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Gross, Histological and Scanning Electron Studies on the Bulbourethral Gland of Donkey (*Equus asinus*) during Different Seasons

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ABSTRACT

Bulbourethral glands (Cowper´s glands) are one of the accessory genital glands, which play an important role in the cleaning and neutralizing of the acidic environment of the urethra prior to ejaculation. The aim of the present work is to study the morphological features of the bulbourethral glands of donkey by using histological and histochemical methods by using different staining methods such as hematoxylin and eosin, trichrome stain, Verhoff's stain and Gomoris reticulin stain, PAS, alcian blue, Combined PAS/ alcian blue and Best's carmine satin. The bulbourethral glands of donkeys were of the combined tubuloalveolar type and lined by principal and basal cells. The activity of the bulbourethral glands varied according to the seasons. It showed maximal activity during spring, which manifested by an increase in the epithelial height and decreasing the nuclear/cell ratio, and the principal cells showed a strong reaction for PAS and aclian blue. Minimal activity was observed in winter, in which the epithelium reaches its minimal height, and the nuclear/cell ratio was increased, and the principal cells showed weak reaction for PAS and aclian blue. In conclusion, this first report describes the histomorphological features of the bulbourethral glands of the donkey. In addition, it showed its different activity during the seasons of the year.

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Introduction

The accessory genital glands include prostate, bulbourethral glands, seminal vesicles, urethral and ampullary glands. They are playing an important role in the reproductive functions and fertility process (Chughtai *et al*., 2005; Flint *et al*., 2016). The bulbourethral glands (Cowper´s glands) are believed to play an important role in the lubrication of the urethra. It produces watery, slightly mucoid secretions, which help in neutralization traces of acidic urine in the urethra (Chughtai *et al*., 2005; Samuelson, 2007; Adebayo *et al*., 2015; Lowe and Anderson, 2015). It is believed to be involved in the process of sperm storage in the llama oviduct (Apichela *et al*., 2014).

The histomorphological structure of the bulbourethral gland was extensively studied in animals; in elephants (Short *et al*., 1967), bucks (Selim, 1974), rams (Abbas, 1976), camels (Ali *et al*., 1976; Mosallam, 1981; Badawy *et al*., 1982; Youssef *et al*., 1984; Aly and Prentis, 1986), buffalo bulls (Moussa *et al*., 1983; Abou-Elmagd and Wrobel, 1989) and Philander opossum (Nogueira *et al*., 1984) and rat (Adebayo *et al*., 2015). Species variation in gross morphology and gross morphometry of the bulbourethral glands of One-humped camel, ram and red Sokoto buck was discussed by Mahmud *et al*. (2016).

In our previous reports, we discussed the histomorphological structure and the seasonal variations of the ampullary, prostate, urethral glands, and seminal vesicles of donkey during different seasons (Abou-Elhamd *et al*., 2013a,b, 2019, 2020). The aim of the present study is to investigate the histological and histochemical structures of the bulbourethral glands of donkey with special reference to the histomorphological changes in the urethral gland during different seasons.

Materials and methods

After Abou-Elhamd *et al*. (2012, 2013a,b, 2019, 2020), the present study was carried out in department of Anatomy and Histology, faculty of veterinary medicine (2005-2007) on 20 sexually mature apparently healthy male donkeys (Jacks) 5 to 9 years old (5 animals for each season). The animals were anesthetized and then thoroughly bled to death by severing the common carotid artery. The jacks were dissected, eviscerated and their accessory genital glands were perfused in-situ through the right and left internal pudendal arteries with the appreciate fixatives. The fixatives included neutral buffered

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formalin, Bouin´s fluid (for routine histological and morphometrical examination), Carnoy´s fixative (for carbohydrates histochemistry) and 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 at 4˚C (for scanning electron microscopy).

The specimens were collected from the bulbourethral glands of 5 jacks in each season. The specimens were further fixed in the same fixatives used for the appreciate time. They were thereafter processed for paraffin embedding. 5-7 µm thick paraffin sections were stained with the following stains: Harris haematoxylin and eosin for general histological examination (Harris, 1900); Crossmon´s trichrome stain for identification of collagenous and muscle fibers (Crossmon, 1937); Verhoeff´s methods for identification of elastic fibers (Verhoeff, 1908); Gomori´s reticulin method for detection of reticular fibers (Gomori, 1937); Periodic acid-schiff (PAS) technique for demonstration of neutral mucopolysaccharides (Mc, 1946); Alcian blue technique (pH 2.5) for demonstration of acidic mucopolysaccharides with or without counter staining with haematoxylin; Combined Alcian blue-PAS technique for acid and neutral mucopolysaccharides; Best´s carmine method for detection of glycogen content (Best, 1906). All the procedures were cited in Suvarna *et al*. (2018).

The morphometrical studies were performed on the stained histological sections of the glands under investigation using Lecia Q 500 MC image analyser. The measurements were carried out on each gland of the 5 jacks in all seasons in the following manner: The height of the glandular epithelium and the nuclear / cell ratio of the principal cells of the glandular epithelium of the bulbourethral glands was calculated from fifteen random fields of the glands under investigation; the interstitial connective tissue/ glandular tissue ratio of the studied glands was calculated from five random fields in each season.

All procedures of the current study have been conducted in accordance with the University guidelines for the care of experimental animals. Ethical approval was obtained from the Committee of Faculty of Veterinary Medicine, Assiut University, Egypt.

Statistical analysis

All the data from the 4 groups during the different seasons were presented as means + SE, which were statistically analysed by using SPSS data analysis software (version 17). The analysis was performed by one-way ANOVA followed by Scheffe and Duncan test, P value <0.05 is considered as significance.

Results

The bulbourethral (Cowper´s glands) (Glandulae bulbourethrales) were paired pear-shaped organs, located dorsolateraly on either side of the pelvic urethra at the level of the ischial arch near the bulb of the penis. They were covered by the bulboglandularis muscle. Each gland had 6-8 excretory duct openings, arranged into two longitudinal rows on the dorsal wall of the membranous urethra close to the median plane. The two glands were separated from each other by a muscular septum (Fig. 1).

Histological observations

The bulbourethral glands of the donkey consisted of stroma and parenchyma. The former was formed of capsule, trabeculae, and interstitial connective tissue. The parenchyma was formed of glandular portions and duct system (Fig. 2).

The capsule was muscular in nature and surrounded the gland entirely. It was formed mainly of intermingled circular

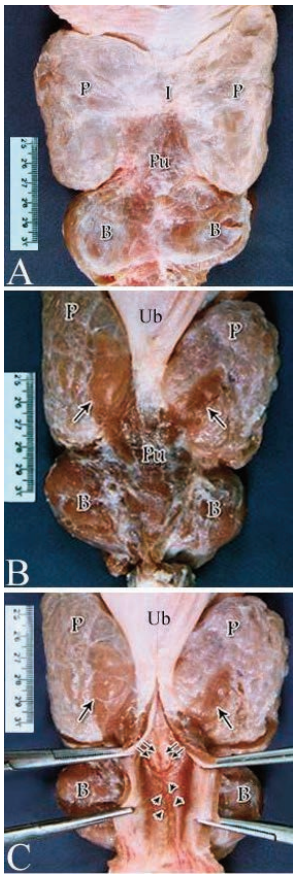

Fig. 1. Photograph showing the bulbourethral glands of the donkey: (A) Dorsal view, (B) ventral view, (C) an incised pelvic urethra at the ventral aspect. Bulbourethral glands (B), prostate gland (P) and its isthmus (I), pelvic urethra (Pu), opening of the ejaculatory ducts (double arrow), bulbourethral gland openings (arrowhead), band of the urethralis muscle (arrow) covers the caudal pole of the prostate (P), Urinary bladder (Ub).

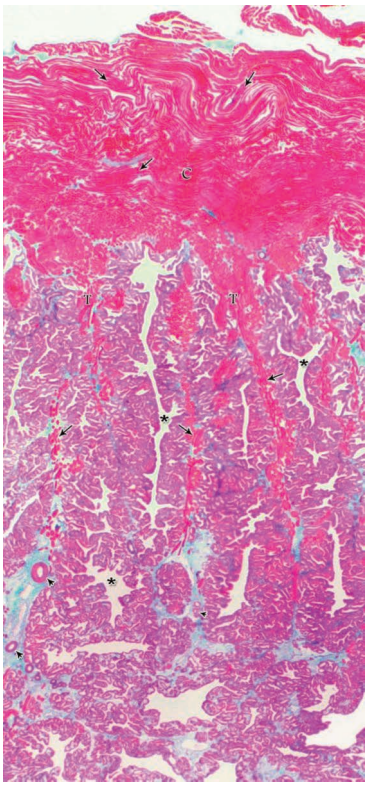

Fig. 2. General structure of the bulbourethral glands of the donkey: Capsule (C), trabeculae (T) of the stroma as well as glandular lobules with their collecting sinuses (asterisk), skeletal muscle fibers (arrows, red colour), collagen fibers (green colour) and blood vessels (arrowheads). Crossmon´s trichrome stain. X 25.

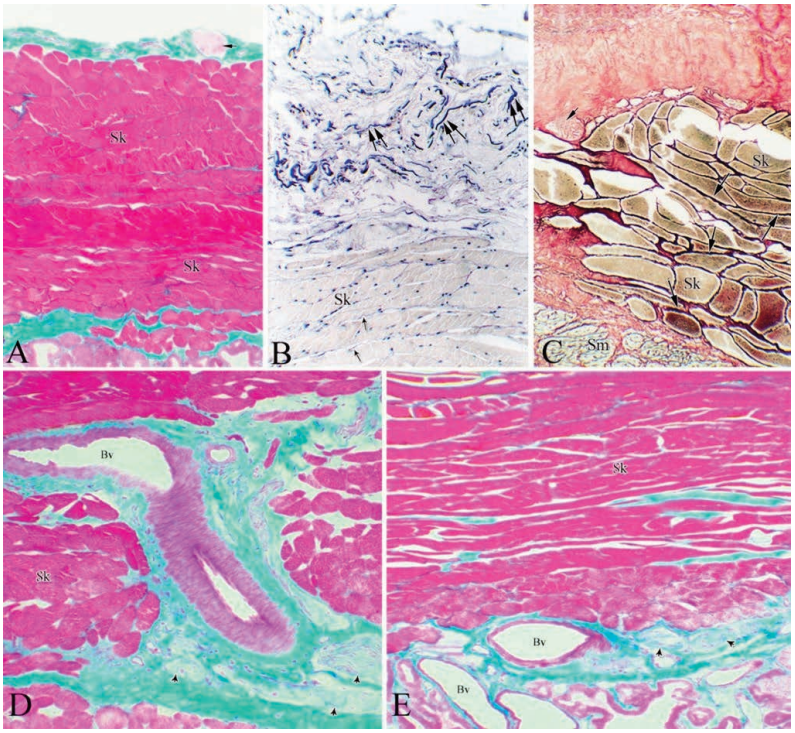

Fig. 3. Fibromuscular capsule and its outer covering dense connective tissue layer of the bulbourethral glands of the donkey: Using Crossmon´s trichrome stain (A, D, E), Verhoff´s stain (B), Gomori´s stain (C). Intermingled bundles of skeletal muscle fibers (Sk), collagen fibers (green colour A, D, E and brown colour, C), elastic fibers (double arrows, B), reticular fibers (arrows), bundle of smooth muscle fibers (Sm), bundles of myelinated nerve fibers (arrowheads), the blood vessels (Bv) and myelinated nerve fiber bundles (arrowhead) traverse the capsule (D) secretory end-pieces (S). A, B, D and E: X100, C: X200.

and longitudinal layers of skeletal muscle fiber bundles (Bulboglandularis muscle), in addition to some oblique ones. These muscular layers were supported mainly by coarse collagenous fibers (Fig. 3). Few fine elastic fibers were observed only in between the muscle fibers (Fig. 3B), while the reticular fibers formed a network surrounding each muscle fiber separately (Fig. 3C). This muscular coat covered externally by a connective tissue layer consisting of coarse collagenous (Fig. 3A) and fine elastic fibers (Fig. 3B). Within this layer, small-sized arteries, and veins, as well as myelinated nerve fiber bundles traversed the capsule mostly in an oblique manner to reach the exterior of the parenchyma (Fig. 3D& E), from which, they extended to its interior accompanying the connective tissue trabeculae.

A thick layer of smooth muscle fiber bundles was demonstrated between the muscular capsule and the parenchyma only at the attachment of the bulbourethral glands with the urethral wall (Fig. 4), which were supported with coarse collagenous (Fig. 4B) and fine elastic fibers(Fig. 4C); in addition to the reticular fiber network (Fig. 3C). This layer decreased gradually in thickness towards the free border of the gland, until it disappeared completely. Some of them accompanied the skeletal ones within the trabeculae for a short distance only in the region where the bulbourethral glands attached to the urethra (Fig. 5A-C).

From the deepest aspect of the muscular capsule, fibromuscular trabeculae extended into the gland, divided it into a variable number of lobules of different sizes and shapes. These trabeculae arose firstly thick, then decreased gradually in thickness towards the center of the gland (Fig. 2). They consisted mainly of skeletal muscle fibers, in addition to the collagenous ones. Smooth muscle fibers were seen only within the trabeculae towards the urethral wall. However, at the opposite side of the urethral wall (free border of the bulbourethral glands), the smooth muscle fibers could not be demonstrated within the trabeculae. Reticular fibers network was observed supporting the muscle fibers, while the elastic

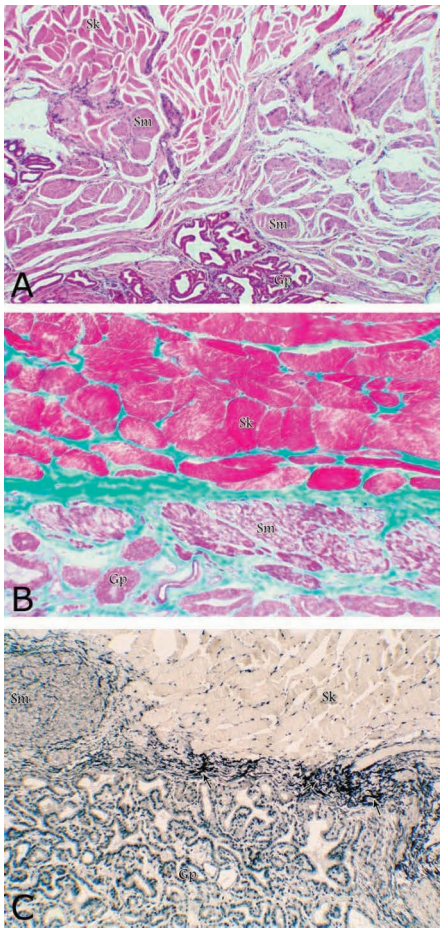

Fig. 4. Area of attachment of the bulbourethral glands with the urethra using Haematoxylin & eosin (A), Crossmon´s trichrome stain (B) and Verhoff´s stain (C). Thick layer of smooth muscle fiber bundles (Sm) between the skeletal muscle fibers of the muscular capsule (Sk) and the parenchyma (Gp). These bundles were supported with collagenous (green colour) and elastic fibers (arrows). A: X50, B: X200, C: X100.

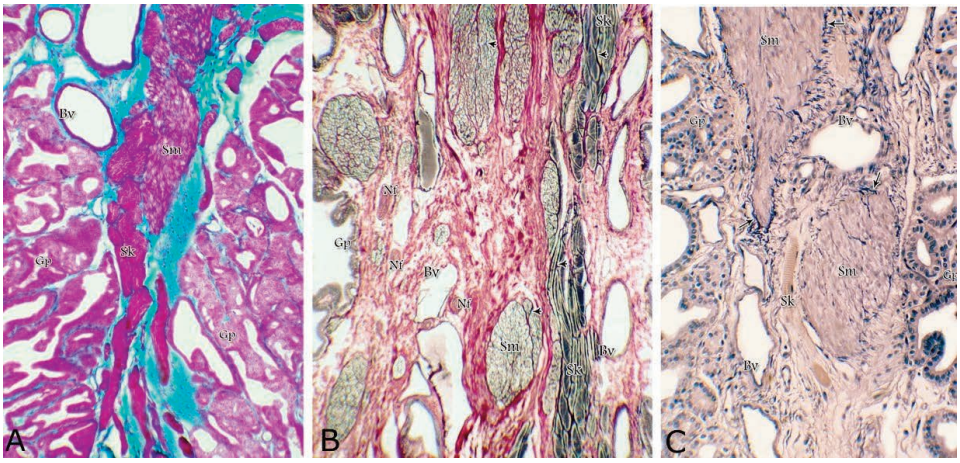

Fig. 5. Fibromuscular trabeculae of the bulbourethral glands of the donkey using Crossmon´s trichrome (A) Gomori´s stains (B), Verhoff´s stain (C). The trabeculae are formed of skeletal (Sk) and smooth (Sm) muscle fibers as well as collagen fibers (green and brown colour), elastic (arrow) and reticular fibers (arrowhead) supporting the skeletal and smooth muscle fibers. Blood vessels (Bv), nerve fiber (Nf). Parenchyma (Gp) on either side of the trabeculae. A and B: X100, C: X200.

ones were more concentrated mainly in relation to the smooth muscle fibers. Blood vessels of variable diameter, bundles of myelinated nerve fibers, lymphocytes, and fibrocytes were commonly seen within the trabeculae (Fig. 5A-C).

The interstitial connective tissue supporting the secretory portions of the bulbourethral glands of donkey was a direct continuation of the interlobular trabeculae. Structurally, it simulated nearly the trabeculae with the absence of the skeletal and smooth muscle fibers. Consequently, it was formed mainly of a network of reticular fibers enclosing the secretory end-pieces (Figs. 6A& B). Few fine elastic (Fig. 6C) and collagen fibers (Fig. 7A-D) were seen between the glandular portions. Arterioles, venules, and blood capillaries were also demonstrated, in addition to the fibroblasts, lymphocytes, and plasma cells.

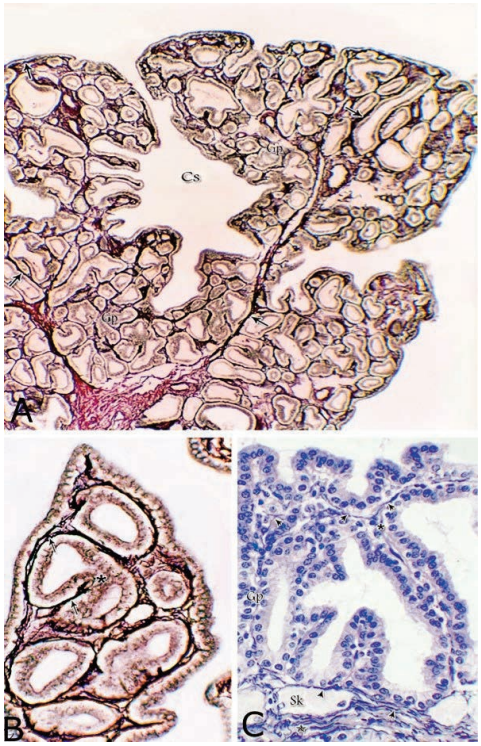

Fig. 6. Connective tissue fibers supporting the glandular parenchyma of the bulbourethral glands of the donkey using Gomori´s stain (A& B) and Verhoff´s stain (C). Glandular epithelium (Gp), reticular fiber network (arrow), few fine elastic fibers (arrowhead) within the interstitial connective tissue (asterisk) and trabeculae (star), central collecting sinus (Cs), skeletal muscle fibers (Sk), Collagen fibers (brown colour). A: X 100. B& C: X 400.

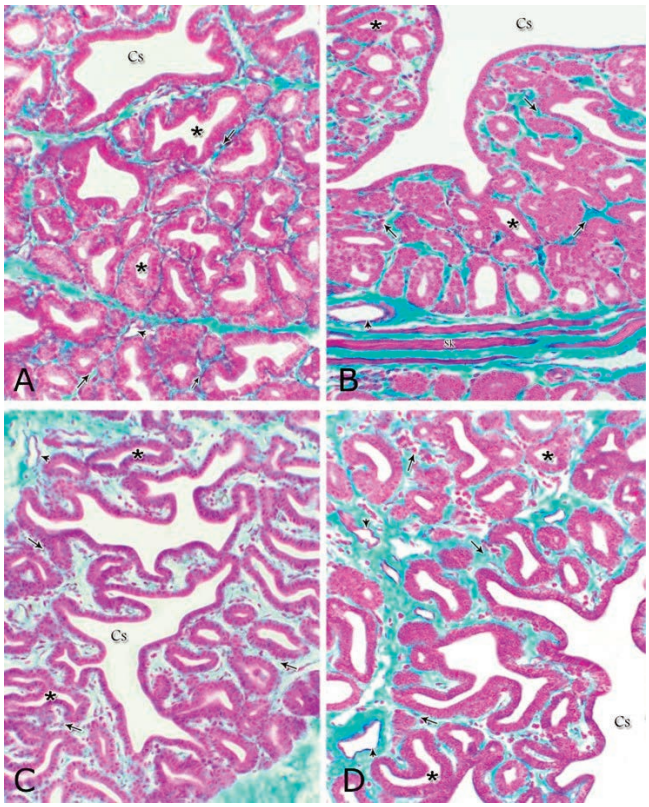

Fig. 7. Seasonal variation of the interstitial connective tissue (arrow) between the secretory end-pieces (asterisk) of the bulbourethral glands of the donkey during spring (A), summer (B), autumn (C) and winter (D) using Crossmon´s trichrome stain. Central collecting sinus (Cs), collagen fibers (green colour), skeletal muscle fibers (Sk), blood vessels (arrowhead). X 200.

The interstitial connective tissue / glandular tissue ratio of the bulbourethral glands of the donkey revealed highly significant ($P<0.01$) seasonal variations. The minimal amount of the interstitial connective tissue was observed during spring, where the secretory end-pieces were somewhat closely packed together. During this season, this ratio reached about 0.235±0.014. This amount increased gradually during the other seasons of the year, where it measured about 0.341±0.019 during summer and 0.398±0.022 during autumn. The maximal amount of the interstitial connective tissue was demonstrated during winter, where the secretory end-pieces appeared somewhat widely separated. In this season, this ratio reached about 0.430±0.029 (Fig. 8).

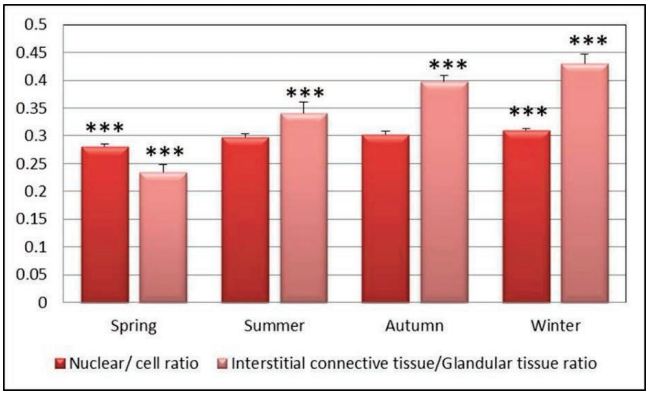

Fig. 8. Histogram showed the nuclear / cell ratio of the principal cells of the glandular epithelium and the interstitial connective tissue / glandular tissue ratio of the urethral glands of the donkey during different seasons of the year.

The parenchyma of the bulbourethral glands formed of glandular portions and duct system. The glandular portions were of the compound tubulo-alveolar variety, forming lobules of variable sizes and shapes, possessing a wide central collecting sinus (Figs. 2, 6 &9). The lining epithelium of the glandular portions (Fig. 9B) was formed of principal and inconstantly basal cells. The principal glandular cells varied from low columnar to high cuboidal with fine granular acidophilic cytoplasm and spherical or ovoid somewhat basely located nuclei possessing dispersed chromatin and mostly one nucleolus. The flat basal cells were small, possessing flattened nuclei. Mitotic activity within these cells was commonly observed (Fig. 9B inset).

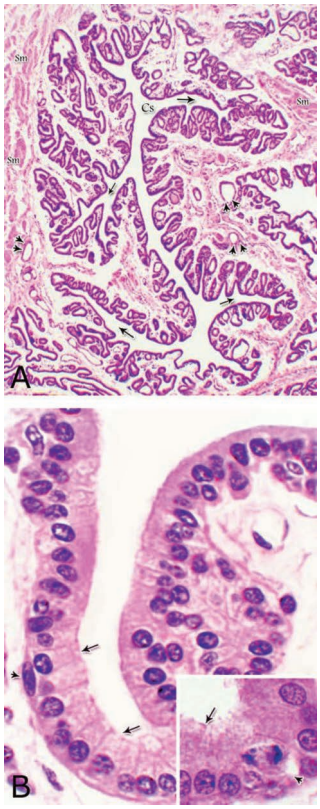

Fig. 9. Glandular lobule and the glandular epithelium of the bulbourethral glands of the donkey using Haematoxylin and eosin stain. Compound tubulo-alveolar glands (arrow) open into central collecting sinus (Cs). Principal cells (arrows), basal cells (arrowheads). Inset: mitotic division within the basal cells. Blood vessels (double arrowheads), smooth muscle fibers within the trabeculae (Sm). A: X 50, B: X 400 and inset: X 1000.

The height of the epithelial lining of the secretory end-pieces of the bulbourthral glands of the donkey showed highly significant (P<0.01) seasonal variations. It reached the maximal height in spring, where it measured about 12.96 µm±0.389. A gradual decreased in the epithelial height was recorded during the summer (about 12.15 µm + 0.290) and autumn (about 11.54 µm±0.424). The lowest height (about 10.77 µm±0.268) was observed during the winter (Fig. 10).

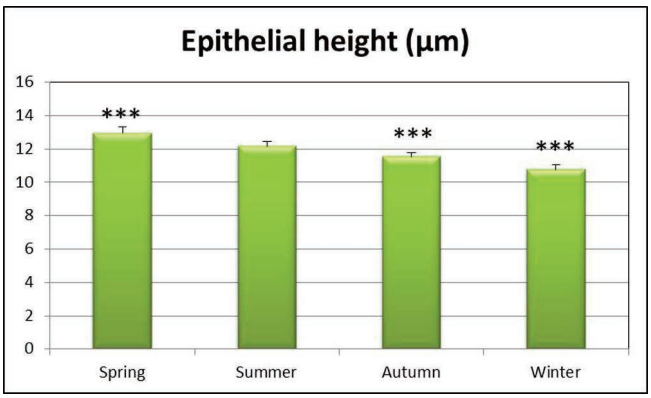

Fig. 10. Histogram showed the epithelial height (µm) of the bulbourethral glands of the donkey during different seasons of the year.

The nuclear/cell ratio of the epithelial lining of the secretory end-pieces of the bulbourethral glands of the donkey showed also highly significant (P<0.01) seasonal variations. The lowest ratio (about 0.280±0.005) was observed during spring. This ratio increased gradually to reach about 0.297±0.006 in summer and 0.302±0.007 in autumn. The highest ratio (about 0.310±0.003) was observed during the winter (Fig. 8).

The duct system of the bulbourethral glands could be organized into tertiary, secondary, and primary ducts. The glandular portions of the bulbourethral glands opened directly by a tertiary duct, which consequently drained into a central collecting sinus or secondary duct (Fig. 11).

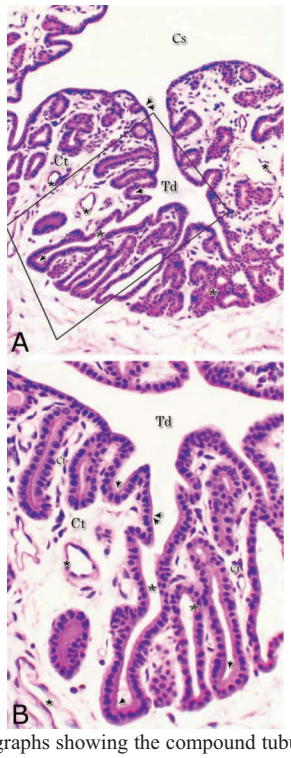

Fig. 11. Photomicrographs showing the compound tubulo-alveolar (star) in (A) and the high magnification of the marked area (B). A tertiary duct (Td), secondary duct or central collecting sinus (Cs), glandular epithelium (arrowhead), ductal epithelium (double arrowhead), interstitial connective tissue (Ct), blood vessels (asterisk). Haematoxylin and eosin stain. A: X 200. B: X 400.

The change from the glandular epithelium to the ductal one (tertiary and secondary duct) occurred gradually, where the principal cells became low cuboidal with slightly basophilic cytoplasm and large spherical or rounded, somewhat darkly stained nuclei occupying most of the cells. The secondary ducts of several lobules or even a single one drained into a long primary duct. The latter was observed accompanied with numerous secretory end-pieces directed towards the membranous urethra, where they penetrated the urethral muscle and opened into its dorsal aspect. At the point of attachment of the bulbourethral glands with the urethra, their outer muscular covering could not be more seen, and the duct became surrounded with a highly vascular dense fiberous connective tissue, containing many cavernous spaces (Fig. 12). The connective tissue layer was formed of coarse collagenous (Fig. 13A), reticular (Fig. 13B), and numerous elastic fibers (Fig. 13C). Reticular fibers formed a network only under the epithelial lining of the duct and the secretory end-pieces accompanied it (Fig. 13B). This connective tissue layer also contained blood capillaries, fibroblasts, fibrocytes, and plasma cells.

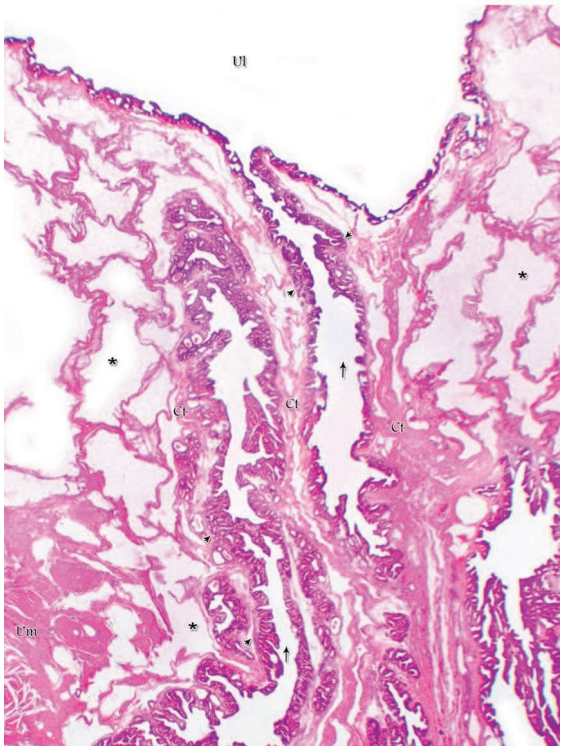

Fig. 12. Primary ducts (arrows) of the bulbourethral gland penetrating the urethral muscle (Um) to open into the urethral lumen (Ul). These ducts are accompanied by numerous secretory end-pieces (arrowhead) and surrounded by dense connective tissue (Ct) containing many cavernous spaces (asterisk).Haematoxylin and eosin stain. X 25.

The epithelial lining of the primary duct simulated firstly that of the secondary one. Near its termination, their principal cells became tall columnar with oval basely located nuclei (Fig. 14A & inset), while at its opening into the urethra, the ductal epithelium changed into stratified cuboidal and columnar one (Fig. 14B).

Scanning electron microscopy of the luminal surface of the membranous urethra during the studied seasons at the level of the bulbourethral glands revealed two rows of openings (Fig. 15A). The latter were appeared mostly as elevated papillae or rarely as dome-shaped elevation possessing a slit-like or rounded opening with irregular indentations (Fig. 15A- C). At high magnification, the luminal surface of their lining cells showed a hexagonal-shaped profile with well-developed cell boundaries and numerous ill-developed microvilli (Fig. 15D).

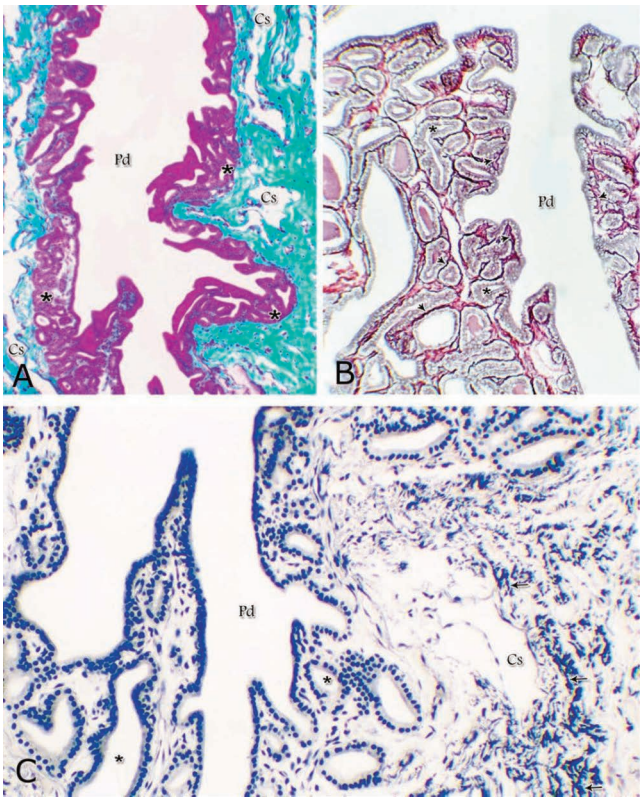

Fig. 13. Photomicrograph showing the collagenous fibers (green colour), reticular (arrowhead) and elastic fibers (arrow) in the wall surrounding the primary duct (Pd) of the bulbourethral glands of the donkey. Cavernous spaces (Cs), secretory end-pieces accompanying the duct (asterisk). A: Crossmon´s trichrome stain. X 100, B: Gomori´s stain, X200 and C: Verhoff´s stain, X 200.

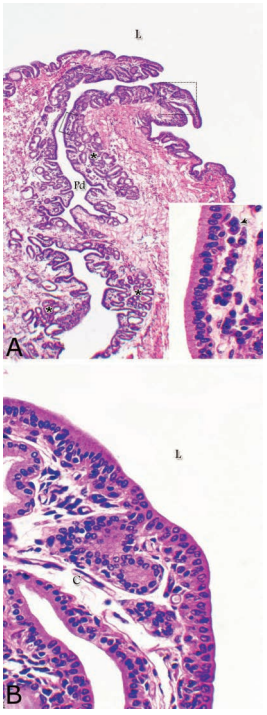

Fig. 14. Photomicrographs of the primary duct (Pd) at its opening into the urethral lumen (L) in (A) and its high magnifications of the marked areas (inset) showed the changing of the low cuboidal principal cells to columnar one (inset) and to stratified columnar and cuboidal (B). Secretory end-pieces accompanied the duct (asterisk), plasma cells (arrowhead), blood capillaries (C). Haematoxylin and eosin stain. (A): X 50. Inset and (B): X 400.

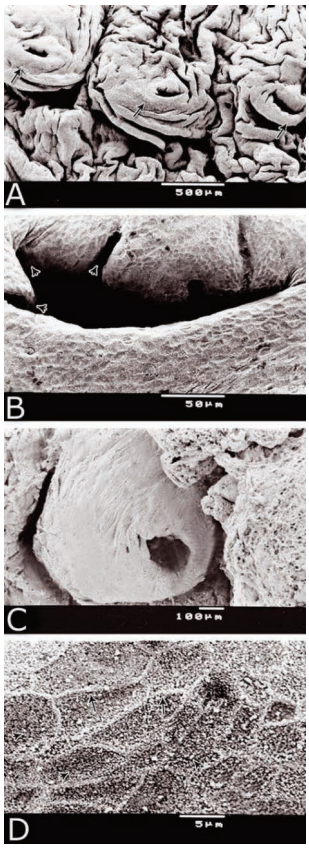

Fig. 15. Scanning electron micrographs of the luminal surface of the membranous urethra (A): the arrangement of the bulbourethral glands openings (arrow). (B): High magnification of the gland opening appeared as papillae surrounding slit-like opening with irregular indentations (arrowhead). C: dome-shaped elevation of the bulbourethral glands opening. D: hexagonal - shaped luminal cell surface of the bulbourethral glands duct opening. These cells had well-developed cell boundaries (arrow) and ill-developed microvilli (arrowhead).

Histochemical observations

Carbohydrates histochemistry

Neutral mucopolysaccharides

During spring, the glandular cells of most of the secretory end-pieces revealed strong diffuse PAS-positive reaction. Few of them were moderately, weakly, or even negatively reacted. It also observed that the PAS reactivity was variable within the cells of the same secretory end-pieces (Fig. 16A). Also, the ductal cell lining as well as the secretory materials reacted strongly positive. In summer, the reactivity of the bulbourethral glands to PAS resembles that of spring, but the intensity of the reaction and the number of the positive reactive units were slightly decreased (Fig. 16B).

During autumn, most of the epithelial cell lining of the secretory end-pieces of the bulbourethral glands was moderately reacted with PAS (Fig. 16C).

During winter, most of the secretory end-pieces as well as the ductal lining of the bulbourethral glands of the donkey revealed a very weak to negative PAS reactivity. However, the secretory materials and few secretory end-pieces exhibited strong PAS positive reaction (Fig. 16D).

In all studied seasons, the skeletal muscle fibers forming the muscular capsule and the fibromuscular trabeculae showed variable degrees of PAS reactivity, which was reflected by dark, intermediate, and light types (Fig. 17A). The smooth muscle fibers, the connective tissue layers outside the muscular coat as well as that of the interstitium, exhibited moderate PAS reaction, while their reticular fibers network as well as that of the parenchyma and the endothelium of the blood vessels reacted strongly positive to PAS (Fig 17B).

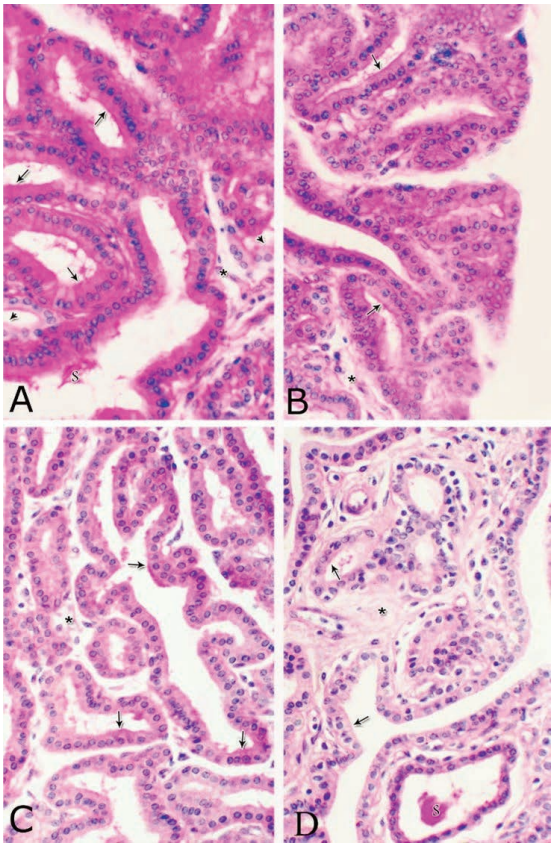

Fig. 16. PAS reaction within the bulbourethral glands during the different seasons. During spring (A), the glandular epithelium (arrow) of most of the secretory end-pieces shows strong diffuse PAS positive reaction, some are negatively reacted (arrowhead). This reaction decreases in intensity during summer (B) and become moderate during autumn (C). During winter (D) most of the glandular epithelium shows very weak reaction with PAS. Connective tissue stroma (asterisk) showed moderate reaction, secretory materials (S) showed strong PAS positive. PAS / haematoxylin stain. X 400.

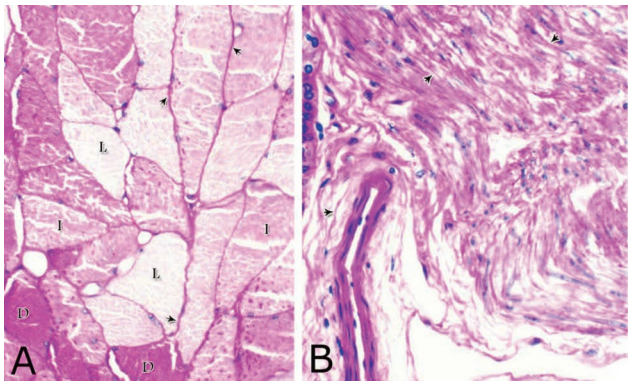

Fig. 17. PAS reactivity of the skeletal (A) and smooth muscle fibers (B) of the fibromuscular capsule of the bulbourethral glands. (A) Variable intensity of the PAS reaction within the skeletal muscle fibers of the fibromuscular capsule, dark (D), intermediate (I) and light (L) muscle fiber types. Smooth muscle showed moderate PAS reactivity. Reticular fibers showed strong PAS reaction (arrowheads). PAS / haematoxylin stain. X Obj. 40 & Oc. 10.

Glycogen

With Best's carmine stain, the glandular epithelial cells of the bulbourethral glands of the donkey, the secretory material, the smooth muscle fibers, and interstitial connective tissue were negatively reacted (Fig. 18A). The skeletal muscle fibers forming the muscular capsule, or the fibromuscular trabeculae

showed strong positive granular substance (Fig. 18B).

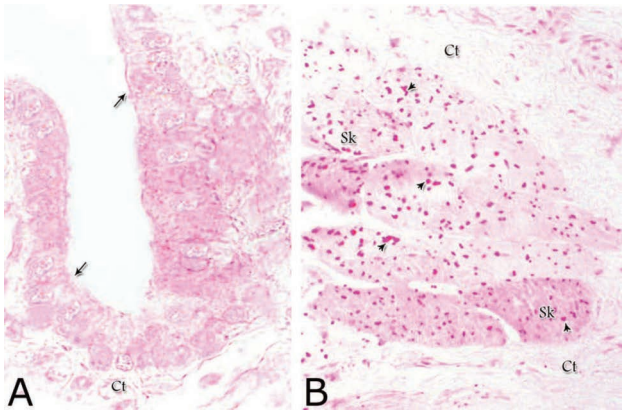

Fig. 18. Best´s carmine reactivity within the bulbourethral glands of the donkey. A: a negative Best´s carmine reactivity within the glandular epithelium (arrow) and interstitial connective tissue (Ct) of the bulbourethral glands of the donkey during spring. B: Positive glycogenic granules (arrowhead) within the skeletal muscle fibers (Sk) of the fibromuscular capsule. A: X 1000, B: X 400.

Acid mucopolysaccharides

During spring, the reactivity of the glandular epithelial cells and interstitial connective tissue as well as the secretory materials of the bulbourethral glands of the donkey to alcian blue was moderate (Fig. 19A). On the other hand, few cells were negatively reacted. The muscle fibers forming the fibromuscular capsule and trabeculae had no alcianophilic substance. The intensity of the alcianophilia deceased gradually during summer (Fig. 19B), which became limited to the apical borders of the principal cells lining the secretory end-pieces during autumn (Fig. 19C). While during winter, the alcian blue reactivity could not be observed within most of the secretory end-pieces (Fig. 19D).

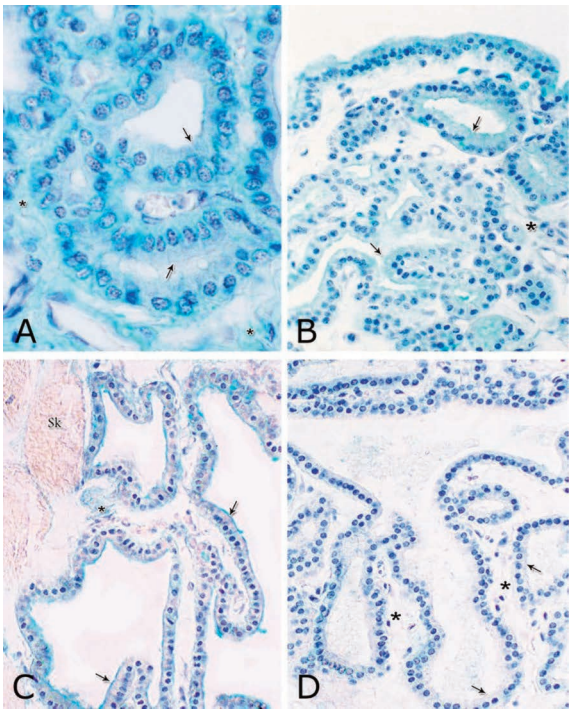

Fig. 19. Alcian blue reactivity of the bulbourethral glands. A moderate alcian blue positive reaction was observed during spring (A). This reaction decreases in intensity during summer (B) and become weak during autumn (C) except the cell apical borders of the principal cells, which showed moderate reaction. During winter, the alcian blue reactivity couldn't be observed (B). Glandular epithelial cells (arrow) and connective tissue stroma (asterisk), Negative reactivity within the skeletal muscle fibers (Sk). Alcian blue / haematoxylin stain. A: X 1000. B-D: X Obj. 400.

During spring, summer, and autumn, the reactivity of the bulbourethral glands of the donkey for alcian blue-PAS combination revealed a mixed reactivity of variable intensity for both stains. During winter, a negative reactivity was the common picture, while few secretory end-pieces revealed variable reactivity for both stains (Fig. 20A-D).

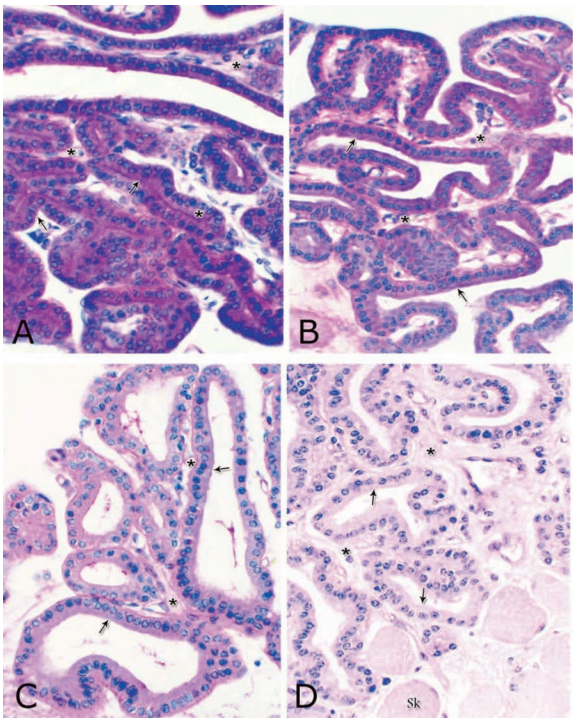

Fig. 20: Alcian blue / PAS reactivity of the bulbourethral glands. This reaction appears strong during spring (A) and decrease in intensity during summer (B). Moderate reaction is observed during autumn (C). While during winter, the reactivity does not differ than that observed with PAS stain (D). Glandular epithelium (arrow), connective tissue stroma (asterisk), skeletal muscle fibers (Sk). Alcian blue/ PAS haematoxylin stain, X 400.

Discussion

The present study revealed that the bulbourethral glands of the donkey were covered by a thick capsule of striated muscle fibers. This why they are not usually palpable per rectum because they are covered by the urethralis and bulboglandularis muscles, which it is a part of ischiourethralis muscle (Budras *et al*., 2009; Chenier, 2009). Similar results were observed by Aitken (1960) in boars, Abbas (1976) in rams, Ali *et al*. (1976) and, Mosallam (1981) and Youssef *et al*. (1984) in camels, Short *et al*. (1967) in elephants, Geuze and Slot, (1978) in rats, Inns (1982) in male tammar wallabies and (Moussa *et al*., 1983) in buffalo bulls. From this muscle, bundles enter the larger trabeculae of the gland as in horses (Sisson, 1975). In rats, contraction of the striated muscle in the capsule of the bulbourethral glands and that of the urethral bulb first force bulbourethral gland secretion out of the lumen (Geuze and Slot, 1978). The present investigation revealed that the trabeculae were formed of skeletal and inconstantly seen smooth muscle fibers, which were supported with collagenous, reticular, and fine elastic fibers. Smooth muscle fibers could not be detected within the trabeculae of the bulbourethral glands of rats (Geuze and Slot, 1978), while that of camels and buffalo bulls was formed mainly of connective tissue fibers and smooth muscle ones (Mosallam, 1981; Moussa *et al*., 1983), respectively).

In accordance with Abbas (1976) and Pawar *et al*. (1986) in rams, Ali *et al*. (1978), Mosallam (1981); Badawy *et al*. (1982); Youssef *et al*. (1984) and Aly and Prentis (1986) in camels, Short *et al*. (1967) in elephants, Selim (1974) in bucks, Moussa

et al. (1983) and Abou-Elmagd and Wrobel (1989) in buffalo, Nogueira *et al*. (1984) in Philander opossum, More (1991) in calves and Adebayo *et al*. (2015) in rat, the bulbourethral glands of the donkey were of tubuloalveolar type. While, in goats (Wrobel, 1970), buffalo bulls (Fahmy and Osman, 1972) and stallions, (Nickel *et al*., 1973), it was tubular in type. However, in rats, it was alveolar in type (Geuze and Slot, 1978). Marei *et al*. (2004) stated that the bulbourethral glands of goats were tubular or tubulo-alveolar in type.

The lining epithelium of the secretory portions of the bulbourethral glands of the donkey was formed of tall cuboidal to columnar cells with inconstant flat basal ones. Similar results were observed in caprine´s (Wrobel, 1970) and rat´s (Geuze and Slot, 1978) bulbourethral glands, . However, the bulbourethral glands of hamster, cats, bucks, boars, Philander opossum, goats, and calves were lined by one type of columnar, cuboidal, or pyramidal cells as reported by Feagans *et al*. (1963); Wrobel (1969); Selim (1974); Nielsen *et al*. (1977); Nogueira *et al*. (1984); Tsukise and Yamada (1987) and More (1991), respectively. The observed mitotic activity within the basal cells revealed that they could be considered as stem cells for the glandular and ductal ones.

The epithelial lining of the secretory end-pieces of the bulbourethral glands of the donkey showed different secretory activities. This might be attributed to seasonal variations. During spring, the glandular epithelium reached its maximal height, and its nuclear / cell ratio reached the lowest value, their cytoplasm was packed with secretory granules and most of the secretory end-pieces showed strong diffuse PAS positive reaction and moderate alcian blue-positive reaction indicating that the cells were in their active stage. While during summer and autumn, the activity of the gland was decreased, this was reflected by the decrease in the cell height and increase in the nuclear/cell ratio, and most of the epithelial lining of the secretory end-pieces was moderately reacted to PAS and the alcian blue reaction became limited to the apical border of the principal cells. During winter, the glandular epithelium appeared less active, where the epithelial height reached its lowest value, and the nuclear / cell ratio was higher than that demonstrated during the other seasons and most of the secretory end-pieces showed very weak to negative PAS and alcian blue reaction. Seasonal variations of the bulbourethral glands were observed in bucks (Selim, 1974), camels (Mosallam, 1981), and white-tailed deer, (Stewart *et al*., 2018), respectively.

The present study showed that the glandular cells of the bulbourethral glands revealed a mixed reactivity of variable intensity to PAS and alcian blue, indicating the presence of neutral and acid mucopolysaccharides. Similar results were observed in rams (Abbas, 1976, Pawar *et al*., 1986), boars (Nielsen *et al*., 1977) and camels (Mosallam, 1981; Youssef *et al*., 1984). Accordingly, the bulbourethral glands of the donkey secreted a fraction of semen rich in mucosubstance because their secretory substances contained abundant amounts of neutral mucopolysaccharides and few amounts of acid mucopolysaccharides. Also, neutral/acid mucosubstances were detected in the cytoplasm of the tubulo-alveolar and ductal cells of the bulbourethral glands of man (Sirigu *et al*., 1993). The bulbourethral glands of hamsters (Feagans and Robertson, 1964), boars (Boursnell *et al*., 1970), Guinea pigs (Nittinger, 1973) and rats (Geuze and Slot, 1978) secrete mucin rich in sialo.

The current study revealed that the glandular epithelium of the bulbourethral glands of the donkey had neither blebs nor brush borders, so the mode of secretion of the glandular epithelium may be merocrine in type. Similar result was recorded by (Nielsen *et al*., 1977) in domestic boars, while in camels, the mode of secretion of the bulbourethral glands of camels was either apocrine or merocrine (Aly and Prentis, 1986).

Similar to that observed by Aitken (1959); Ali *et al*. (1976); Gupta and Singh, (1982) and Moussa *et al*. (1983) in the bulbourethral glands of rams, camels, goats and buffalo bulls, respectively, the bulbourethral glands of the donkey were free from glycogenic substances after Best´s carmine staining. These results were in the contrary with that observed in the bulbourethral glands of camels, Philander opossum and goats, in which there were glycogen laden cells in some end-pieces and ducts (Mosallam, 1981; Nogueira *et al*., 1984; Youssef *et al*., 1984; Tsukise and Yamada, 1987).

The secretory substance of the bulbourethral glands of rat, rodent, and boar played a role in semen coagulation to form the copulatory plug at ejaculation (Hart, 1968; Dunker and Aumuller, 2002; Badia *et al*., 2006). It possibly involved in the lubrication of the urethra and vagina (Geuze and Slot, 1978) and occlusion of the cervix to prevent loss of sperms (Eurell and Frappier, 2006) and recently they play a role in the sperm storage in the llama oviduct (Apichela *et al*., 2014). The acidic nature of the glucoconjugate substance of the bulbourethral glands of goats gave a positive reaction for sulfates and carboxyl groupings, including sialic acid residues (Tsukise and Yamada, 1987). The possible concept may be postulated that acidic glucoconjugates with sialic acid residues play an important role for the activity and metabolism of spermatozoa (Yamada, 1985). The sulfated glucoconjugates could prevent the proliferation of the urethral pathogenic microorganisms and protect spermatozoa from them (Tsukise and Yamada, 1987). The immunological role of the bulbourethral glands in preventing the entry of antigens was confirmed by Migliari *et al*. (1992). Chughtai *et al*. (2005) reported that the function of the Cowper´s gland secretion was to neutralize traces of acidic urine in the urethra. In stallion, the bulbourethral glands, have considerable lipase enzyme activity, which can be potentially affect the sperm motility over time under cooled storage conditions (Chenier, 2009).

Conclusion

The present study demonstrates the gross, histological and histochemical structures of the bulbourethral gland of donkey with special reference to its variable activity during the different seasons.

Conflict of interest

The authors have not declared any conflicts of interest

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