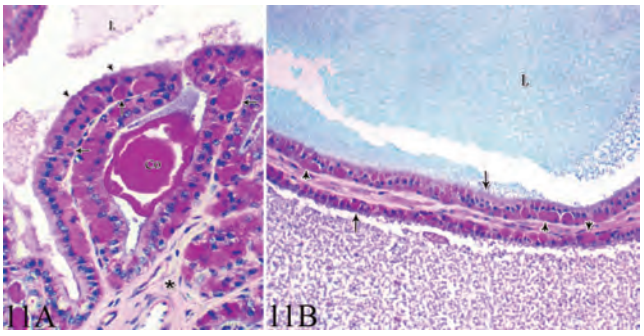


Fig. 11. 11A) Photomicrographs of the lamina epithelialis of the ampulla of the deferent duct of the donkey after combined alcian blue/ PAS technique during spring showing the same picture after PAS except the luminal contents and the secretory substances attached to the apical border of the principal cells (arrowhead) which reveal a mixed reaction. Basal cells (arrow), moderately PAS reacted connective tissue (asterisk), blood vessels (double arrow), lumen (L), concretes (C). Alcian blue/ PAS/ haematoxylin stain. (X400). 11B) The glandular epithelium of the ampulla of the deferent duct of the donkey showed the same PAS positive picture within the principal cells (arrow) and basal cells (arrowhead) after alcian blue / PAS staining. The luminal contents (L) were either alcian blue or PAS positive. Alcian blue/ PAS/ haematoxylin stain. (X200).

Discussion

The present study revealed that the accessory genital glands of the male donkey were formed of ampulla of the deferent duct, seminal vesicles, prostate, bulbourethral glands in addition to the urethral glands. In addition, it described the morphological structure of the ampulla ductus deferentis of donkey by using histological and histochemical methods.

Similar to that reported in red deer, stallions and camels (Aughey, 1969, Ellery, 1971, Goswami *et al*., 1990, respectively), the ampulla of the deferent duct was formed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa or adventitia.

The ampulla of the deferent duct of the donkey had a folded mucosa and its lumen contained spermatozoa and homogenous acidophilic materials, similar to that observed in the ampulla of the deferent duct of rams (Abbas, 1976), red deer (Aughey, 1969), men (Fawcett and Jensh, 1997), camels (Goswami *et al*., 1990) and goats (Suri *et al*., 2008).

The lamina epithelialis of the ampulla of the deferent duct of the donkey was formed of two types of cells; principal columnar and basal cells. Similar results were observed in the ampulla of the deferent duct of bucks as well as men (Selim, 1974; Riva *et al*., 1982). However, the lining epithelium of the luminal surface of the ampulla of red deer (Aughey, 1969), men (Dym, 1983), dogs (Murakami *et al*., 1986) and goats (Suri *et al*., 2008) was pseudostratified columnar in type.

The apical portion of the principal cells of the ampulla of the deferent duct of the donkey possessed brush borders. This might help in the movement of the secretory materials and spermatozoa in the lumen of the duct. Bleb-like protrusions were also observed at the apical borders of the principal cells, indicating an apocrine mode of secretion in the ampulla ductus deferentis of the donkey. Similar finding was observed in rabbits, dogs, golden hamster and camels (Kunkelmann and Kühnel, 1984; Murakami *et al*., 1986; Chow and Pang, 1989; Goswami *et al*., 1990). On the other hand in dogs, bulging apex and the irregularity of the cell surfaces were probably related to the release of the secretory materials from the cell or may be an artifact inflicted on these rather delicate-looking structures by fixatives (Chow, 1988). In addition to the

secretory function of the principal cells, they are capable of phagocytose abnormal, degenerated or even in rare cases intact spermatozoa (Murakami *et al*., 1986).

The present investigation revealed that the basal cells were small and did not form a continuous layer. They appeared nearly rounded, flattened with spherical or flat nuclei and vacuolated or granular acidophilic cytoplasm. Similar findings were observed in rams, dogs, and camels (Abbas, 1976; Murakami *et al*., 1986; Goswami *et al*., 1990). According to the presence of strongly PAS positive globules, it may suggest that these cells possessed secretory activity. However, in men's ampulla these cells did not have any secretory function and their cytoplasm showed numerous filaments (Riva *et al*., 1982).

The present study revealed that the elastic fibers in the lamina propria-submucosa formed a subepithelial network, which agrees with that observed in the ampulla of the deferent duct of camels (Mosallam, 1981; Goswami *et al*., 1990) and in the vas deferens of the donkey (Salem, 2003). On the basis of the great elasticity of the elastic fibers and their capability for expansion about 150 times its original length, to which they return when released (Liebich, 1990). Salem, 2003 suggested that, this specific girdle-like arrangement of these fibers gives the ampulla of the deferent duct great elasticity to counteract the mechanical stress exerted by the luminal contents (secretory materials and spermatozoa) from one side and the powerful muscular contraction from the opposite side. Consequently, this arrangement probably acts as absorbing shock for stretch and compression. This suggestion is acceptable, where this subepithelial network of the elastic fibers within the ampulla of the deferent duct of the donkey is a direct continuation of those of the vas deferens of the same animal.

In accordance with that observed in stallions and camels (Ali *et al*., 1978; Ellery, 1971; Goswami *et al*., 1990; Mosallam, 1981; Wrobel and Dellmann, 1993) the tunica propria/ submucosa of the ampulla of the deferent duct of the donkey contained smooth muscle fibers which extended from the inner muscular layer to base of the mucosal folds. It could be speculated that, the presence of smooth muscle fibers in this manner probably acts as supporting element of the densely packed ampullary glands. It could also be suggested that, contraction of these muscle helps in evacuating the secretory substances from the glands at the appreciate time.

Similar to that observed in stallions (Ellery, 1971), goats (Wrobel, 1971; Marei *et al*., 2004; Suri *et al*., 2008), camels (Goswami *et al*., 1990), ruminants and dogs (Wrobel and Dellmann, 1993), the ampullary glands of the donkey composed tubulo-alveolar while they were tubular in elephants (Short *et al*., 1967), rams (Perera, 1974) and camels (Ali *et al*., 1978) .

The ampullary glands were lined with principal columnar and inconstant basal cells. Similar finding was observed in goats (Selim, 1974; Wrobel, 1971). While, in elephants (Short *et al*., 1967) and camels (Goswami *et al*., 1990), the glandular epithelium varied from cuboidal to columnar. It was simple columnar and pseudostratified in stallions (Ellery, 1971). In rams, the tubules in proximal parts were lined by pseudostratified and basal cells, but those at the bottom were lined by low cuboidal to columnar epithelium (Abbas, 1976).

The lining epithelium of the ampullary gland of donkey had similar characteristics as the lamina epithelialis. The principal columnar cells of the ampullary glands of the donkey carried brush borders as well as bleb-like apical protrusions, indicating apocrine activity. Similar finding was observed in camels and rabbits (Kunkelmann and Kühnel, 1984; Mosallam, 1981).

The lumina of the glandular tubules and alveoli contained variable amounts of secretory substances, concretes and spermatozoa. Similar findings were observed in elephants, stallions and camels (Short *et al*.; 1967; Ellery, 1971; Mosallam, 1981; Badawy *et al*., 1982; Goswami *et al*., 1990). The presence of a large amount of the spermatozoa in the lumina of the tubules and alveoli of the ampulla of the deferent duct of the donkey indicates that it may store spermatozoa for a considerable period of time as reported in rams (Perera, 1974) and camels (Ali *et al*., 1978). The lumen and the diverticula of the ampulla of the deferent duct of rabbits were filled with spermatozoa only at sexual rest, however after ejaculation, the diverticula were usually empty (Kunkelmann and Kühnel, 1984). It could be accepted that the sperms accept their maturation within the ampulla of the deferent duct of bulls (Bergerson *et al.*, 1994).

The muscular coat of the ampulla of the deferent duct of the donkey was formed of inner circular

and outer longitudinal layers of smooth muscle fibers, in addition to some oblique bundles within these layers. This result was contrary to that observed in stallions, where the tunica muscularis was composed of intermingled layers of longitudinal and circular smooth muscle fibers (Ellery, 1971). In domestic animals, the tunica muscularis was consisted of variably arranged smooth muscle bundles (Wrobel and Dellmann, 1993).

The cranial two thirds of ampulla of the deferent duct of the donkey were covered with serosa, while the caudal third (retroperitoneal part) was covered with adventitia. A network of elastic fibers was seen in the inner portion of this layer near the muscular layer. The probable function of this elastic network was pulling the adventitia during contraction of the ampulla and releasing it during relaxation.

The PAS reactivity within the glandular epithelial cells of the ampulla of the deferent duct of the donkey indicated the presence of neutral mucopolysaccharides. This suggests that the ampullary secretions are involved in altering the plasma-membrane glycoconjugates of spermatozoa by which it contribute to their maturation (Parillo and Verini Supplizi, 2008).

The present study revealed that only the luminal contents and the secretory substances attached to the apical border of the principal cells, showed moderate alcian blue positive reactivity. In red deer (Aughey, 1969), camels (Mosallam, 1981) and goats (Marei *et al*., 2004), the ampulla of the deferent duct was free from alcian blue reactive substance, except for luminal contents, and some cells lining large excretory duct in case of red deer. While in camels it showed very weak alcainophilia, suggested a very small amount of sialic acid (Ali *et al.*, 1978). On the other hand, most sialic acid in the seminal plasma of stallions and jacks was derived from ampulla and epididymis (Mann, 1964). This may solve the problem of negatively reacted surface and glandular epithelial cells of the ampulla ductus deferentis of the donkey.

In equines, the ampullary secretion was largely associated with the presperm fraction. It contributed ergothionine, a sulpher containing nitrogenous base to the ejaculate. Ergothionine, an antioxidizing agent is protecting susceptible chemicals in semen from oxidation (Mann, 1975; Phillis, 1976; Davies Morel, 2003).

Conclusion:

In conclusion, this report presents the first morphological and histochemical studies on the ampulla ductus deferentis of donkey, which might help in understanding the ultrastructural features of this gland.

Acknowledgment

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