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Histomorphology and histochemistry study of ampulla of vas deferens in adult indigenous gazelle (*Gazella subgutturosa*)

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Abstract

The present study was conducted during the period from September to November 2016 to record the histomorphology and the histochemistry of the ampulla of vas deferens of gazelle one of the most important breeds in Iraq. Eight adult animals were utilized to this study. Ampulla showed distinct four layers, tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia. Tubulo-alveolar glandular was present in the propria-submucosa. The lining epithelium of the tunica mucosa is a pseudostratified columnar epithelium between glandular end-pieces consisted of collagen fibers. The tunica muscularis consisted of two distinct layers, inner and outer circular. The longitudinal mainly consisted of smooth muscle fibers, collagen fibers and few reticular fibers. The tunica adventitia consisted of the loose arranged collagen, elastic and reticular fibers. The diameter of the alveoli was $31 \mu\text{m} \pm 1.23$ while the epithelial height was $52 \mu\text{m} \pm 0.48$. The muscular layer thickness was $735 \mu\text{m} \pm 0.21$. The secretions stained intensely with PAS indicating the presence of mucopolysaccharides that serve the nutrition to ejaculated spermatozoa.

Keywords: ampulla vas deferens; histochemistry, histomorphology, gazelle

1. Introduction

The *Gazella subgutturosa*, commonly known as Goitered Gazelle, Black-Tailed Gazelle are members of the family *Bovidae* and the sub family *antilopinae* ^[1]. The goitered gazelles are middle-sized gazelles, which live in semi-deserts, deserts on the Asian Continent and Iraq. Goitered gazelles are herbivores and generally grasses ^[2]. The male genital system is very important in the reproduction of the animals because it produces the spermatozoa that which are necessary in the fertilization to increase the numbers of animals, which have economic importance to the world countries, so the veterinarians establish the artificial insemination technique ^[1]. The ampulla is glandular enlargements associated with the terminal parts of the ductus deferens ^[3]. They are well developed in the stallion, bull, and ram and absent in tom cats, and boar. These glands of fusiform ampulla are empty into the vas deferens and contribute volume to the semen ^[4]. This study was undertaken to record the histomorphology and histochemistry of ampulla of the vas deferens in gazelle and determine the nature of the secretory products that secreted by the epithelial tissue and mucosal glandular tissue.

2. Materials and methods

The present study was conducted during the period from September to November 2016 on eight healthy adult male indigenous Gazelle (*Gazella subgutturosa*) average weight (15-19 kgs) aged 1.5-2 years used for histomorphological and histochemical study. The samples were taken immediately after slaughtering within 15-20 minutes. The ampullae of vas deferens were collected, washed with normal saline and fixed in 10% neutral buffered formalin. After complete fixation the specimens for 48hrs, washing by using continuous tap water for 6 hours to remove the formalin solution from the tissue. The sections were stained with counter stained Harris haematoxylin and eosin (H&E) for general constructions of tissue. Periodic acid-schiff (PAS) stain for mucopolysaccharides, glycoprotein and basement membrane, the polysaccharides appeared red or purple color in positive reaction and Masson's Trichrome Stain for collagenous fibers and smooth muscles. Stained slides were examined by using light microscope with digital camera USB connected with the computer slides which were pictured directly from computer at various adjustment powers (100X, 40X and 10X).

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2.1 Statistical analysis

The micrometric values were analyzed according to statistical package for the social sciences (SPSS) VERSION13.00. The significant differences between right and left ampulla means were assessed by t-test. The data were expressed as Mean \pm Standard Errors (SE) and p-value < 0.05 was considered statistically significance [5].

3. Results and Discussion

The histological results of ampulla in gazelle showed that it was branched tubuloalveolar gland. The alveoli and tubules were lined with pseudostratified columnar epithelium with spherical nuclei enclosed a wide lumen duct and has a folded mucosa, its lumen contained spermatozoa and homogenous acidophilic materials (Fig.1). This observation is similar to results reported by Suri *et al.* [6]. Ampulla has distinct four layers, tunica mucosa, tunica sub mucosa, tunica muscularis and tunica adventitia (Fig. 2). Nissar *et al.* [7] described similar layers for ampulla in all domestic animals. Tunica mucosa showed folds projected into the lumen of gland protrusions which were also observed at the apical borders of the principal cells, indicating an apocrine mode of secretion in the ampulla ductus that is deferent in the gazelle. Similar finding was observed in the rabbits, dogs, golden hamster and camels [8]. The propria of submucosa was rich in collagen and elastic fibers which give positive with masson's trichrom stain (Fig. 3 and 4). The arrangement of these fibers give the ampulla of the deferent duct great elasticity to counteract the mechanical stress by the luminal contents secretory materials and spermatozoa from one side and the muscular contraction from the other side. This arrangement probably acts as absorbing shock for stretch. These results observed in stallions and camels [9]. The elastic fibers were absent in ruminant as reported by Alkafafy *et al.* [10]. The smooth muscle fibers were well vascularized as many small blood vessels were seen. It extended from the inner muscular layer to base of the mucosal folds (Fig. 5). The presence of smooth muscle fibers in this manner probably acts as supporting element of the ampullary glands. Similar results were found in stallions, goats and camels [11]. The duct of these glands was lined by pseudostratified columnar epithelium (Fig.6 and 7). Similar findings were observed in Black Bengal goat [11], whereas, Archan *et al.* [12] described branched tubular glands in propria- submucosa of ampulla as they were opened on the mucosal surface without special excretory ducts. This was in contrast with the findings of Pyne and Chauhan [13]. Occasional basal cells with rounded nucleus, droplets of lipid were present. The present study revealed that the basal cells were small and did not form a continuous layer. They appeared nearly rounded, flattened with spherical or flat nuclei and vacuolated or granular acidophilic cytoplasm. Similar findings were observed in rams, dogs, and camels [14]. Chaves *et al.* [15] supported the observation in which they found that the basal cells and lipid may present in the epithelia of bull. The luminal secretions were also found sometimes these secretory in bluish or pink color with H&E staining. This may be due to metachromatic reaction by sulphated mucopolysaccharides [16]. These secretions were stained with PAS (Fig.8), that indicating the presence of either neutral mucin or weakly acidic sulphated mucin [17]. Other concretions like crystals secretions were found in ampulla and this result is similar to the finding obtained by Abou-Elhamd *et al.* [18] in horses and ruminant. There was

presence of sperms in alveolar and tubular lumen (Fig. 9). This was in agreement with Mahmud *et al.* [19] in domestic animals and in goat. The presence of a large amount of the spermatozoa in the lumina of the tubules and alveoli of the ampulla of the deferent duct of the gazelle indicates that it may store spermatozoa for a considerable period of time as reported in rams, and camels or it could be accepted that the sperms complete their maturation within the ampulla of the deferent duct of bulls [20].

The micrometric data for ampulla gland (table 1) showed that the diameter of alveoli in right ampulla was $31 \mu\text{m} \pm 0.022$ while in the left ampulla was $32 \mu\text{m} \pm 0.013$. Epithelial height also recorded in right ampulla was $52 \mu\text{m} \pm 0.081$ and in left ampulla was $49 \mu\text{m} \pm 0.084$. The muscular layer thickness in right ampulla ($735 \mu\text{m} \pm 0.054$) and in left ampulla was $740 \mu\text{m} \pm 0.46$. No significant difference was recorded in the alveoli diameter, epithelial height and muscular layer thickness between right and left ampulla. Sudhakar [21] described that the epithelial cells vary in height in the functional glandular unit and their duct in domestic animals. In the present investigation the epithelial height and diameters of alveoli did not show significant differences. If the differences present this could be due to the increased secretory activity under the influence of increased concentration of testosterone in the breeding season [22].

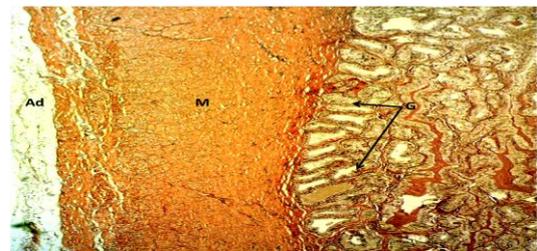


Fig 1: Histological section in Ampulla showing the gland (G), Muscularis mucosa (M) and tunica Adventitia (Ad) (H&E 40X)

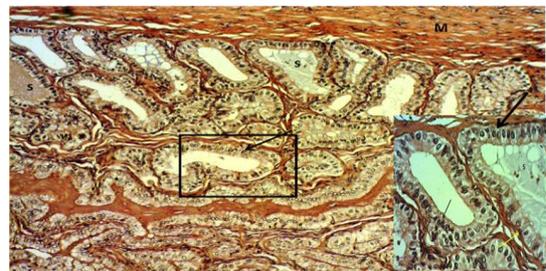


Fig 2: Histological section in Ampulla showing the pseudostratified epithelia of the gland (black arrow), Muscularis mucosa (M) and gland secretion (S) (H&E 100X)

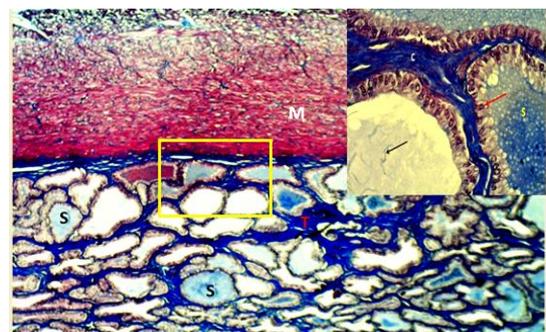


Fig 3: Histological section in Ampulla showing the Muscularis mucosa (M), gland secretion (S), trabeculae of collagen fiber (T) pseudostratified columnar epithelium (red arrow) and spermatozoa (black arrow) (Masson's trichrom 100X)

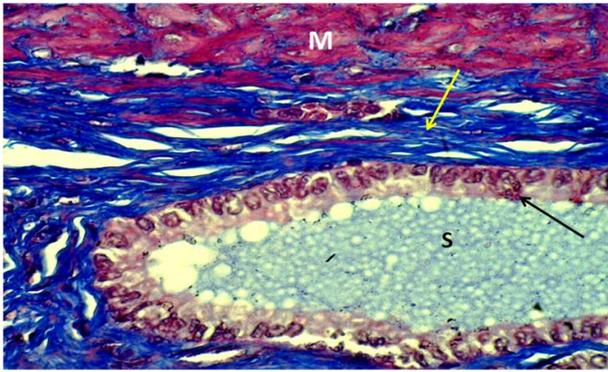


Fig 4: Histological section in Ampulla showing the pseudostratified epithelia of the gland (black arrow), gland secretion (S), muscular layer and collagen fiber (yellow arrow) (Masson's trichrom 400X)

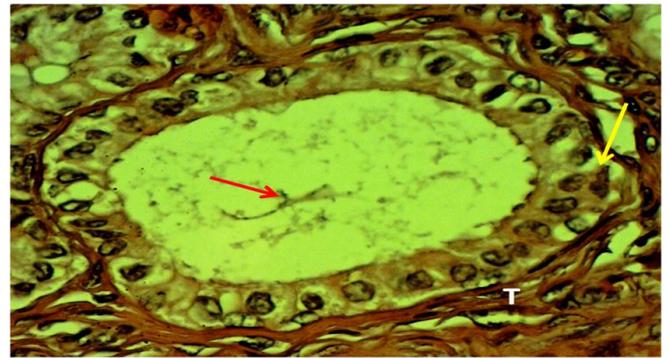


Fig 9: Histological section in Ampulla showing the pseudostratified epithelia of the gland, basal cells (yellow arrow), trabeculae (T) and spermatozoa (red arrow) (H&E 400X)

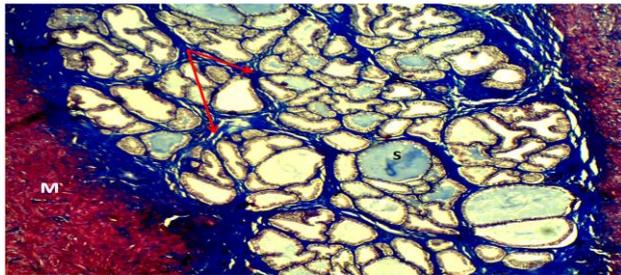


Fig 5: Histological section in Ampulla showing the gland lobule, collagen fiber septa (red arrow), gland secretion (S), muscular layer (M) (Masson's trichrom 40X)

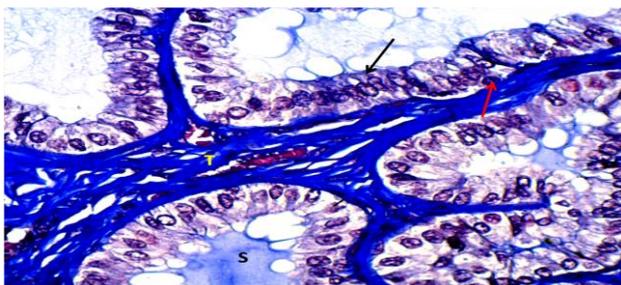


Fig 6: Histological section in Ampulla showing basal cell (red arrow), gland secretion (S), trabeculae (T) pseudostratified columnar epithelia of the gland (black arrow) (Masson's trichrom 400X)

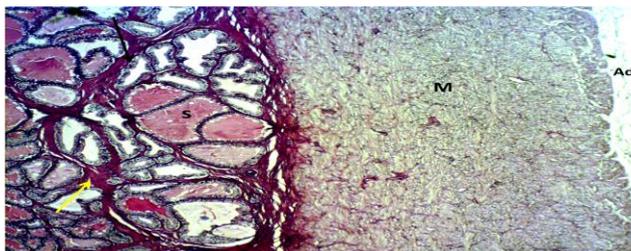


Fig 7: Histological section in Ampulla showing the gland lobule (black arrow), gland secretion (S), muscular layer (M), tunica adventitia (Ad) and septa (yellow arrow) (PAS 40X)

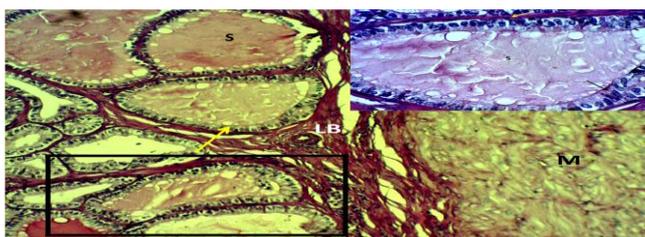


Fig 8: Histological section in Ampulla showing the pseudostratified epithelium (yellow arrow), gland secretion positive to PAS stain (S), muscular layer (M), lamina propria (B) (PAS 100X)

Table 1: The histological parameters for Ampulla gland:

	Right ampulla/ μm	Left ampulla/ μm
Epithelial height	52.01 \pm 0.081	49.00 \pm 0.084
Diameter of alveoli	31.00 \pm 0.022	32.21 \pm 0.013
Muscular layer thickness	735.11 \pm 0.054	740.05 \pm 0.46

Value are given as mean \pm SE, n=8 (All differences were not significant)

4. Conclusion

In the present study, results confirmed the possibility of the determination the histomorphological, histochemical and biometric characteristics of the accessory sexual glands ampulla of the vas deferens which could help in understanding the ultrastructural features of this gland in the indigenous Gazelle.

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