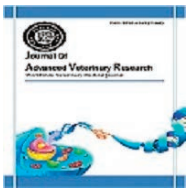
Journal of Advanced Veterinary Research

https://advetresearch.com



Histological and Histochemical Studies on the Seminal Vesicles of Donkey (*Equus asinus*): with Special Reference to their Seasonal Variations

Alaa S. Abou-Elhamd[1,2*], Yousria A. AbdelRahman[1], Aziza A. Selim[1]

[1]*Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Assiut University, Egypt.*
[2]*Department of Respiratory care, Faculty of Applied Medical Sciences, Jazan University, KSA.*

ARTICLE INFO

Original Research

Received:
25 March 2020

Accepted:
20 June 2020

Keywords:

Seminal vesicles,
Seasonal variations,
Donkey

ABSTRACT

The objective of this study was to describe the histological and histochemical structure of the seminal vesicles during different seasons of the year. The specimens were collected from the seminal vesicles of 24 sexually mature apparently healthy male donkeys (5 to 7 years of age) during different seasons of the year. The seminal vesicles (Vesiculae seminales) of the donkey were paired pear-shaped sacs. The wall of the seminal vesicles of the donkey was consisted of tunica mucosa, tunica muscularis and tunica serosa or adventitia. The tunica mucosa of the seminal vesicle was highly folded, surrounding a large irregular oval central lumen. These folds carried many lateral secondary branches with numerous tubular invaginations into the underlying connective tissue. The lamina epithelialis of the seminal vesicles consisted of principal and basal cells. The activity of seminal vesicles of donkey varied during different seasons of the year. It reached maximal activity during spring which was manifested by increasing in the epithelial height of the glandular epithelium, decreasing the nuclear/ cell ratio and the interstitial/ glandular tissue ratio and increasing the secretory activity. This activity of the seminal vesicles decreased gradually during summer and autumn to reach its minimal during winter. In conclusion, the seminal vesicles of donkey have more pronounced activity in spring than in other season of the year.

J. Adv. Vet. Res. (2020), 10 (3),126-134

Introduction

The mammalian accessory genital glands include prostate, seminal vesicles, bulbourethral glands, ampulla ductus deferens and urethral glands (Banks, 1993; Liebich *et al*., 2004; Abou-Elhamd, 2005; Eurell and Frappier, 2006; Abou-Elhamd *et al*., 2012; Abou-Elhamd *et al*., 2013; Abou-Elhamd *et al*., 2019; Skonieczna *et al*., 2019). The accessory genital glands play an important role in the reproductive function by secreting several factors that nourishing the spermatozoa and clear the urethra prior to ejaculation (Marieb and Hoehn, 2003; Ricardo, 2018; Noda and Ikawa, 2019).

The histomorphological features of the seminal vesicles were studied in many mammalian species such as stallion (Ellery, 1971), red deer (Aughey, 1969), buck (Selim, 1974; Marei *et al*., 2004), ram (Abbas, 1976) and bull (Amselgruber and Feder, 1986; Abou-Elmagd and Kelany, 1992).

Surveying the available literature revealed that seasonal variation of accessory genital glands was extensively investigated in mammals (Abbas, 1976; Inns, 1982; Youssef *et al*., 1984; Abou-Elhamd, 2005; Suri *et al*., 2008). From the vast volume of literature on these glands, little attention has been paid to those of equidae (Ellery, 1971). To the best of the authors' knowledge, this study presented first description of the histological and histochemical structure of the seminal vesicle of donkey with special reference to its seasonal variations.

Materials and methods

The present study was carried out in 20 sexually mature apparently healthy male donkeys (Jacks) 5 to 9 years old. Animals were anesthetized by using chloroform (Sigma Aldrich, Merck, Germany) to pain relief 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. They included neutral buffered formalin, Bouin´s fluid (for routine histological and morphometerical examination), Carnoy´s fixative (for carbohydrates histochemistry). The specimens were collected from the seminal vesicles 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

*Corresponding author: Alaa S. Abou-Elhamd
E-mail address: Alaa88@yahoo.com

ISSN: 2090-6277/2090-6269/

stains: Harris haematoxylin and eosin for general histological examination; Crossmon´s trichrome stain for identification of collagenous and muscle fibers; Verhoeff´s methods for identification of elastic fibers; Gomori´s reticulin method for detection of reticular fibers; Periodic acid-schiff (PAS) technique for demonstration of neutral mucopolysaccharides (McManus, 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 (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 lamina epithelialis and the glandular epithelium of the seminal vesicle was calculated from fifteen random fields of the glands under investigation; the nuclear / cell ratio of the principal cells of the lamina epithelialis and the glandular epithelium was calculated from fifteen random fields in all studied glands; the interstitial connective tissue / glandular tissue ratio of the studied glands was calculated from five random fields.

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 significant.

Results

The seminal vesicles (Vesiculae seminales) of the investigated donkeys were paired pear-shaped sacs. Each one possessed a large central lumen and located dorsolateral to the corresponding ampulla of the deferent duct. They were partially enclosed within the genital folds and connected caudomedially to the cranial edge of the prostate. Each seminal vesicle had an excretory duct, which ran caudally beneath the isthmus of the prostate, opened with the corresponding terminal part of the deferent duct forming the ejaculatory ducts at the Colliculus seminalis (Fig. 1).

Histological observations

The wall of the seminal vesicles was consisted of tunica mucosa, tunica muscularis and tunica serosa or adventitia (Fig. 2).

The tunica mucosa of the seminal vesicle was highly folded, and surrounding a large irregular oval central lumen often containing lightly stained acidophilic substance with deeply stained variable sized acidophilic bodies. The primary mucosal folds appeared shorter at the dorsal aspect of the seminal vesicle (Fig. 2B), and then they increased gradually in height along their lateral sides to reach their maximal height at the ventral aspect (Fig. 2C). These folds carried many lateral secondary branches with numerous tubular invaginations into the underlying connective tissue.

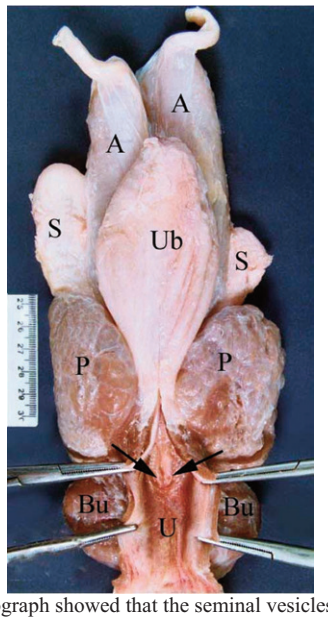


Fig. 1. Photomicrograph showed that the seminal vesicles (S) were located dorsolateral to the corresponding ampulla of the deferent duct (A) and opened with the corresponding terminal part of the deferent duct forming the ejaculatory ducts (arrows). P, prostate; Bu, bulbourethal gland; Ub, urinary bladder.

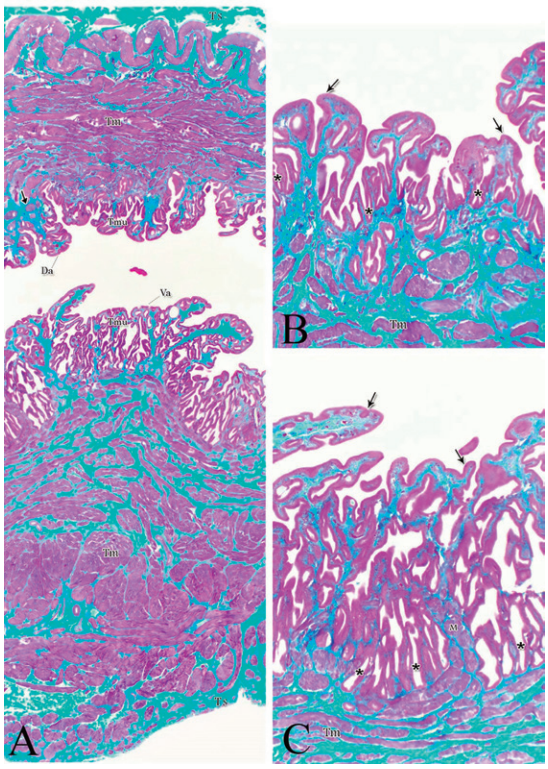


Fig. 2. A: Photomicrograph of the wall of the seminal vesicle of the donkey during winter showing the arrangement of its layer into; tunica mucosa (Tmu), tunica muscularis (Tm), tunica serosa (Ts) as well as the variation in the height of their mucosal folds and thickness of tunica muscularis at both dorsal (Da) and ventral aspects (Va); extension of muscular bundles into the mucosal folds (arrows). B& C: High magnification photomicrograph of A showing the variation in the height of the mucosal folds of the seminal vesicle during winter. Mucosal folds appeared short at the dorsal aspect (A) and tall at the ventral aspect (B). Lamina epithelialis (arrows), branched tubular glands (asterisks), muscular bundles of the mucosal fold (M), tunica muscularis (Tm), collagen fibers (green colour). Crossmon´s trichrome stain. A: X 25, B& C: X 50.

The lamina epithelialis of the seminal vesicles consisted of principal and basal cells. The principal cells varied from high cuboidal to columnar. They often possessed brush border and

they were characterized with acidophilic cytoplasm and oval or rounded nuclei with dispersed chromatin with one or two distinct nucleoli. Few vacuoles of variable sizes were observed in some principal cells. The flat basal cells were few and occasionally seen at the basement membrane. They possessed somewhat flat or oval nuclei and faintly stained acidophilic or sometimes vacuolated cytoplasm. Some clear cells were also observed among the principal ones. They were characterized by their clear cytoplasm with few fine acidophilic granules dispersed elsewhere in it, well-distinct cell boundaries and rounded vesicular nuclei at variable locations. The number of these clear cells did not vary within the lamina epithelialis in the different season of the year (Fig. 3).

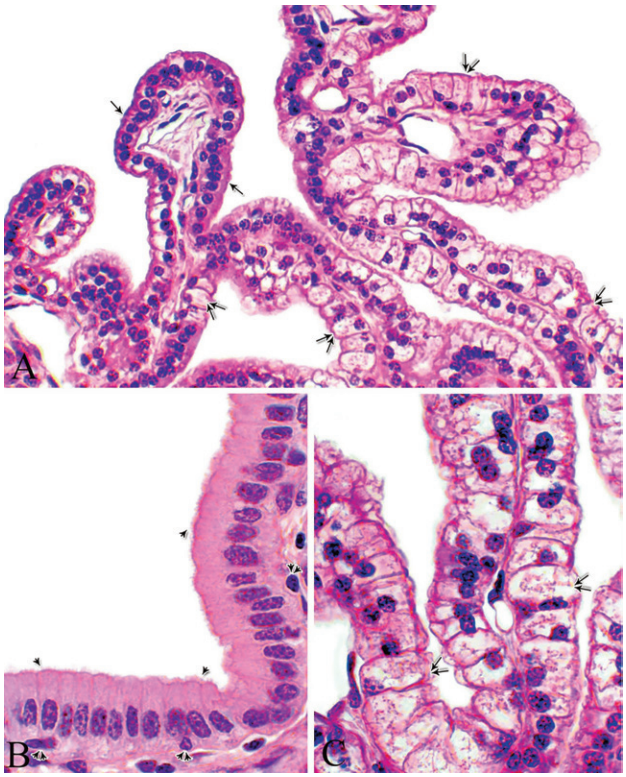


Fig. 3. Photomicrograph showing the surface epithelium of the seminal vesicle of the donkey during spring. It is formed of principal cells (arrows) with brush border (arrowheads) and acidophilic cytoplasm, containing oval vesicular nuclei. Basal cells (double arrowheads), clear cells (double arrows) with few fine dispersed acidophilic granules, with distinct cell boundaries and variably located nuclei. Haematoxylin & eosin stain, A: X 400, B & C: X 1000.

The height of the lamina epithelialis revealed highly significant (P<0.01) seasonal variations. At spring, the epithelium reached its maximal height, which is decreased during summer and autumn, the lowest epithelial height was recorded during winter (Fig. 4).

The nuclear / cell ratio of the lamina epithelialis of the seminal vesicles of the donkey showed also highly significant (P<0.01) seasonal variations. This ratio reached its minimal value during spring, where it increased gradually during summer and autumn, where the maximal value was measured during winter (Fig. 5).

The glands of the seminal vesicle of the donkey were branched tubular in type present in the lamina propria (Figs. 1, 2). Their lining epithelium was formed of principal and basal cells. They simulating more or less those of the lamina epithelialis, but their height were slightly lower than that of the surface epithelium (Fig. 6). Some secretory portions were lined completely with the clear cells (Figs. 3A, C).

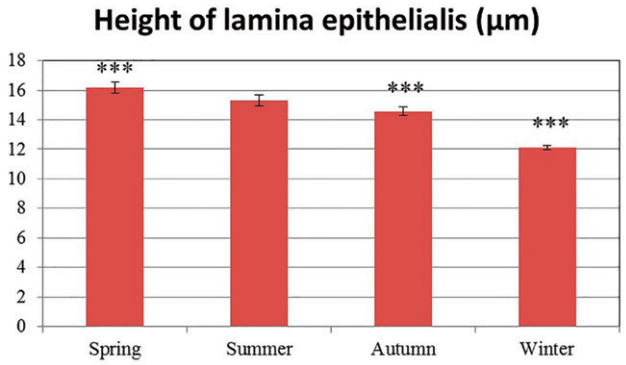


Fig. 4. Height of the lamina epithelialis of seminal vesicle of donkey showed highly significant (P< 0.01) seasonal variation. It reached maximal height in spring and minimal height in winter.

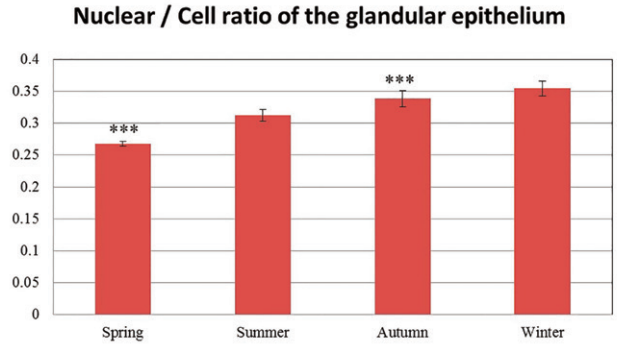


Fig. 5. Nuclear cell ratio of the principal cells glandular epithelium showed highly significant (P<0.01) difference between spring, autumn and winter seasons.

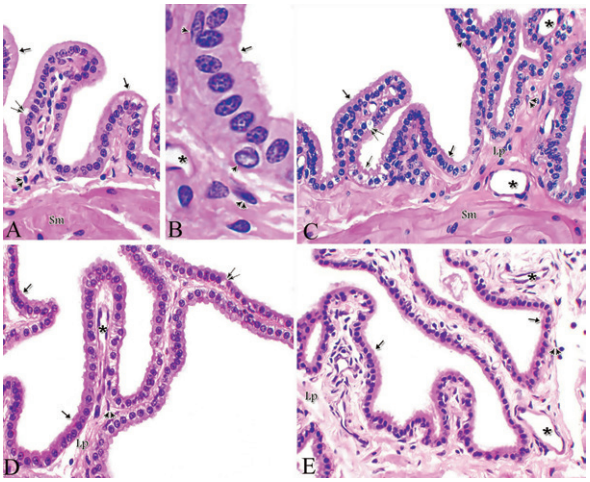


Fig. 6. Photomicrograph showing the variations in the height of the glandular epithelium of the seminal vesicles of the donkey during spring (A&B), summer (C), autumn (D) and winter (E). Principal cell (arrows), basal cells (arrowheads); lamina propria (Lp) containing blood vessels (asterisks), fibroblasts (double arrowheads). Smooth muscle fibers (Sm), vacuoles (double arrows). Haematoxylin & eosin stain, A& C-E: X 400. E: X1000.

The height of the lining epithelium of the tubular glands showed highly significant (P<0.01) seasonal variations. The maximal height was recorded in spring, which is decreased gradually during summer, autumn and winter (Figs. 7, 8).

The nuclear / cell ratio of the glandular epithelium showed highly significant (P<0.01) seasonal variations. The lowest ratio was recorded in spring, this ratio showed also a gradual increase during summer and autumn. The highest ratio was observed during winter (Fig. 8).

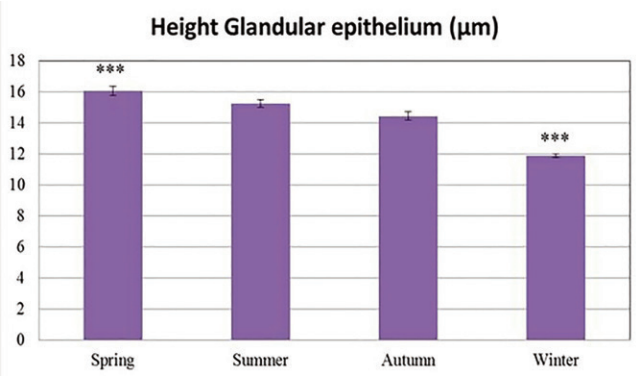


Fig. 7. Height of the principal cells of the glandular epithelium (µm) showed highly significant (P<0.01) seasonal variations between spring, autumn and winter seasons.

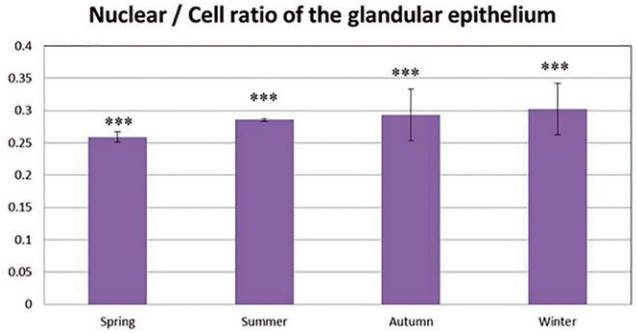


Fig. 8. Nuclear / Cell ratio of the principal cells of the glandular epithelium (µm) showed highly significant (P<0.01) seasonal variations between spring, autumn and winter seasons.

The lamina propria was formed of dense connective tissue layer containing mainly collagenous fibers (Fig. 9A). Reticular fibers (Fig. 9B) were also present supporting the surface and glandular epithelium as well as the wall of the blood vessels. Network of elastic fibers was observed only in relation to the smooth muscle fiber bundles, which extended from the inner circular layer into the large mucosal folds (Figs. 9C, D). In addition, blood vessels of various caliber, fibroblasts and lymphocytes were seen.

The interstitial connective tissue / glandular tissue ratio of the glands of the seminal vesicles showed highly significant (P<0.01) seasonal variations. The lowest ratio is recorded during spring, where the connective tissue was of its minimal amount, and then it increased during the rest of the seasons. The highest ratio was observed during winter, where the connective tissue reached its maximal amount (Figs. 10, 11).

The tunica muscularis was thinner at the dorsal aspect of the seminal vesicle, then it increased gradually in thickness along their course towards the ventral one (Fig. 2). It was arranged into two layers of variable thickness; an inner circular and an outer longitudinal smooth muscle fiber layers. Oblique bundles were commonly demonstrated in between these layers (Figs. 2,12).

The muscular layers supported by dense connective tissue layer, which formed of collagenous, reticular, and elastic fibers. Collagenous fibers were the most prominent type, which surrounded the muscular bundles (Figs. 2, 12C). Reticular fibers network was observed surrounding each muscle fiber separately (Fig. 12C). Elastic fibers were observed in large amount between the muscular bundles, although fine ones were also demonstrated around the muscular cells (Fig. 12D). Between these muscular layers, various sized blood vessels, and bundles of myelinated nerve fibers and fibroblasts were observed (Figs. 2, 12B).

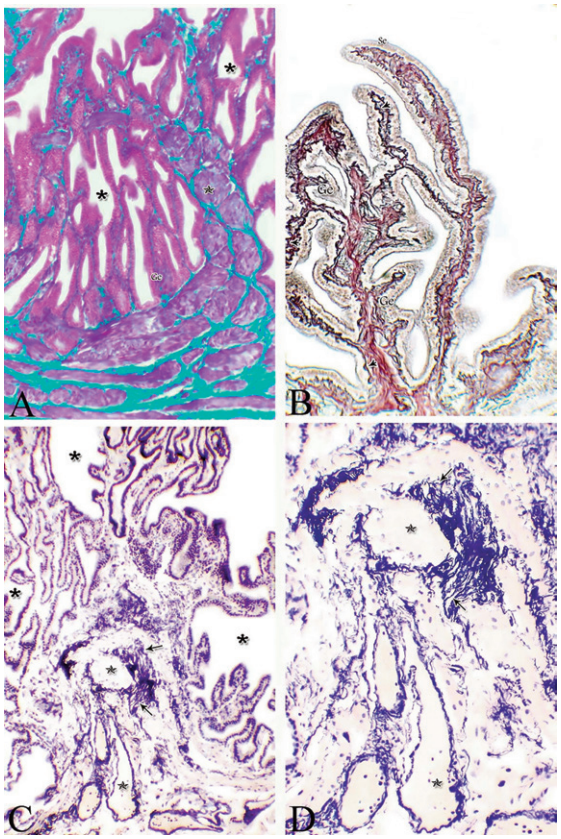


Fig. 9. Photomicrographs of the lamina propria of the seminal vesicle of the donkey during winter (A) and summer (B) showing collagenous fibers (green & brown colour) and reticular fibers network (arrowheads) supporting the surface (Se) as well as glandular epithelium (Ge) and elastic fibers (C& D, arrows) . Secretory end-pieces (asterisks), smooth muscle fiber bundles within the mucosal folds (stars). A: Crossmon´s trichrome stain. X 100, B: Gomori´s stain. X: 100. C& D: Verhoff´s stain. C: X 50 and D: X 400.

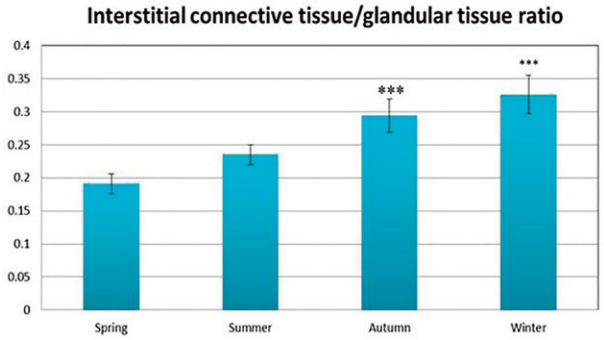
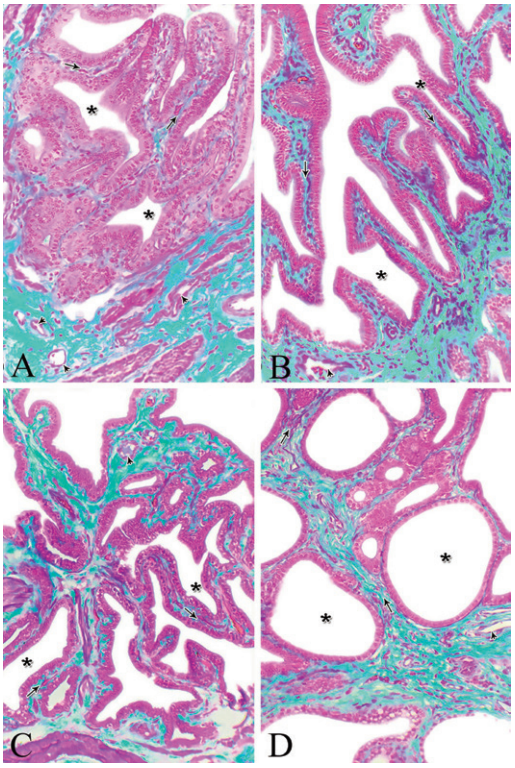


Fig. 10. Interstitial connective tissue/glandular tissue ratio showed highly significant (p<0.01) seasonal variations between spring, autumn and winter

The tunica serosa covered approximately the cranial two third of the seminal vesicle (Figs. 2, 13A-C) while the rest, the retroperitoneal part, was invested by tunica adventitia (Fig. 12D). Flat mesothelial cells of the tunica serosa covered a submesothelial connective tissue layer. The latter or the adventitia was formed of connective tissue layer containing collagenous and fine elastic fibers (Figs. 2,13B) in addition to fibroblasts, lymphocytes, blood vessels of variable caliber and bundles of myelinated nerve fibers as well as intramural ganglion (Figs. 13A, D).

The excretory duct of each seminal vesicle was larger than that of the deferent duct and located lateral to it. It possessed an irregular lumen with cyst-like invaginations, containing mostly acidophilic secretory materials and surrounded by highly vascular dense fibrous connective tissue layer (Figs. 14).



Figs. 11. Photomicrographs showing the variation in the amount of connective tissue supporting the glands (arrows) during spring (A), summer (B), autumn (C) and winter (D). Secretory portions (asterisks); blood vessels (arrowheads); collagen fibers (green colour). Crossmon´s trichrome stain. X 200.

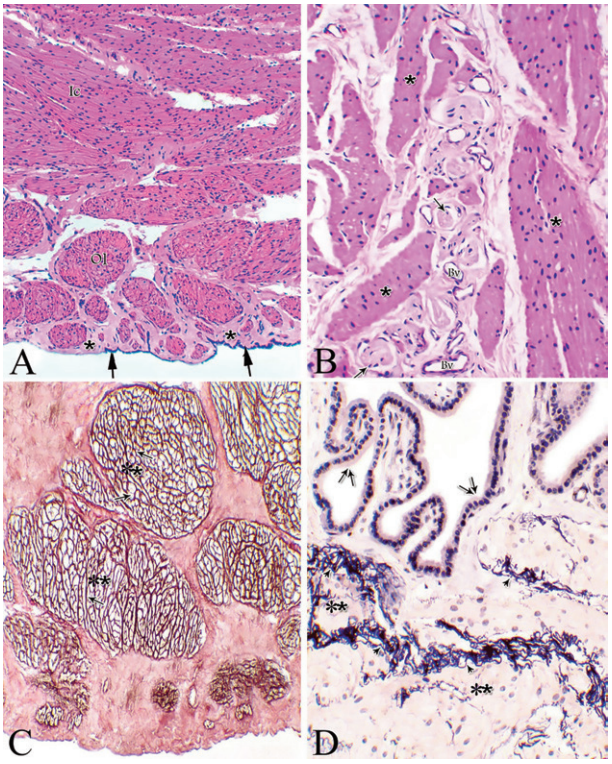


Fig. 12. Photomicrograph showing the tunica muscularis and serosa of the seminal vesicle of the donkey during summer. Inner circular (Ic), outer longitudinal (Ol), mesothelium (Thick arrows), submesothelial connective tissue (asterisk). Collagenous fibers (brown colour) and reticular fibers network (arrows) elastic fibers (arrowheads) muscular bundles (double asterisks), glandular epithelium (double arrows). Bundles of myelinated nerve fibers (thin arrows) and blood vessels (Bv) are present in between the muscular bundles (double asterisks) of the tunica muscularis of the seminal vesicle. A& B: Haematoxylin & eosin stain X200, C: Gomori´s stain and D: Verhoff´s stain. A& D: X100, B: X200 and D: X 400.

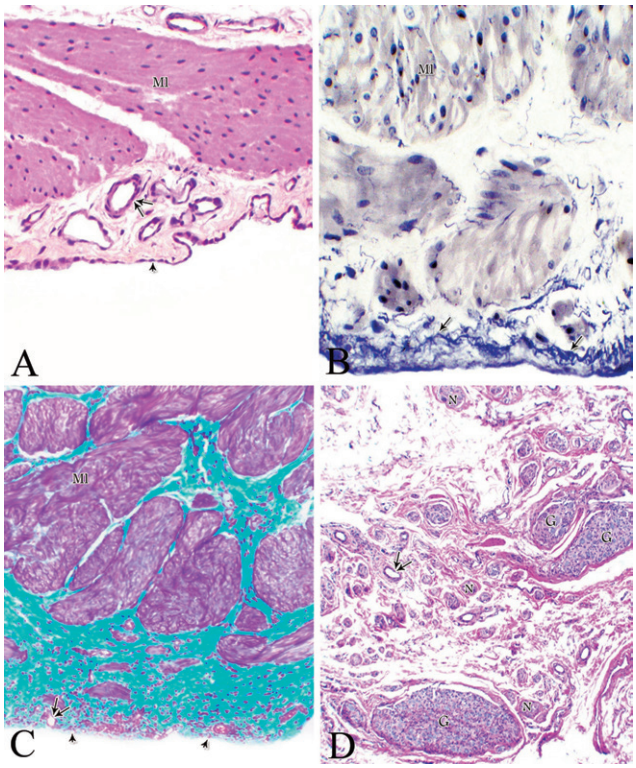


Fig. 13. Photomicrographs of the tunica serosa and adventitia of the seminal vesicle of the donkey during spring showing mesothelium (arrowhead), collagen fibers (green colour), elastic fibers (arrow), blood vessels (double arrow), intramural ganglia (G), bundles of myelinated nerve fibers (N) and muscular layer (Ml). A & D: Haematoxylin & eosin stain, B: Verhoff´s stain, C: Crossmon´s trichrome stain. A: X 200. B& C: X400, D: X50.

The epithelial lining of the excretory duct was formed firstly of bi-layered epithelium with patches of stratified columnar and cuboidal one. The bi-layered type consisted of superficial columnar or cuboidal cells with deeply stained acidophilic cytoplasm and somewhat oval nuclei. Its basal cells simulated nearly the basal cells, lined the glands of seminal vesicle. Also, cyst-like invaginations were observed simulating nearly that of the deferent duct, where they were lined with principal cuboidal cells, with lightly stained foamy acidophilic cytoplasm and rounded vesicular nuclei, as well as basal cells with flattened nuclei (Fig. 14).

Histochemical observations

Neutral mucopolysaccharides

During spring, the surface and glandular epithelium as well as the luminal content of the seminal vesicles of the donkey strongly reacted for PAS. This reaction was represented by diffuse homogenous PAS positive substance filling mostly the cell cytoplasm (Fig. 15A). Also the apical borders of these cells were strongly reacted for PAS. Some cells were moderately or even negatively reacted. Most of the clear cells contained small amount of weakly reacted PAS positive substance, while the luminal content which attached to their apical borders were strongly reacted (Fig. 15 inset).

During summer, the strong PAS positive reaction became mostly limited to the cell apical regions; although a PAS moderate reactivity was observed in some secretory portions (Fig. 15B). During autumn, weak PAS positive reactivity could be detected within most cells of the surface and glandular epithelium of the seminal vesicles. The apical borders of some cells were moderately or strongly reacted (Fig. 15C). During winter, the majority of the surface and glandular epithelium of the seminal vesicles were nearly negatively reacted for PAS

except the secretory substances related to their apical borders, which were strongly reacted. Some secretory portions were either weak or moderately reacted (Fig. 15D).

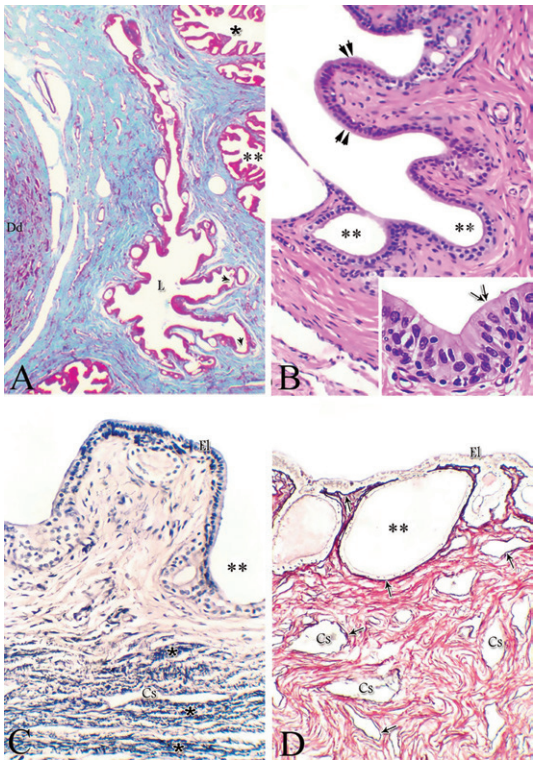


Fig. 14. A: Photomicrograph showing the right excretory duct of the seminal vesicle of the donkey with its irregular lumen (L) and cyst-like invaginations (arrowheads) during spring. Part of right deferent duct wall (Dd), parts of the prostatic ducts (asterisks). Collagen fibers (green colour). B& inset: bi-layered epithelium (double arrowheads), cyst-like invaginations (double asterisks) of the excretory duct of the seminal vesicle of the donkey during spring. Inset: the stratified columnar epithelium (double arrow) lining the duct at its termination. C & D: Elastic fibers in the outer portion of the connective tissue (asterisk), reticular fibers network (arrow) under the epithelial lining (El) and cavernous spaces (Cs) and collagen fibers (brown colour). A, Crossmon´s trichrome stain, X: 25. B: Haematoxylin & eosin stain, X: 200. Inset: X: 400. C: Verhoff´s stain: X: 200. D: Gomori´s stain. X: 200.

In all studied seasons of the year, the luminal contents were strongly PAS positive. While, the collagenous fibers of the lamina propria as well as the tunica muscularis and tunica adventitia showed moderate PAS reaction except the reticular fibers of the previous layers and those supporting the muscle fibers were strongly reacted (Fig. 15).

Glycogen

With Best's carmine stain, the surface and glandular epithelium, the connective tissue and muscular layers of the seminal vesicles of the donkey did not show any significant reaction for glycogen during the studied seasons (Fig. 16).

Acid mucopolysaccharides

After alcian blue staining, all layers forming the seminal vesicles of the donkey were negatively reacted during the four season of the year, except the luminal content and the secretory materials attached to the apical border of the principal cells of the surface and glandular epithelium which were strongly reacted. Most of the clear cells contained a small amount of moderately reacted alcianophilic granules, which were mostly attached to the cell membrane or distributed elsewhere in the cytoplasm (Fig. 17).

With alcian blue-PAS technique during all studied seasons of the year (Fig. 18), no differences could be observed concerning the pattern of distribution and the intensity of reaction beyond that demonstrated for PAS stain except for the luminal content and the secretory materials attached to the apical borders of the principal and clear cells, which revealed a mixed staining appearance for both stains. On the other hand, the clear cells themselves had a mixed staining picture, which was mostly alcianophilic. Some of the luminal content was either alcian blue or PAS positive.

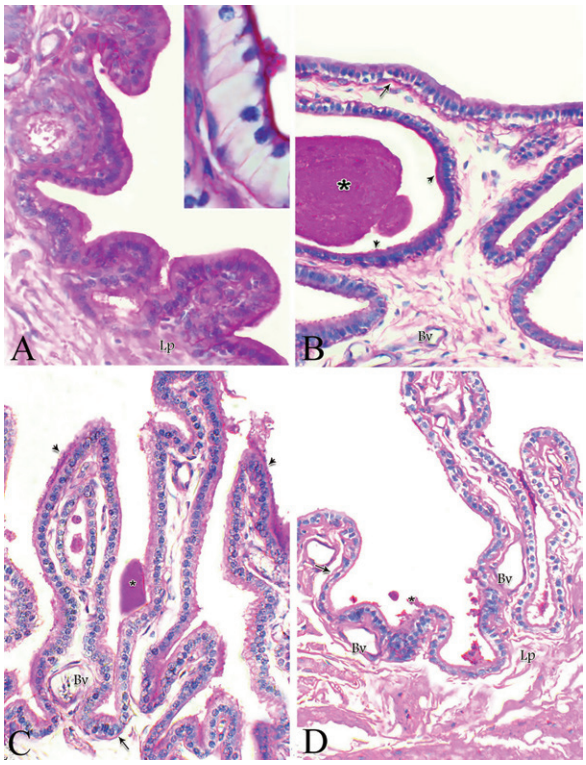


Fig.15. Photomicrographs showing the PAS reactivity within the glandular epithelium of the seminal vesicle of the donkey. During spring (A) diffuse homogenous PAS positive substance fill most of the cell cytoplasm. A weakly reacted PAS positive substance within the clear cells and strongly reacted luminal contents attached to their apical borders (inset). During summer (B) strong PAS positive reaction is detected in the cell apical regions and moderate PAS reactivity in some secretory portions (arrowhead). During autumn (C) weak reaction is observed within most cells and moderate reaction within few ones (arrowhead). During winter (D) most of the cells were negatively reacted except their apical borders. Strongly reacted luminal contents (asterisk). Moderately reacted collagenous fibers of the lamina propria (Lp) and blood vessels (Bv), strongly reacted reticular fibers (arrow). PAS / haematoxylin stain. X400.

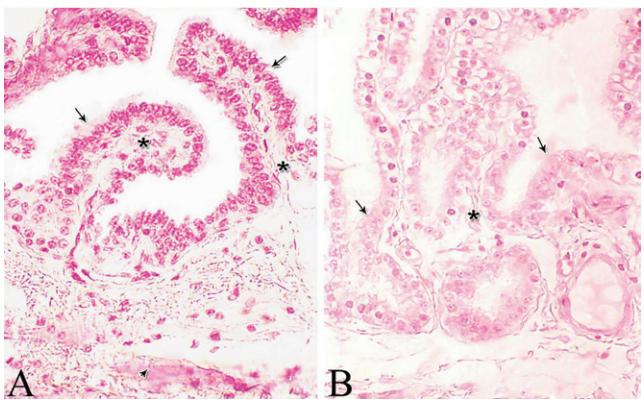


Fig. 16. Photomicrographs showing a negative Best's carmine reactivity within the glandular epithelium (arrow), connective tissue stroma (asterisk) as well as the smooth muscle fibers (arrowhead) of the seminal vesicle of the donkey during spring. Best's carmine stain. X: 400.

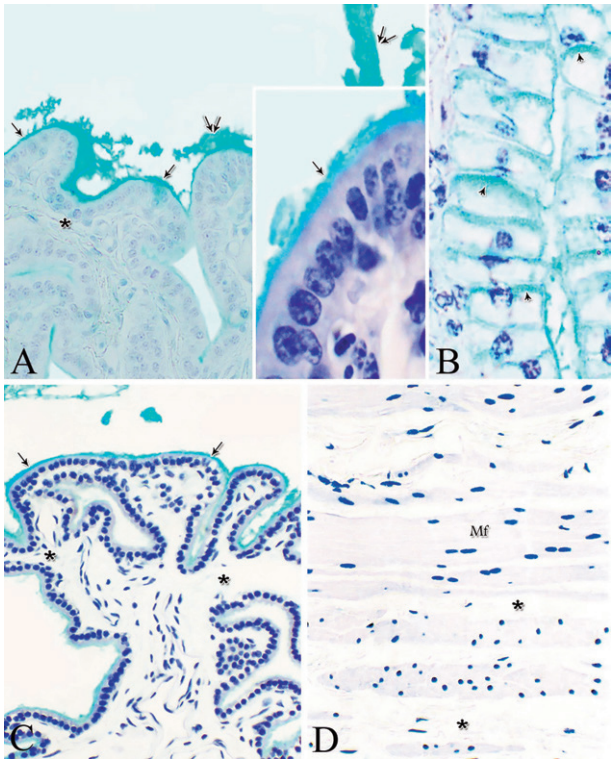


Fig. 17. Photomicrographs of the seminal vesicle of the donkey during spring (A, inset & B) and winter (C & D) showing strong alcian blue positive reaction in the secretory materials attached to the apical border of the principal cells (arrows) and luminal contents (double arrows). Few alcianophilic granules are observed within the clear cells (arrowheads). Negative reaction within the connective tissue (asterisks) of the lamina propria and inbetween the muscular bundles as well as the muscle fibers themselves (Mf). Alcian blue / haematoxlin stain A, C& D, X: 400; Inset & B: X: 1000.

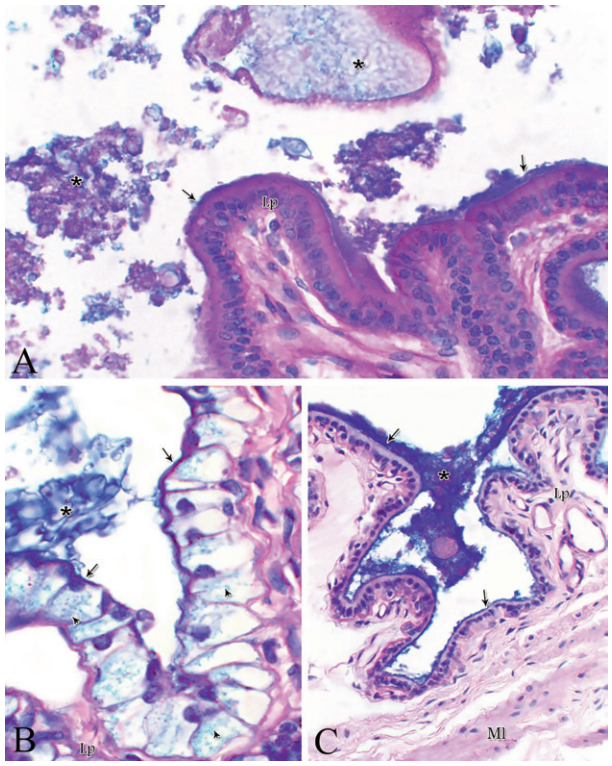


Fig.18. Photomicrographs of the glandular epithelium of the seminal vesicle of the donkey during spring showing mixed alcian blue / PAS staining in the secretory materials attached to the apical borders of the principal and clear cells (arrows) as well as in the luminal contents (asterisks). Clear cells with alcianophilic granules (arrowheads), Lamina propria (Lp), muscular layer (Ml). Alcian blue / PAS / haematoxylin stain. A& C: X 400, B: X1000

Discussion

The present investigation revealed that the donkey had true paired seminal vesicles. Each one located dorsolateral to the corresponding ampulla of the deferent duct and possessed a large central lumen. Similar results was recorded in horses (Banks, 1993) and men (Cormack, 1987; Fawcett and Jensh, 1997; Young and Heath, 2000).

Similar to that observed in the seminal vesicles of elephants (Short *et al*., 1967), common marmoset (Miraglia *et al*., 1970), men (Dym, 1983; Young and Heath, 2000) and rats (Yuri, 1990), the mucosa of the seminal vesicles of the donkey was highly folded. These folds carried many lateral secondary branches with numerous tubular invaginations into the underlying connective tissue.

In accordance with Ellery (1971) in stallion and Riva (1967) and Insler and Lunenfeld (1993), the glands of the seminal vesicle of the donkey were of the branched tubular variety. However, they were branched tubulo-alveolar in stallions (Eurell and Frappier, 2006), branched alveolar in bulls, compound tubulo-alveolar in buffalo bulls, (Fahmy and Osman, 1972; Moussa *et al*., 1983; Sudhakar *et al*., 1986), equines (Sisson, 1975) and rats (Yuri, 1990) and compound tubular in red deer (Aughey, 1969) and rams (Abbas, 1976).

In accordance with Riva (1967) and Insler and Lunenfeld (1993) in men, (Aughey, 1969) in red deer, (Bidwai and Bawa, 1972) in Indian hedgehogs, (Eurell and Frappier, 2006) in stallions, Wrobel (1969) in the seminal glands of boar, Wrobel (1970) in goats, (Moussa *et al*., 1983; Amselgruber and Feder, 1986; Abou-Elmagd and Kelany, 1992) in buffalo bulls and (Kamel *et al*., 1987) in lambs, the glandular epithelium of the seminal vesicles of the donkey were formed of principal and inconstant basal cells. However, that of the vesicular glands of goats (Yao and Eaton, 1954), elephants (Short *et al*., 1967), common marmoset (Miraglia *et al*., 1970), rats (Flickinger, 1974) as well as that of the seminal vesicles of men (Dym, 1983; Fawcett and Jensh, 1997; Young and Heath, 2000) was pseudostratified in nature. In stallions (Ellery, 1971) and equines (Sisson, 1975), the glandular epithelium of the seminal vesicles was predominantly simple columnar in type. Contrary to that observed by Kamel *et al*. (1987) and Abou-Elmagd and Kelany (1992) in the vesicular glands of lambs, buffalo bulls, respectively, the principal columnar cells of the seminal vesicles of the donkey showed no cytoplasmic protrusions. It was thus reasonable to assume that these cells released their secretory substances by merocrine mode as described by Marei *et al*. (2004) in goats. These cells were provided with brush border as that observed in the seminal vesicles of rats (Flickinger, 1974) as well as the seminal glands of goats (Gupta and Singh, 1982) and bovines (Agrawal and Vanha-Perttula, 1987).

The present study revealed that clear cells were observed among the principal ones of the seminal vesicles of the donkey, where some secretory end-pieces were completely lined with them. These cells were not previously described in the seminal vesicles or glands in the available literature. The epithelial lining of the seminal vesicles of elephants (Short *et al*., 1967), buffalo bulls (Fahmy and Osman, 1972) and rams (Abbas, 1976) contained intra-cytoplasmic vacuoles. In latter animal, the degree of vacuolation showed pronounced variations according to the secretory activity of the cells.

The present study showed marked seasonal variations in the secretory activities of the vesicular glands, which indicated by variation in the glandular epithelium height, the nuclear / cell ratio and the interstitial connective tissue / glandular tissue ratio. The maximal activity was observed during spring, where the epithelium reached its maximal height. The nuclear / cell ratio and the interstitial connective tissue / glandular tis-

sue ratio reached their lowest values and PAS reaction reached its maximal intensity. In winter the glands reached their minimal activity, where the epithelium reached its minimal height, while nuclear / cell ratio and the interstitial connective tissue/ glandular tissue reached their maximal values and the secretory cells became nearly PAS negative. In the other two seasons, the activity of the glands was fluctuated between spring and winter. Seasonal variations were observed in the seminal vesicles of red deer, stallions, Indian hedgehogs and seminal glands of bucks and rams.

In accordance with that observed in the seminal vesicles of red deer (Aughey, 1969) and men (Riva and Stockwell, 1969), as well as in the seminal glands of buffalo bulls (Moussa *et al*., 1983), the seminal vesicles of the donkey showed no significant reaction for glycogen. Gupta and Singh (1982) stated that the concentration of glycogen decreased in concentration at 20 days post castration in goats.

Similar to the current study, the seminal vesicle of stallions, showed different activity during the year, where the glandular epithelium reached its maximal height in June specimens and the cell cytoplasm appeared to be engorged, causing the free border of the cells to bulge towards the lumen. In March, the height of the glandular epithelium was lower. However, in September and December, the height of the glandular epithelium was much lower and the cytoplasm of the cells did not bulge towards the free border (Ellery, 1971). From the before mentioned parameter, Ellery (1971) observed that the highest activity of these glands in stallions was detected in June, which corresponds to the high activity during spring season in the present study. In red deer, Aughey (1969) found that during August (pre-rut), the connective tissue septa were particularly prominent. The glandular tubules were lined with short columnar cells and numerous basal cells. While, in October (rutting period), there was a marked increase in the amount of the glandular tissue, which was accompanied by a corresponding decrease in the interglandular tissue and the columnar cells became taller with a more uneven surface. These seasonal changes were considered to be due to the endocrine status of the interstitial cells of the testis. The interstitial cells showed a fibroblast appearance in the pre-rut and highly reactive cells in the rut. Also, Bidwai and Bawa (1972) stated that the Indian hedgehog is sexually active between late April and late September; thereafter, its testes and associated sex glands abruptly retrogressed and remained quiescent throughout winter. They added that this animal showed regression of the seminal vesicles in the non-breeding season when the secretory activity at its lowest ebb. By contrast, at the beginning of the breeding season, the epithelial cells of these glands showed intense secretory activity.

The before mentioned seasonal variations of the activity of the seminal vesicles were considered to be in accordance to the activity of the interstitial cells of testes, because this gland is androgen-dependant. Short and Mann (1966) reported that the inactive testis of non-rut roebuck possessed small interstitial cells with low testosterone, while the active testis of the rut possessed eosinophilic interstitial cells with high testosterone level.

A Network of elastic fibers was observed in the lamina propria, which accompanied the smooth muscle bundles and extended into the primary mucosal folds. Similar results were observed in the seminal vesicles of stallions (Eurell and Frappier, 2006). It could be accepted that, smooth muscle fibers of the interstitial tissue during their contraction help in delivering the secretion into the pelvic urethra at the time of ejaculation (Nickel *et al*., 1973). Contraction and relaxation of the smooth muscle fibers may help in the lengthening and shortening of these mucosal folds with the subsequent change in their luminal diameters according to the secretory state of the seminal vesicle. Also the presence of a network of elastic fibers around these smooth muscle fibers gives these folds more flexibility during tension.

The muscular layer was formed of inner circular and outer longitudinal smooth muscle fibers with some oblique bundles. This agrees with that observed in the seminal vesicles of red deer (Aughey, 1969) and men (Insler and Lunenfeld, 1993). In common marmoset (Miraglia *et al*., 1970), stallions (Ellery, 1971; Banks, 1993) and men (Dym, 1983; Fawcett and Jensh, 1997; Young and Heath, 2000), the tunica muscularis was made only of inner circular and outer longitudinal layers. The latter authors added that this muscular layer was supplied by sympathetic nerves; during ejaculation, muscular contraction also forced secretion from the seminal vesicles into the urethra.

In accordance with Aughey (1969); Riva and Stockwell (1969) and Bidwai and Bawa (1972) in the seminal vesicles of red deer, men, Indian hedgehogs, respectively as well as in the seminal glands of goats (Gupta and Singh, 1982; Marei *et al*., 2004) and lambs (Kamel *et al*., 1987), the glandular epithelium of the seminal vesicles of the donkey showed PAS positive reaction, indicating the presence of neutral mucopolysaccharides. This result disagrees with that observed in the vesicular glands of buffalo bulls (Moussa *et al*., 1983).

The seminal vesicles of the donkey were negatively reacted for alcian blue, except the luminal content and the secretory materials attached to the apical border of the surface and glandular epithelium, which were strongly reacted. Most of the clear cells contained a small amount of alcianophilic substance indicating that these cells may secrete few acid mucopolysaccharides. The presence of alcianophilic materials within the lumen of the seminal vesicle may come from the secretion of the clear cells. The seminal vesicles of men (Riva and Stockwell, 1969) as well as the seminal glands of rams (Abbas, 1976), goats (Gupta and Singh, 1982) and buffalo bulls (Moussa *et al*., 1983), showed no alcian blue reactivity. In red deer, the reaction was confined only to some cells lining the large excretory duct and the secretion within the duct lumen (Aughey, 1969).

Conclusion

The seminal vesicles are paired sacs located dorsolateral to their corresponding ampulla ductus deferens. The mucosa of the seminal vesicles is highly folded and the lamina epithelialis is formed of principal and basal cells which they showed different activity during the different seasons. The seminal vesicles showed their maximal activity during spring which is manifested by increased the epithelial height, the secretory activity of the epithelium and decrease the nuclear/ cell ratio and intestinal/ glandular tissue ratio. This activity decreased during other seasons of the year tom reach minimal activity during winter.

Conflict of interest

The authors declare no conflict of interest exist.

References

Abbas, A.A., 1976. Histological and histochemical studies of the accessory genital glands of the balady ram with special reference to age and seasonal variations. M. V. Sc. Thesis, Fac. Vet. Med., Zagazig University, Egypt.

Abou-Elhamd, A., 2005. Morphological studies on the accessory genital glands of donkey "*Equus asinus*" in different seasons. MSc, Assiut University, Egypt.

Abou-Elhamd, A.S., AbdelRahman, Y., Selim, A., 2019. Pelvic Urethra and its Associated Glands in Donkey (*Equus asinus*): Histolog-

ical and Histochemical Findings with Special Reference to their Seasonal Variations. Journal of Advanced Veterinary Research 9, 134-143.

Abou-Elhamd, A.S., Salem, A.O., Selim, A.A., 2012. Histological and Histochemical Studies on the Ampulla of the Deferent Duct of Donkey (*Equus asinus*). Journal of Advanced Veterinary Research 2, 261-270.

Abou-Elhamd, A.S., Salem, A.O., Selim, A.A., 2013. Histomorphological Studies on the Prostate Gland of Donkey Equus Asinus during Different Seasons. Journal of Histology 2013, 1-19.

Abou-Elmagd, A., Kelany, A.M., 1992. Electron microscopical observation on the active vesicular glands of buffalo. Assiut Vet. Med. J. 28, 68-80.

Agrawal, Y., Vanha-Perttula, T., 1987. Effect of secretory particles in bovine seminal vesicle secretion on sperm motility and acrosome reaction. J. Reprod. Fertil. 79, 409-19.

Amselgruber, W., Feder, F.H., 1986. Light and electron microscopic studies of the seminal vesicular gland (Glandula vesicularis) of the bull. Anat. Histol. Embryol. 15, 361-79.

Aughey, E., 1969. Histology and histochemistry of the male accessory glands of the red deer, Cervus elaphus L. J. Reprod. Fertil. 18, 399-407.

Banks, W.J., 1993. Male reproductive system. St. Louis. Baltimore. Boston. Chicago. London. Philadelphia. Sydney. Toronto: Mosby Year book.

Bidwai, P.P., Bawa, S.R., 1972. Histochemical study of the seminal vesicle secretion of the Indian hedgehog with particular reference to the crystalloid bodies. J. Reprod. Fertil. 30, 219-23.

Cormack, D.H., 1987. The male reproductive system. In Ham´s Histology. 9th ed. J. B. Lippincott. Com. philadelphia.

Dym, M., 1983. The male reproductive system. In histology, cell and tissue biology. 5th ed. Edited by Weiss, L. The Mac Millan Press.

Ellery, J.C., 1971. Spermatogenesis, accessory sex gland, histology and the effects of seasonal change in the stallion. Ph D. Thesis. Fac. Graduate School, University Minnesota.

Eurell, J.A., Frappier, B.L., 2006. Male reproductive system. In Dellman's textbook of veterinary histology. 6th edn. State Avenue, Ames, Iowa, USA: Blackwell Publishing.

Fahmy, M.F.A., Osman, A.M.H., 1972. Certain morphological investigations on the male genital organs of water buffalo as guide for andrological diagnosis. IV. Accessory glands. Egypt. J. Vet. Sci. 9, 56-65.

Fawcett, D.M., Jensh, R.P., 1997. Concise histology. 1st ed. Chapman & Hall. International Thomson publishing. New York. Albany. Bonn. Boston. Cincinnati. Detroit. London. Madrid. Melbourne. Mexico city. Pacific Grove. Paris. San Francisco. Singapore. Tokyo.Toronto. Washington.

Flickinger, C.J., 1974. Synthesis, intracellular transport, and release of secretory protein in the seminal vesicle of the rat, as studied by electron microscope radioautography. Anat. Rec. 180, 407-25.

Gupta, A.N., Singh, Y., 1982. Effect of castration on the cytology of the vesicular glands in goats. Indian J. Anim. Sci. 52, 72-79.

Inns, R.W., 1982. Seasonal changes in the accessory reproductive system and plasma testosterone levels of the male tammar wallaby, Macropus eugenii, in the wild. J. Reprod. Fertil. 66, 675-80.

Insler, V., Lunenfeld, B., 1993. Functional anatomy of the male accessory organs. In infertility; male and female. 2nd ed. P. Churchill Livingstone. Edinburgh. London. Madrid. Melbourne. New York and Tokyo.

Kamel, G., Hassan, A.H.S., El-Hommosi, F., Mostafa, N.K., 1987. Quantitative morphological study on the seminal gland of lambs after zeranol implantation. Assiut Vet. Med. J. 18, 51-59.

Liebich, H.K.H.-G., Bragulla, H., Budras, K., Cerveny, C., Konig, H., Liebich, H., Moierl, J., Mulling, C., Reese, S., Ruberte, J., 2004. Veterinary Anatomy Of Domestic Mammals-Textbook And Colour Atlas.

Marei, H.E.S., El-Habback, H.A., Abou-Esa, K.F., 2004. Histology, fine structure and carbohydrate histochmistry of the epithelium of the accessory male genital glands of the Egyptian balady goat. Vet. Med. J. Giza. 52, 165-182.

Marieb, E.N., Hoehn, K., 2003. The reproductive system. Human Anatomy and Physiology, 1056-1061.

McManus, J., 1946. Histological demonstration of mucin after periodic acid. Nature 158, 202-202.

Miraglia, T., Telles Filho, M., Branco, A.L., 1970. The male reproductive system of the common marmoset (*Callithrix jacchus*). Acta Anat (Basel) 76, 594-611.

Moussa, M.H., Badawy, A.B., Kandil, M.H., Shahin, Y.M., 1983. Histological and histochemical studies of the accessory genital glands of buffalo-bull (*Bubalis bubalis*). Anat. Anz. 153, 429-39.

Nickel, R., Schummer, A., Seiferle, E., 1973. Male genital organs. In the viscera of the domestic mammals. 1st ed. Translated and revised by Sack W.O. Verlag Paul Parey Berlin. Hamburg.

Noda, T., Ikawa, M., 2019. Physiological function of seminal vesicle secretions on male fecundity. Reproductive Medicine and Biology 18, 241-246.

Ricardo, L.H.J., 2018. Male Accessory Glands and Sperm Function. Spermatozoa: Facts and Perspectives, 101.

Riva, A., 1967. Fine structure of human seminal vesicle epithelium. J. Anat. 102, 71-86.

Riva, A., Stockwell, R.A., 1969. A histochemical study of human seminal vesicle epithelium. J. Anat. 104, 253-62.

Selim, A., A., 1974. Histological and histochemical studies on the accessory genital glands of balady buck with special references to seasonal changes. M.V.Sc. , Assiut University, Egypt.

Short, R.V., Mann, T., 1966. The sexual cycle of a seasonally breeding mammal,the roebuck (*Capreolus capreolus*). J. Reprod. Fertil. 12, 337-51.

Short, R.V., Mann, T., Hay, M.F., 1967. Male reproductive organs of the African elephant, Loxodonta africana. J. Reprod. Fertil. 13, 517-36.

Sisson, S., 1975. Urogenital system. In Sisson, S and Grossman, J. D. The anatomy of domestic animals. Rev. by Getty, R. Vol. I. 5th ed. W.B. Saunders C. Philadelphia. London. Toronto.

Skonieczna, J., Madej, J.P., Będziński, R., 2019. Accessory genital glands in the New Zealand White rabbit: a morphometrical and histological study. Journal of Veterinary Research 63, 251.

Sudhakar, L.S., Dhingra, L.D., Sharma, D.N., 1986. Postnatal histomorphology of the vesicular gland in Murrah buffalo bull. Indian, J. Anim. Sci. 56, 866-869.

Suri, S., Sudhakar, L.S., Bhardwaj, R.L., 2008. Seasonal variation in the histomorphology and histochemistry of ampulla of the vas deferens of Gaddi goat and Gaddi sheep. Int. J. Morphol. 26, 131-136.

Suvarna, K.S., Layton, C., Bancroft, J.D., 2018. Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sciences.

Wrobel, K.H., 1969. The fine structure of the epithelium of the porcine seminal vesicle. Zentralbl Veterinarmed A 16, 400-415.

Wrobel, K.H., 1970. Morphology of the epithelium of the seminal vesicle in the goat. Zentralbl Veterinarmed A 17, 634-643.

Yao, T.S., Eaton, O.N., 1954. Postnatal growth and histological development of the reproductive organs sin male goats. American Journal of Anatomy, 95, 401– 431.

Young, B., Heath, J.W., 2000. Male reproductive system. In Wheater´s Functional Histology. 4th ed. . Edinburgh. London. New York. Philadephia. St. Louis. Sydney. Toronto.

Youssef, H.I.A., Kandil, H.H., Mosallam, S.M., 1984. Seasonal changes in the bulbourethral gland of one humped camel. J. Egypt. Vet. Med. Assoc. 44, 101-110.

Yuri, K., 1990. Immunohistochemical and enzyme histochemical localization of peptidergic, aminergic and cholinergic nerve fibers in the rat seminal vesicle. J. Urol. 143, 194-8.