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Histological and Morphometrical Studies on the Ampulla of the Deferent Duct of Donkey (*Equus Asinus*) in Different Seasons

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Abstract

The objective of this study is to describe the histological and histochemical structure of the ampulla ductus defrentis during different seasons of the year. The experiment was carried out 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. Both the lamina epithelialis and the epithelium lining the ampullary glands of the donkey showed highly significant ($P < 0.01$) seasonal variations. The interstitial tissue/ glandular tissue ratio of the ampullary glands showed as seasonal variations. The glandular epithelium of the ampullary glands of donkey had strong PAS positive reaction. This reaction decreased gradually during summer and autumn to reach its minimal amount during winter. In conclusions, the ampullary glands of donkey appeared more active during spring and this activity decreased gradually during the summer and autumn to reach it lowest activity during winter.

Keywords: Ampulla ductus deferentis; Donkey; Seasonal variations

Introduction

The accessory genital glands are include the ampullary, vesicular, prostate, bulbourethral and urethral glands (Banks, 1993; Davies Morel, 2003). They are a series of glands situated between the vas deferens and the root of the penis. These glands are responsible for the secretion of the seminal plasma, which provides the substrate for conveying the sperm to the female and ensuring final maturation.

The accessory genital glands of donkey also are formed of ampulla ductus deferentis, seminal vesicles, prostate gland, bulbourethral glands and urethral glands. The wall of the ampulla of the deferent duct was formed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa or adventitia. The lamina epithelialis was formed of two types of cells; principal columnar and basal cells (Abou-Elhamd *et al*., 2012).

In a previous report (Abou-Elhamd, *et al*., 2012) we discussed the histological and histochemical structure of the ampulla ductus defrentis of donkey. Therefore the present investigation was performed to describe the effect of seasons on the histological and histochemical features of this gland. This is to provide a basic knowledge on it for both academics and reproductive reasons as well as to compare it with the accessory genital glands of the other mammals.

Materials and methods

After Abou-Elhamd *et al*. (2012), 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. Their age ranged between 5 to 7 years.

The animals were anesthetized and then thoroughly bled to death from the common carotid artery. The 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

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formaldehyde, Bouin´s fluid (for routine histological and morphometerical examination) and cold formol-calcium (for lipids).

The fixed specimens from the ampulla of the deferent duct were further 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) and Periodic acid-schiff, PAS (Mc, 1946).

For demonstration of lipids, Sudan black staining after Bayliss and Adams (1972), we used the accessory genital glands of another 2 jacks each season.

The morphometrical studies were performed on the stained histological sections of the glands under investigation using Leica Q 500 MC image analyser. The measurements were carried out on each gland of the 6 jacks in all seasons in the following manner: the height of the surface and the glandular epithelium, the nuclear / cell ratio of the principal cells of the surface and glandular epithelium 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. All the data were presented as means + SE, which were statistically analysed using SPSS (one way ANOVA and Scheffe tests).

Results

Histological observations

The height of the lamina epithelialis of the ampulla of the deferent duct of the donkey revealed highly significant (P<0.01) seasonal variations. In spring, the epithelium reached its maximal height (20.10 µm + 0.363). A gradual decrease in the epithelial height was observed during summer (about 17.98 µm + 0.979) and autumn (about 16.76 µm + 0.404), The lowest epithelial height (about 15.92 µm + 0.354) was recorded during winter (Figs. 1, 2). In accordance, the nuclear / cell ratio of the principal cells of the lamina epithelialis of the ampulla of the deferent duct also revealed highly significant (P<0.01) seasonal variations. It reached the lowest value (about 0.271 + 0.004) during spring. A gradual increase in this ratio was recorded during summer (about 0.276 + 0.009) and autumn (about 0.278 + 0.008), while the highest ratio (about 0.314 + 0.003) was measured during winter (Fig. 3).

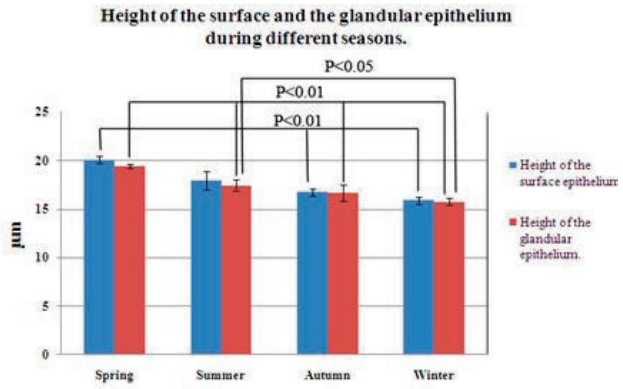

Fig. 1. The height of the surface and the glandular epithelium of the ampulla ductus deferentis showed significant seasonal variations.

The height of the glandular epithelium of the ampullary glands showed highly significant (P<0.01) seasonal variations. It reached its maximum during spring (about 19.44 um + 0.209). It decreased gradually to about 17.48 um + 0.545 and 16.74 um + 0.845 at summer and autumn, respectively. The lowest measurement (about 15.77 um + 0.384) was recorded in winter (Figs. 1, 4).

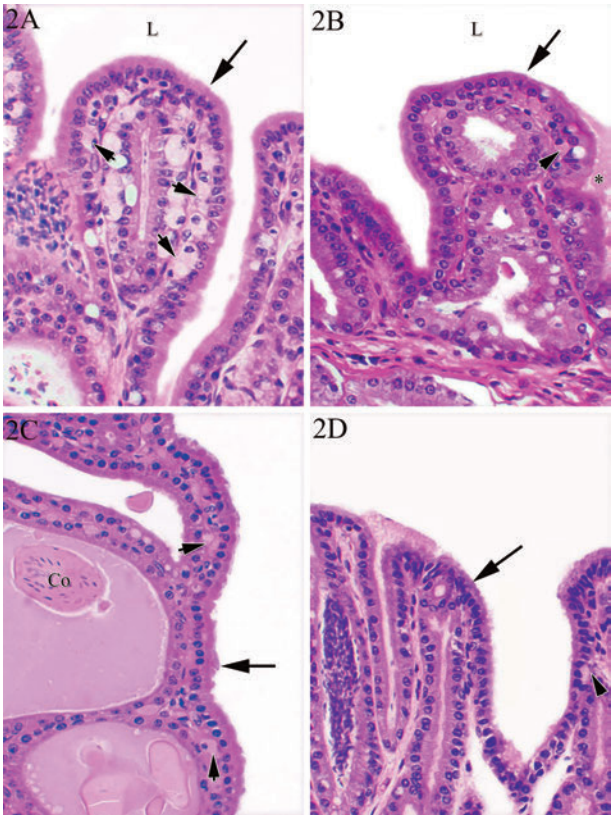

Fig. 2 (A- D). Photomicrographs showing the variation in the height of the lamina epithelialis (arrow) of the ampulla of the deferent duct of the donkey during spring (2A), summer (2B), autumn (2C) and winter (2D). Lumen (L), basal cells (arrowhead), concerates (Co), Secretory material (asterisk). Haematoxylin & eosin stain. X400.

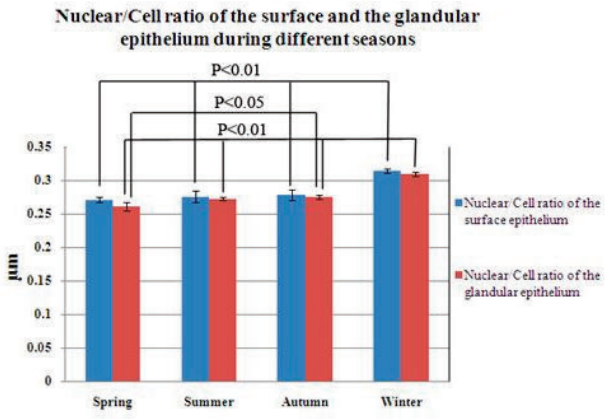

Fig. 3. The nuclear/ cell ratio of the surface and the glandular epithelium of the ampulla ductus deferentis showing significant seasonal variations.

The nuclear / cell ratio of the principal cells of the glandular epithelium of the ampullary glands also revealed highly significant (P<0.01) seasonal variations. The lowest ratio (about 0.261 + 0.006) was recorded during spring. This ratio increased gradually during the other seasons of the year. During summer, it was about 0.273 + 0.003 while during autumn it reached about 0.275 + 0.003. The highest ratio (about 0.310 + 0.003) was recorded during winter (Fig. 3).

The interstitial connective tissue / glandular tissue ratio of the ampulla ductus deferentis of donkey varied among the studied seasons (P<0.01). The lowest ratio (0.066 + 0.006) was observed during spring. This ratio showed a gradual increase during the rest of the year, where it reached about 0.103 + 0.014 during summer and 0.147 + 0.006 during autumn. However, the highest ratio (about 0.175 + 0.017) was recorded during winter (Figs. 5, 6).

Histochemical observations

Carbohydrate histochemistry

Neutral mucopolysaccharides

During spring, strong PAS positive reactions were observed within the majority of the principal cells of the lamina epithelialis and the glandular epithelium of the ampulla of the deferent duct (Fig. 7). 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. Others were moderately or even negatively reacted.

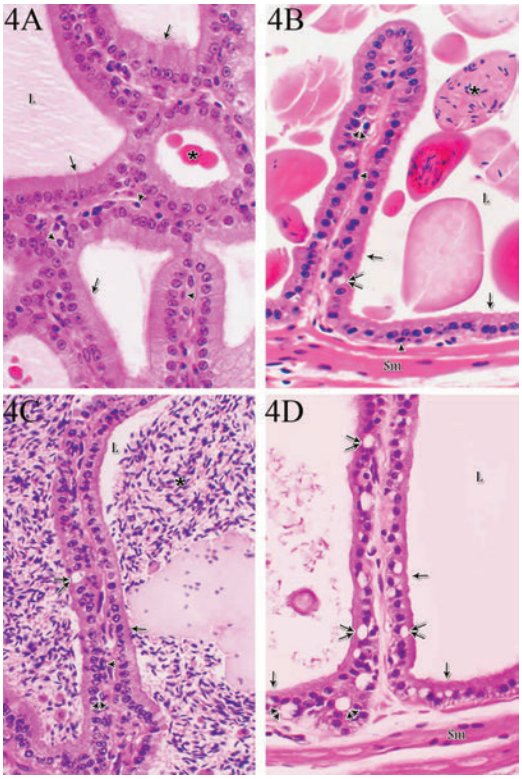

Fig. 4 (A-D). The epithelial lining of the ampullary glands showed seasonal variations. It reached its maximal height during spring (4A), which is decreased gradually during summer (4B), autumn (4C) to reach its minimal value during winter (4D). Principal cells (arrow), which may contain vacuoles (double arrow); basal cells (arrowhead), which may be vacuolated (double arrowhead). Smooth muscle fiber (Sm), lumina of the secretory end-pieces (L) containing spermatozoa, secretory materials and concretes (asterisk). Haematoxylin & eosin stain. (X 400).

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. 7A-B). The bleb-like apical protrusions of the principal cells were strongly PAS positive (Fig. 7C).

The glandular epithelium of some secretory portions showed nearly the same PAS positive reactivity of the surface epithelium although some of them were weakly or evenly negatively reacted (Figs. 7B).

During summer and autumn, differences in the distribution of the PAS positive reactivity in the surface and glandular epithelium could not be detected beyond that recorded in spring but the inten-

sity of the PAS reaction was somewhat less especially during autumn (Figs. 8A, 8B).

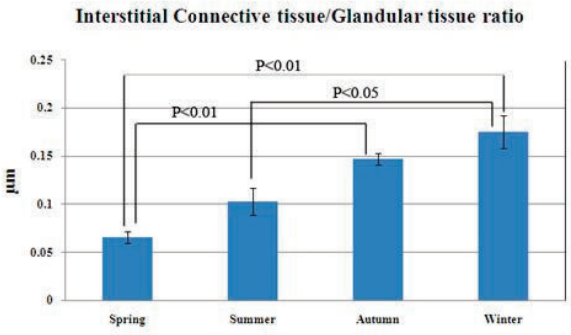

Fig. 5. The intersitial connective tissue/ Glandular tissue ratio of the ampulla ductus deferentis showing significant seasonal variations.

During winter, the lamina epithelialis and the glandular epithelium revealed few fine PAS positive granules, which scattered elsewhere in the cytoplasm of some principal cells. The majority of the principal cells were weakly or negatively reacted. The intensity of PAS positive reaction within the basal cells was somewhat lower than that during spring (Fig. 8C-D).

During all the studied seasons of the year, all the connective tissue elements of the lamina propria-submucosa and the smooth muscle fibers were weakly reacted with PAS, while their supporting reticular fibers were strongly PAS positive (Fig. 8C-D).

Acid mucopolysaccharides

Nevertheless the luminal content and the secretory materials attached to the principal cells of the epithelial lining the ampulla ductus deferentis of donkey, all the layers of the ampulla ductus defernts of donkey were negatively reacted. This positive reactivity showed no seasonal variations (Data not shown).

Lipids

During spring, some principal cells of the lamina epithelialis showed few fine sudanophilic granules, which were distributed in their cytoplasm. Other cells were negatively reacted. Some basal cells contained globular sudanophilic mass of variable sizes. The larger one occupied mostly the whole cell. The sudanophilia of the glandular epithelium of the secretory portions of the ampullary glands was nearly the same as that of the surface epithelium (Figs. 9A -B).

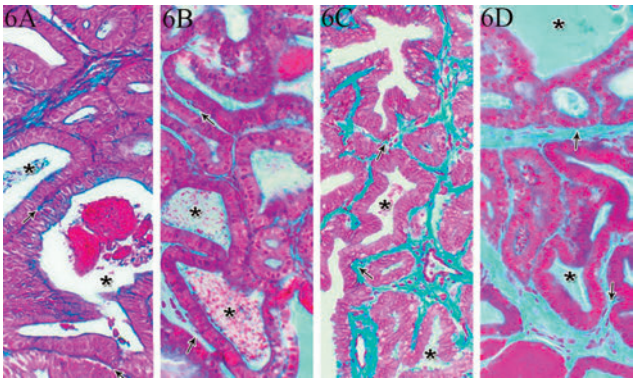

Fig. 6 (A-D). Photomicrographs showing the variation in the amount of the interstitial connective tissue (arrow) inbetween the ampullary glands (asterisk) during spring (6A), summer (6B), autumn (6C) and winter (6D). Crossmon´s trichrome stain. (X200).

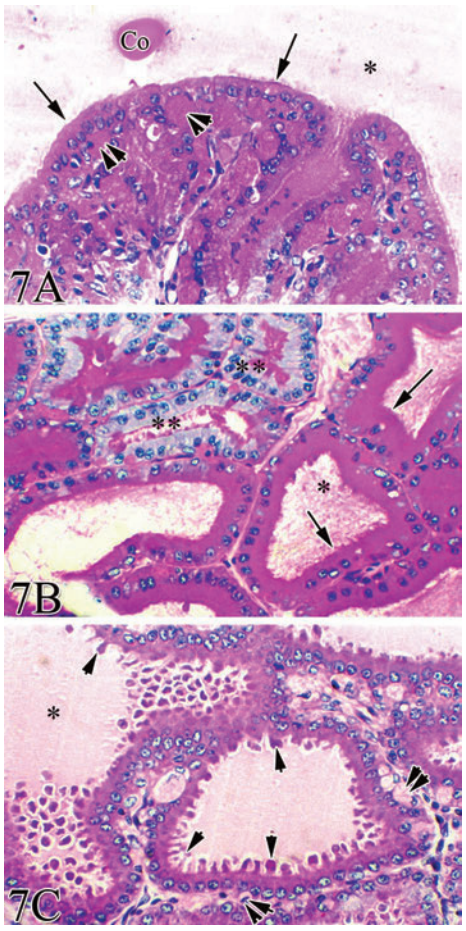

Fig. 7. Photomicrographs showing strong diffuse PAS reactivity in the surface (7A) and glandular epithelium (7B- 7C) of the ampulla of the deferent duct of the donkey during spring. Strong diffuse PAS reactivity within the principal cells with the brush border (arrow) or with bleb-like apical protrusions (arrowhead); basal cells (double arrowhead), negatively reacted secretory end-pieces (double stars), secretory materials (asterisk), concretes (Co). PAS / haematoxylin stain. (X400).

During summer, the intensity and pattern of distribution of the sudanophilic materials within the surface and glandular epithelium of the ampulla of the deferent duct increased than those observed during spring. A further increase in these pictures was recorded during autumn. While during winter, the sudanophilia in the surface and glandular epithelium (Fig. 9A -B) reached its maximal intensity and distribution.

The luminal content, the lamina propria-submucosa, the muscular layer and the tunica serosa or adventitia was negatively reacted for Sudan stain (Fig. 9A-D).

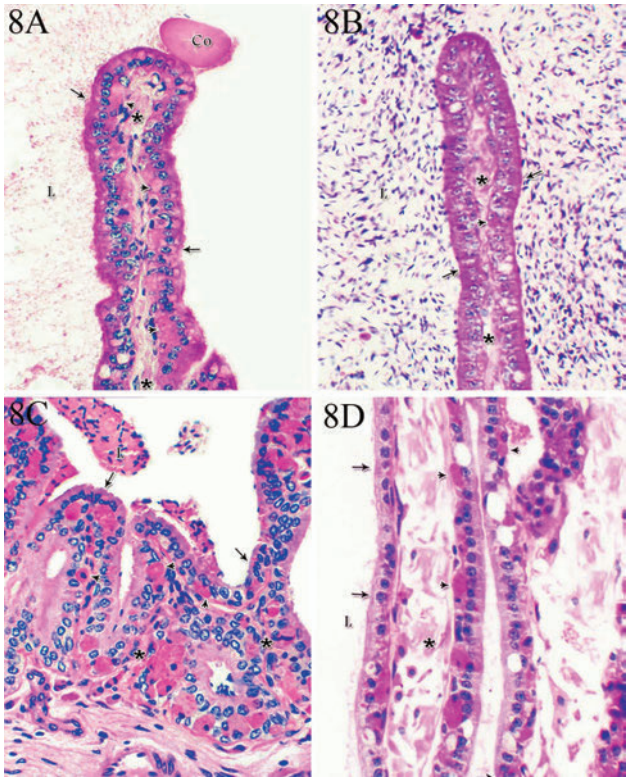

Fig. 8 (A- D). PAS reactivity in the glandular epithelium of the ampulla of the deferent duct during summer (8A) and autumn (8B) was less intense than that observed during spring (7B). Photomicrographs showing During winter, the PAS reactivity in the surface (8C) and glandular epithelium (8D) of the ampulla of the deferent duct of the donkey was less than observed in the other seasons. Principal cells (arrow), basal cells (arrowhead), weakly reacted connective tissue fibers (asterisk), lumen (L) contain secretory material, spermatozoa and concretes (Co), lumen (L) contains secretory materials and spermatozoa. PAS / haematoxylin stain. (X400).

Discussion

In this report we revealed that the ampulla ductus deferentis of donkey showed different activity during different seasons of the year. This manifested by seasonal variations in the morphometrical parameters of the surface and glandular epithelium, connective tissue glandular tissue ratio and in the PAS reactivity of the ampulla ductus deferentis.

The lamina epithelialis of the ampulla of the deferent duct of the donkey showed seasonal variations. Similar result was observed in the ampulla of the deferent duct of camels, where the epithelial lining was seen packed with granules during spring and reduce granulation in the lining cells was observed during summer (Mosallam, 1981).

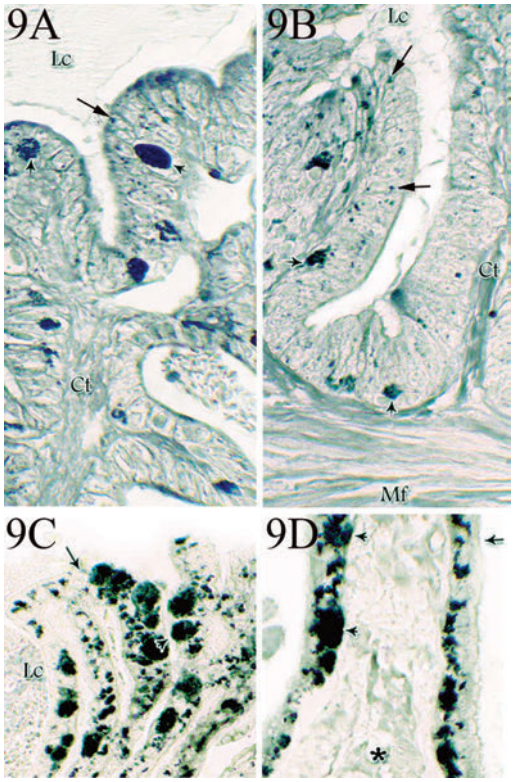

Fig. 9 (A-D). Photomicrographs of the surface epithelium (9A) and glandular epithelium (9B) of the ampulla of the deferent duct of the donkey during spring showing few sudanophilic granules within the principal cells (arrow) and globules within the basal cells (arrowhead). During winter, sudanophilic granules were abundant within the principal cells (arrow) and sudanophilic globules were increased within the basal cells (arrowhead) of the lamina epithelialis (9C) and glandular epithelium (9D). Connective tissue fibers (asterisk) as well as the luminal contents (Lc) were negatively reacted. Luminal contents (Lc), the connective tissue fibers (Ct) as well as the muscle fibers (Mf) were negatively reacted. Sudan black stain. X400.

Seasonal variations in the ampulla of the deferent duct of the donkey were observed during the different seasons. Maximal activity was observed during spring. During this season, the lining epithelium of the surface and glandular epithelium were reached their maximal height and revealed numer-

ous apical protrusions and their cytoplasm appeared less vacuolated. During winter, the ampullary gland reached its lowest activity, which was manifested by an increase in the intra-cytoplasmic vacuoles and the apical protrusions were few. In summer and autumn, the picture was inbetween that of spring and winter. Seasonal variations have also been observed in the ampulla of the deferent duct of stallions (Ellery, 1971), bucks (Selim, 1974), rams (Abbas, 1976) camels (Mosallam, 1981) and Gaddi goat and Gaddi sheep (Suri *et al*., 2008) during different seasons of the year.

The PAS reactivity within the ampullary glands of the donkey showed seasonal variations. During spring, most of the glandular cells, the apical protrusions as well as luminal contents were strongly PAS positive. During winter, few PAS positive granules were observed in some glandular cells, while most of them negatively reacted. During summer and autumn, there was no difference in the distribution of PAS positive reactivity in the surface and glandular epithelium compared to spring, but the intensity of the reaction was somewhat less especially during autumn. Also seasonal variations with PAS technique were observed in the ampulla ductus deferentis of red deer, bucks and camels (Aughey, 1969; Selim, 1974; Mosallam, 1981). 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 glandular epithelium of the ampulla of the deferent duct of the donkey showed Sudan black positive reaction, and seasonal variations were observed. During spring, the sudanophilic materials within the surface and glandular epithelium were few, which increased during summer and autumn, while during winter; the sudanophilia reached its maximal intensity and distribution. A supporting evidence for the beforementioned seasonal variations was observed by Aughey (1969) and Selim (1974). In pre-rut period of red deer, the lipid droplets were present together with few small scattered ones in the supranuclear cytoplasm. During the rut, lipid droplets were abundant (Aughey, 1969). In bucks, during winter season, diffuse sudanophilic materials were observed at the apical fourth of the columnar cells, while the basal cells showed no lipid substances. At spring, the sudanophilic materials were lower in intensity. In summer, these sudanophilic materials were demonstrated and gradually increased both in amount and intensity towards the end of this season. In autumn (active season), these sudanophilic substances occupied the supra- and infranuclear zones of the columnar cells, which decreased in intensity at the end of this season (Selim, 1974).

Some basal cells of the ampullary gland of the donkey contained globular sudanophilic mass of variable sizes; the larger one occupied mostly the whole cell. This result was to some extent similar to that observed in the basal cells of the ampullary glands of ruminants, where lipid droplets of variable size coalesce giving the appearance of fat cells (Wrobel and Dellmann, 1993).

In goats, the lipid contents of the ampullary glands were few, the principal cells contained sometimes fine lipid particles, which were often observed within the basal cells (Wrobel, 1971). In camels (Ali *et al*., 1978) demonstrated abundant large and small lipid droplets in the cytoplasm of columnar cells of the ampullary glands and stated that it was possible that the lipid in the seminal plasma may originate from ampulla. Chow and Pang (1989) reported that the columnar cells of the ampullary glands of golden hamster contained lipid droplets, which were discharged into the lumen. They added that this gland might produce both fatty and proteinaceous products.

The response of golden hamster ampullary gland secretory cells to melatonin administration was manifested by accumulation of lipid droplets and increased numbers of lipofuscin granules. This reflected a decline in the release of and increase in the intracellular disposal of lipids by the cell, manifesting reduced secretory output. The further degeneration of epithelial cells suggests that melatonin had an adverse effect on the function of these secretory cells. Thus confirming the suppressive effects of melatonin on reproductive structures of rodents (Chow and Pang, 1989).

Conclusion

In conclusion, this report presents the first morphological and morphometerical studies on the ampulla ductus deferentis of donkey during different seasons, which might help in understanding the ul-

trastructure features of this duct. The ampulla ductus deferntis of the donkey was active all over the year, but this activity was more pronounced during spring which was manifested by increasing epithelial height, decreasing nuclear / cell ratio, decreasing interstitial connective tissue / glandular tissue ratio and increasing the cellular secretory activity. This activity decreased gradually during summer and autumn to reach its minimal level during winter. This was supported by McDonald and Pineda, 1989) who observed that, the behavioral response in stallions is more intense and pronounced during spring and summer (the highest androgen levels) and become lowest during fall and winter (Hafez and Hafez, 2000). Also the libido in male donkey was more intense in spring and summer than in autumn and winter (Gastal, *et al*., 1996).

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.

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.

Ali, H.A., Tingari, M.D., Moniem, K.A., 1978. On the morphology of the accessory male glands and histochemistry of the ampulla ductus deferentis of the camel (Camelus dromedarius). Journal of Anatomy 125, 277-292.

Aughey, E., 1969. Histology and histochemistry of the male accessory glands of the red deer, *Cervus elaphus L*. Journal of Reproduction and Fertility18, 399-407.

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

Bayliss, O.B., Adams, C.W., 1972. Bromine-Sudan Black: a general stain for lipids including free cholesterol. Histochem J 4 505-515.

Chow, P.H., Pang, S.F., 1989. Ultrastructure of secretory cells of male accessory sex glands of golden hamster (*Mesocricetus auratus*) and effect of melatonin. Acta Anatomica (Basel) 134, 327-340.

Crossmon, G., 1937. A modification of Mallory's connective tissue with a discussion of principles involved. The Anatomical Record 69, 33-38. Cited by Böck, P. (1989): In Romeis Mikroskopishe Technik. 17 Aufl. Urban und Schwarzenberg. München-Wien-Baltimore.

Davies Morel, M.C.G., 2003. Equine reproductive physiology, Breeding and Stud management. 2nd ed. CABI publishing.

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.

Gastal, M.O., Henry, M., Beker, A.R., Gastal, E.L., Goncalves, A., 1996. Sexual behaviour of donkey jacks: Influence of ejaculatory frequency and season. Theriogenol 46, 593-603.

Hafez, B., Hafez, E.S.E., 2000. Reproduction in farm animals. 7th ed, Lippincott Williams & Wilkins. Philadelphia. Baltimore. New York. London. Buenos Aires. Hong Kong. Sydney. Tokyo.

Harris, H. F., 1900. On the rapid conversion of haematoxylin into haematin in staining reactions. Journal of applied microscopy and laboratory methods 3, 777.

Mc, M.J., 1946. Histological demonstration of mucin after periodic acid. Nature 158 202.

McDonald, L.E., Pineda, H.H., 1989. Veterinary endocrinology and reproduction 4th ed., Lea & Febiger. Philadelphia. London.

Mosallam, E.S.M., 1981. Histological and histochemical studies of the male accessory glands of the adult camel (Camelus dromedarius) in the different seasons of the year: Cairo.

Parillo, F., Verini Supplizi, A., 2008. Characterization of glycoconjugates in the secretory epithelium of the equine ampulla ductus deferentis. Histology and histopathology 23, 341-349.

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

Suri, S., Sudhakar, L.S., Bhardwaj, R.L., 2008. Seasonal Variation in the Histomorphology and Histochemistry of Ampulla of Vas Deferens of Gaddi Goat and Gaddi Sheep. International Journal of Morphology 26, 131-136.

Wrobel, K.H., 1971. The ampulla of the vas deferens ingoats. Zentralbl Veterinarmed A 18(3), 250-263.

Wrobel, K.H., Dellmann, H.D., 1993. Male reproductive system. In textbook of veterinary histology. 4th. ed. Edited by Dellmann, H.-D.P, Lea & Febiger. Philadelphia.