Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(4): 598-604

Received: 16-05-2017
Accepted: 17-06-2017

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Histological features of Vomer nasal organ in Indigenous Gazelle (*Gazella subgutturosa*)

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Abstract
The present investigation was carried out to study the histological features of vomer nasal organ in Indigenous Gazelle. Six adult Gazelle were used. The results indicate that, the Vomer nasal organ was symmetrical tubular organ included two parts: Conducting and olfactory parts. Olfactory part involved vomer nasal duct. The conducting part involved the incisive duct and incisive papilla which lined by stratified squamous epithelium, the incisive duct measured (623.8±12.1µm) in diameter and lined by ciliated pseudo stratified columnar epithelium. The vomer nasal duct has significant widest diameter (1040±50.9µm) and oval- circular lumen with two walls; medial wall was lined with neuro-epithelium which has significant epithelial height (83.0±2.4µm), lateral wall has lined with respiratory epithelium of height (43.4±3.6µm). The lamina propria- submucosa beneath medial wall was loose connective tissue contained many of wide veins and axons. The lamina propria- submucosa beneath incisive duct and lateral wall possessed compound tubuloalveolar mucous glands.

Keywords: Vomernasal organ, Jacobson gland, Nasopalatine duct, neuroepithelium.

Introduction
Most mammals and many reptiles and amphibians possess a secondary olfactory system, the vomer nasal system, which apparently detects particular classes of chemical signals [1]. The vomer nasal organ composed many functional components included; the accessory olfactory bulb, the vomer nasal amygdala, and the nerves and olfactory tracts, all these components are connected to do their function [2]. The vomer nasal organ composed of vomer nasal cartilage and the vomer nasal duct which surrounded by vascular soft tissue and involves of both sensory and respiratory epithelium and forms a hollow tube that is ended blind caudally and opened rostrally wit outer environment [3]. Vomer nasal organ is considering an auxiliary olfactory organ in most mammals and has an important function in sexual pheromones sensation [4, 5]. It narrow tubular structures located at the base of nasal septum of nasal cavity composed [6, 7]. Vomer nasal organ of different mammals have been described by several authors like [8] in Sheep, [9] in canine, [10] in Ox, and [11] in horse. The *Gazella subgutturosa* is endemic and spread in several Asian countries; Azerbaijan, Georgia, Iran, Pakistan, Afghanistan, Uzbekistan including Iraq. In Iraq, the historical distribution of Gazella subgutturosa was in the north to southern regions of Iraq [12-14]. *Gazella subgutturosa* has saved in several nature reserves located in many provinces including, the district of AL Madaen in the outskirts of the capital Baghdad, an area of (157) acres, where ksiab-reservoir to save the species and varieties from the risk of Extinction, includes Gazella subgutturosa which involved in this study. This study was aimed to investigate the histological structure of vomer nasal organ in indigenous Gazelle (*Gazella subgutturosa*).

Materials and Methods
A six heads of adult, healthy indigenous male Gazelle were used for this study. The animals were obtained from (AL-Madaen Animal Reservoir) in Baghdad-Iraq, and the study was conducted in laboratory of Dept. of anatomy, histology & embryology at College of Veterinary Medicine-University of Baghdad, during a period extended from October – December 2016. The animals were euthanized by slaughterer. After slaughtering, the animals heads have been removed immediately from the body and the incisive papilla were infused with 10% formalin throughout the orifices of incisive papillae, then the nasal region included the hard palate was immersed in 10% formalin for seven days. The nasal region has been cutting up into five transversal sections (Each was about 0.75-1.5cm). The sectioning has made up through the

hard palate from the level of the incisive papilla into the 12th transverse palatine ridge that included the vomer nasal organ as the following: The 1st section represented the incisive papilla. The 2nd section represented the incisive duct (At level of 1 into 4 palatine ridges). The 3rd section represented the rostral portion of vomernasal duct (At level of 5 into 7 palatine ridges). The 4th section represented the middle portion of vomernasal duct (At level of 8 into 9 palatine ridges). The 5th section represented the caudal portion of vomernasal duct (At level of 10-11 Palatine ridges). After well fixation, each section has been trimmed to achieve the region which include the basal part of nasal septum that housed both tubes of vomernasal organ, then the sections transformed into decalcified solution which composed of equal parts of Formic acid solution [250 ml formic acid (98%) + 250 ml Distal water] and Sodium citrate solution [50 gr. Sodium citrate +250 ml Distal water]. The decalcified solution haven been used for 10 days and the solution changed every five days to achieve well decalcification, [15]. Then the sections processed upgrading with ethanol alcohol for paraffin technique and sectioned serially at (5-6) µm. The prepared tissue sections were stained with the Hematoxylin and Eosin stain, Masson's trichrom stain [16]. The Histometrical measurements included the diameters of vomernasal duct and the height of the epithelial lining in all portions of vomernasal organ.

Statistical analysis

Statistical analysis has been done by using The IBM/SPSS statist version (24) to analyze the estimated data, the results were representing by Means and Standard Error (M±SE). One way analysis of Variance (ANOVA) has been used to detect age related variation. The value (P<0.05) was considered to be significant.

Results and Discussion

The results showed that the vomer nasal organ was divided into two parts: conducting part and olfactory part.

The conducting part; was included the incisive papilla and incisive duct. Incisive papilla had two orifices which were lined by thick stratified squamous epithelium (Fig.1&2). The sub epithelial connective tissue showed well vascular, dense irregular connective tissue. The Nasopalatine ducts were pass dorsomedial to the orifice of papilla before they open into the incisive papilla (Fig.2 &3). The present results are agreed with results of [10] in Ox and [17] in Buffalo. Incisive ducts were measured (623.8±12.1µm) in diameter, had an oval shaped lumen which enclosed by C- shape hyaline cartilage that was incomplete laterally (Fig.4), the incisive ducts showed two walls (lateral & medial walls), both walls were lined by ciliated pseudo stratified columnar epithelium (respiratory epithelium). The epithelium has composed of three types of cells; ciliated columnar, non-ciliated goblet cells and basal cells (Fig.5). At initial part of incisive duct the sub epithelial connective tissue was loose connective tissues invaded by many of wide veins and glandular acini which appear within the lamina propria submucosa of the dorsal aspect and lateral wall (Fig.6) while at the caudal part the duct was heavily invaded by glandular tissue (Fig.7), the present study revealed that, the vomer nasal organ was started at the end of incisive duct which connected the vomernasal duct with the oral cavity through the incisive papilla, so that the incisive papilla and incisive duct were considered as a connecting part which connects the vomer nasal organ with the outer environment [10] in Ox and [17] in Buffalo.

Olfactory part: this part was represented the vomernasal duct.

Rostral portion of vomernasal duct: this portion was extended from level of the 5th into 7th palatine ridges. The lumen of vomer nasal duct has significantly increased at level of (P<0.05) and measured (1040±50.9µm) in diameter in compared with other portions of vomer nasal duct, the vomernasal duct had crescent - oval shaped lumen which incompletely surrounded by hyaline cartilage (Fig.8), the present result suggest that the incomplete status of cartilage helps to gives the vomernasal duct a suitable space to distend during pumping mechanism, this suggestion has supported by results of [8] & [18] in sheep, on other hand the variable shape of the lumen of vomernasal duct, suggests that, the action of pumping status of vomer nasal organ is responsible for this variability (when the lumen of duct emptying from mucus fluids the lumen shape is crescent and becomes typically oval shape when the lumen of duct distended with mucus fluids),the incomplete vomernasal cartilage represented by cleft which lead to entrance of blood supply into the soft tissue of the vomer nasal organ from nasal mucosa, this agrees with results [19] in cattle, [14] in cat, dog, pig, cow, and horse, [6] in sheep, [20] in pig and [10] in Ox. The vomernasal duct was displaying two walls (medial & lateral). The respiratory epithelium was lined the lateral wall and part of medial wall, while the medial wall was lined with pseudo stratified columnar epithelium (neuro-epithelium) (Fig.9&10), this result agrees with the results of all authors except the result of [21] in Iranian goat, the present result revealed that, the vomer nasal duct started at this portion, that related with the presence of neuro-epithelium which lined the medial wall that considered the functional units of vomernasal organ, this result is consistent with the result of Vaccarezza who remember that the vomernasal duct started at the middle portion of organ in rat [22]. Also in horse, Okano, Salazar and Lee, remembered that the starting of vomer nasal organ in horse was at the body portion of vomer nasal organ because this portion bore both respiratory epithelium and neuro-epithelium [19], [11] & [23]. Also in canine, Sano mentioned that, the vomernasal duct has started at the body portion [24]. In cat, the vomernasal duct has started at the middle portion [25, 26]. The present results showed that, the neuro-epithelium had significant epithelial height which measured (83.0±2.4µm) that composed of three types of cells (Sustantecular cells, bipolar neurons & basal cells). The Supporting cells (sustantecular cells) were restricted in the upper region of neuro-epithelium and most of them had rounded shaped nuclei (Fig.10). The bipolar neurons were large cells, had eosinophilic cytoplasm and their nuclei were large spherically shaped & located at the center of epithelium. The apical surface of bipolar neurons showed brush border (microvilli) (Fig.10). The basal cells were a distinct layer of little cells located at the basement membrane and had flattened horizontally oriented nuclei (Fig.10), the result is incompatible with results of Zuri and Uraih whom refereed that, the olfactory epithelium in rat has characterized by abundant of cilia and microvilli [27, 28]. Also Korean goats [29] showed that the sensory epithelium has four types of basal cells can be distinguished morphologically, two of which are attached to the basement membrane. The lamina propria-submucosa beneath neuro-epithelium was well vascular loose connective tissue characterized by presence of many of wide

veins, capillaries and axons (Fig.11). The lateral wall with parts of the dorsal and ventral commeasures of vomernasal duct were lined by ciliated pseudo stratified columnar epithelium (respiratory epithelium) which showed few of goblet cells and measured about (43.4±3.6µm) (Fig.9&12). The respiratory epithelium consists of 4 types of cells (Ciliated columnar cells, non- ciliated columnar cells, goblet cells & basal cells), the present observation showed that, the cilia are distributed in lateral epithelium of vomernasal duct (Fig.12&13), this suggest that the cilia serve a purpose in mixing the mucus fluids along the lumen of duct, so that the cilia improved good contact between molecule of pheromones and the microvilli of bipolar neurons and this opinion is supported by [9] in canine. The present result of lining epithelium of lateral wall was in doesn't converge with the results of [22] in rat showed that the rostral portion begins with epithelium of flattened stratified squamous epithelium. In horse [19] referred for absence of neuro epithelium in this portion, also in dog [24] refereed for presence of non keratinizes stratified squamous epithelium in this portion, and [23] in horse referred for this result, which suggested that all mammals which their vomer nasal organ has open into outer environment throughout the nasal cavity have the longest conducting part which extended into rostral portion and lined with protected epithelium (stratified squamous epithelium). The lamina propria- submucosa beneath respiratory epithelium was characterized by the presence of clusters of Jacobson's glands. These glands have been dispersed among a loose connective tissue (Fig.13), the present study revealed that, although the respiratory epithelium at various points present few numbers of excretory ducts that reach to the lumen through its epithelium, but the secretion primarily discharges through the main excretory ducts which were opened at the dorsal and ventral commissures of the vomernasal duct (Fig.7), this result is supported by the results of [8] in sheep· [30] in horse, [7] in cattle, [31] in buffalo, [20] in pig, [21] in Iranian goats· [32] in dog.

Middle portion of vomernasal duct: This portion was located at level of the 8th & 9th palatine ridges. The lumen of vomernasal duct has measured (874.0±47.4µm) in diameter, had oval- shape. The vomernasal duct was completely surrounded by vomernasal cartilage (Fig.14). The medial wall and most of lateral wall were lined by neuro-epithelium, while the little surface area of lateral wall was lined by respiratory epithelium (Fig.15). The neuro-epithelium had epithelial height which measured (76.2±1.7µm), the cellular composition of neuro-epithelium showed that the olfactory bipolar neurons consisting of 1-2 rows of darkly stained spherical nuclei. The Sustantecular (supporting cells) cells have been displayed the thickest cells rows which had more darkly stained vertically oriented elliptical nuclei and tended to be near the apical surface of neuro-epithelium. The row of basal cells was as well as that seen in the rostral portion (Fig.16), these results agrees with the results of [8] in sheep, [9] in canine, [30] in cattle and horse, [7] in cattle and [33] in Angora goats. Lateral wall of vomer nasal duct was lined by respiratory epithelium displaying heavy population of goblet cells which measured (41.4±1.6µm) (Fig.17), the present result suggest that, the undulating feature of vomernasal duct helps in distension and increasing the lumen of duct that may draw the fluids from incisive canal and help including more of mucus fluids containing pheromones within the lumen of vomernasal duct so, during this distention the lumen will be oval in shape, this feature acts as a part of mechanical apparatus of pumping system, this observation is supported by results of [8] in sheep, [33] in Angora goats. The lamina propria-submucosa beneath the both neuro-epithelium and respiratory epithelium was loose connective tissue which heavily invaded by wide veins, more of glandular tissue and number of axons (Fig.18), this is agreed with [9] in canine, and [10] in Ox, the present results suggest that the increased population of glands in this portion is important, the secretion of these glands acts as dissolving media for pheromones which sniffed and passed through the vomernasal duct, the pheromones easily dissolved in the sero-mucus which covered microvilli [34].

Caudal portion of vomernasal duct: This portion was located at level of the 10th & 11th palatine ridges. The lumen of vomernasal duct has measured (551.0±139.6 µm) in diameter, had oval- circular shape and narrower lumen than that of rostral and middle portions, this result are agreement with results of [7] in cattle, [11] in horse, the present study found that the vomernasal duct which had medial and lateral wall was lined with the same different types of epithelium which mentioned in the previous portions, the neuro-epithelium has measured (57.8±3.6) and respiratory epithelium measured (32.6±1.5), both types of epithelia have been extend into the caudal portion of vomer nasal organ, this is an important feature, this result is paralleled with the results of [22] in rat, [19] in horse and cattle, [24] in canine, [20] in pig, [25] in cat, in these animals the caudal portion of vomer nasal duct, the epithelium gradually changed into simple columnar epithelium and ciliated pseudo stratified columnar epithelium. The statistical analysis showed significant mean value of diameters at level (P<0.05) in the incisive papilla and at the rostral portion of vomer nasal duct of vomer nasal organ. On other hand the significant mean value of epithelium height at level (P<0.05) for neuro epithelium was recorded at the rostral and middle portions of vomernasal organ. The height of respiratory epithelium showed non-significant values at level (P<0.05).

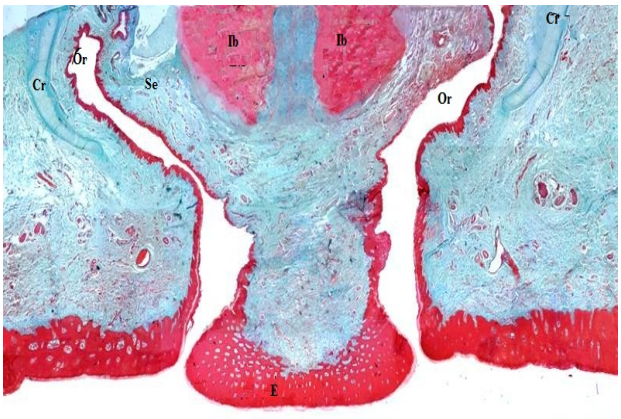

Fig 1: Transverse section of incisive papilla shows: Incisive bone (Ib), orifice of papilla (Or), cartilage (Cr), sub epithelial connective tissue (Se) and epithelium (E). Masson's trichrom stain.40x

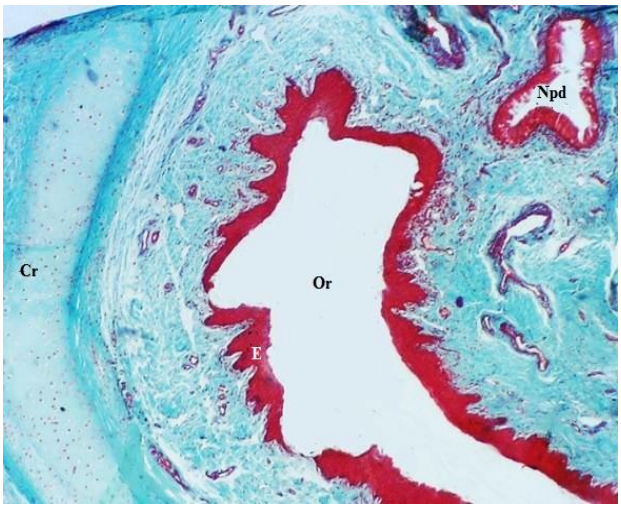

Fig 2: Section of the orifice of incisive papilla shows: Orifice of papilla (Or), cartilage (Cr), sub epithelial connective tissue (Se), epithelium (E) & nasopalatine duct. Masson's trichrom stain.100x

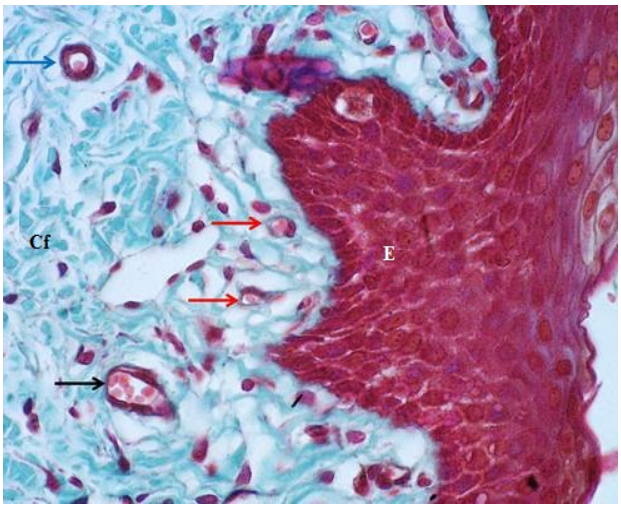

Fig 3: Magnified section of incisive papilla shows: Collagen fibers (Cf), arteriole (Blue arrow), venule (Black arrow), capillaries (Red arrows) and epithelium (E). Masson's trichrom stain.400x.

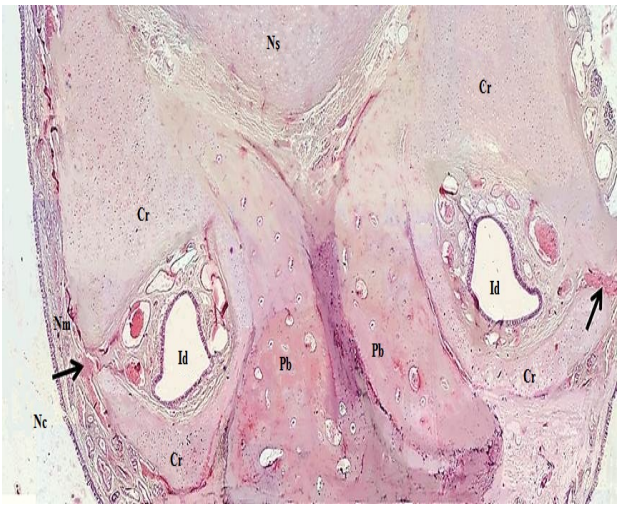

Fig 4: Transverse section at initial part of the incisive duct shows: Incisive duct (Id), cartilage (Cr) & incomplete status of cartilage at lateral aspect (Arrows), nasal cavity (Nc), nasal mucosa (Nm) nasal septum (Ns), palatine bone (Pb) H&E stain.40x

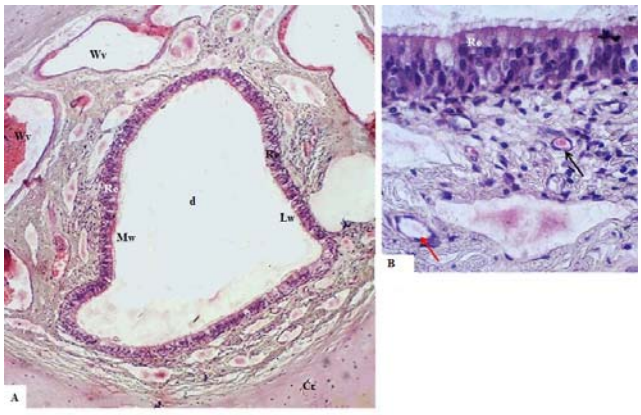

Fig 5: (A) &(B) are magnified sections at initial part of the incisive duct shows: duct (d), cartilage (Cr), medial wall of duct (Mw), lateral wall (Lw), wide veins (Wv), respiratory epithelium (RE), capillary (black arrow) & venule (red arrow). H&E stain. 100x & 400x.

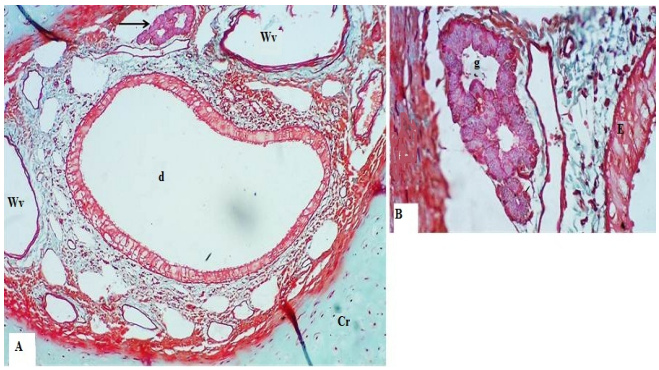

Fig 6: (A) & (B) are magnified sections of initial part of incisive duct shows: duct (d), cartilage (Cr), wide veins (Wv), epithelium (E), glandular acini (g) & epithelium (E). Masson's trichrom stain. 100x & 400x.

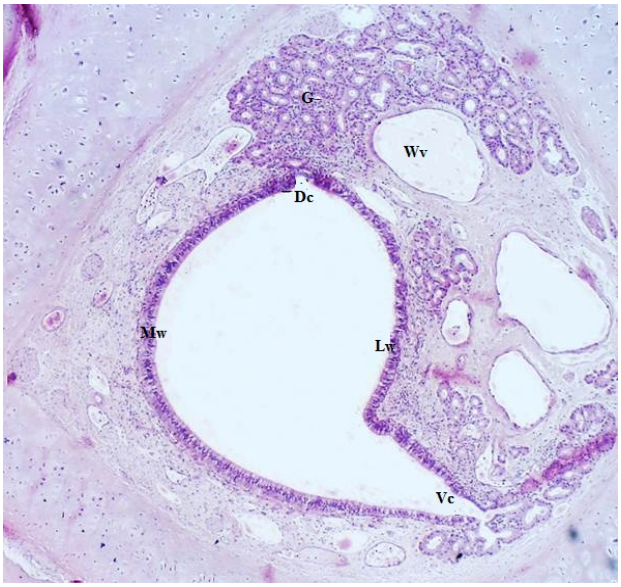

Fig 7: Transverse section at caudal part of the incisive duct shows: medial wall of duct (Mw), lateral wall (Lw), wide vein (Wv), glandular tissue (G), dorsal commissure (Dc) & ventral commissure (Vc). H&E stain.100x

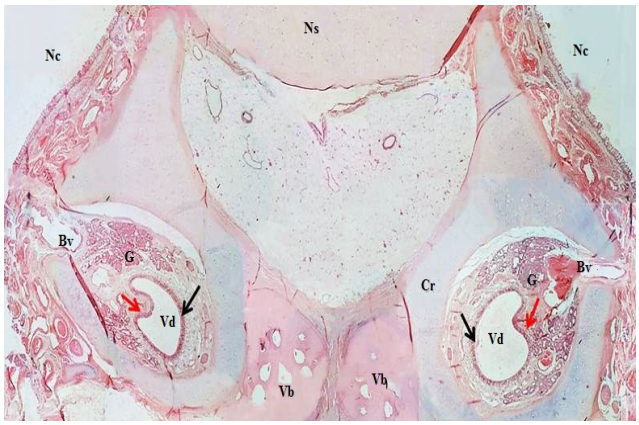

Fig 8: Transverse histological section at rostral portion of VNO shows: Nasal septum (Ns), vomer nasal duct (Vd), medial wall (black arrows), lateral wall (Red arrows), cartilage (Cr) & incomplete status of cartilage at lateral aspect indicate blood vessel (Bv), nasal cavity (Nc), glandular tissue (G) & vomer bone (Vb) H&E stain.40x

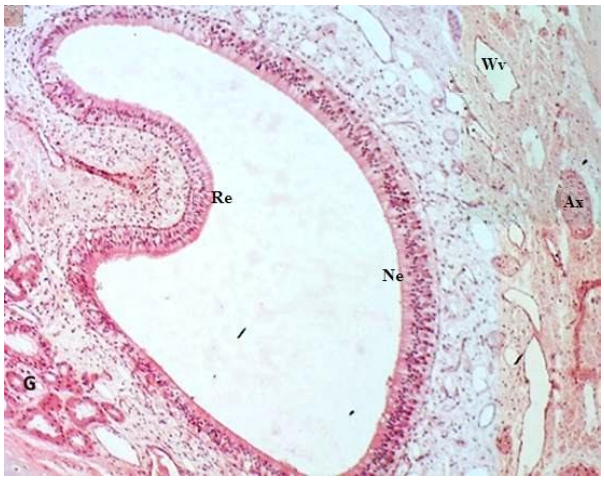

Fig 9: Section of Vomer nasal duct (Rostral portion) shows: neuro epithelium (Ne), respiratory epithelium (Re), wide vein (Wv), axon (Ax) & glandular tissue (G). H&E stain.100x

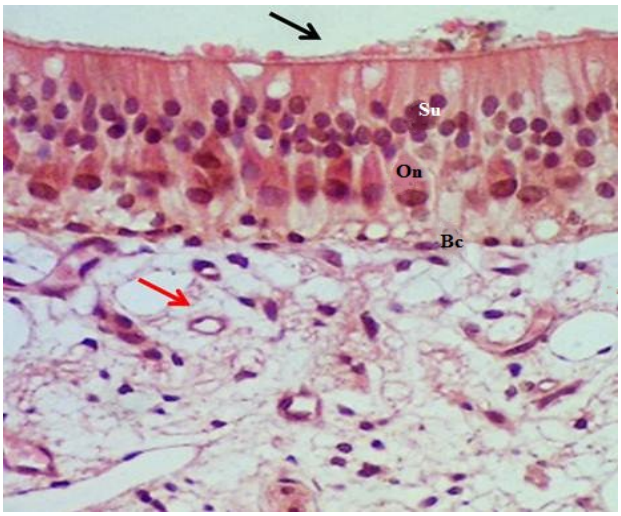

Fig 10: Magnified section of neuro-epithelium (Rostral portion) shows: microvilli (black arrow), nuclei of sustantecular cells (Su), nuclei bipolar neurons layer (On) nuclei of basal cells (Bc), capillary (Red arrow). H&E stain.400x

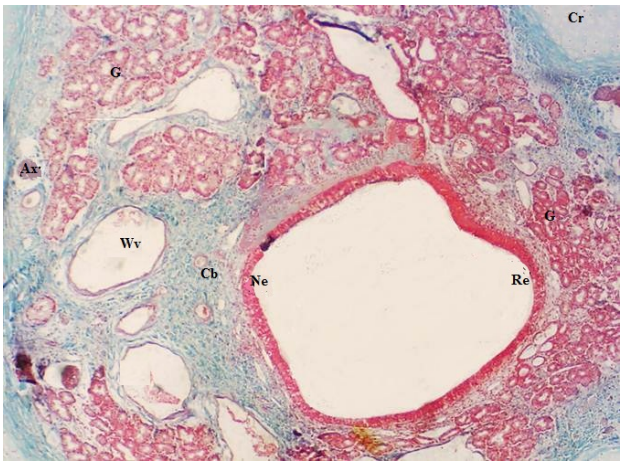

Fig 11: Section of Vomer nasal duct (Rostral portion) shows: neuro epithelium (Ne), respiratory epithelium (Re), collagen bundles (Cb), wide vein (Wv), axon (Ax) H&E stain. Masson's trichrom stain.40x

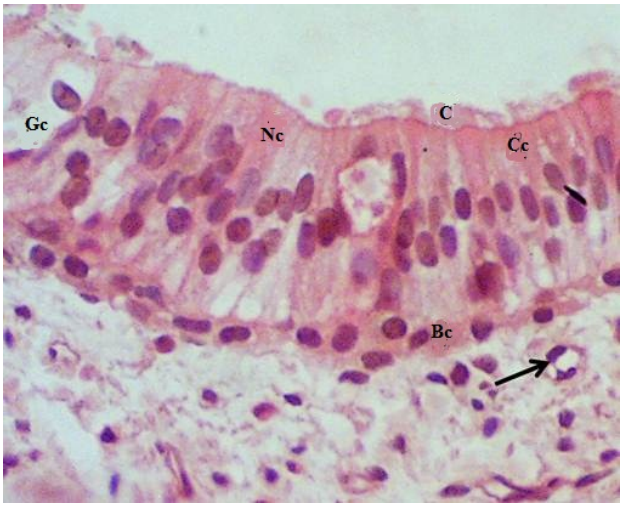

Fig 12: Magnified section of respiratory epithelium (Rostral portion) shows: cilia (C), ciliated cells (Cc), non-ciliated cells (Nc), goblet cells (Gc) basal cells (Bc) & capillary (arrow). H&E stain.400x

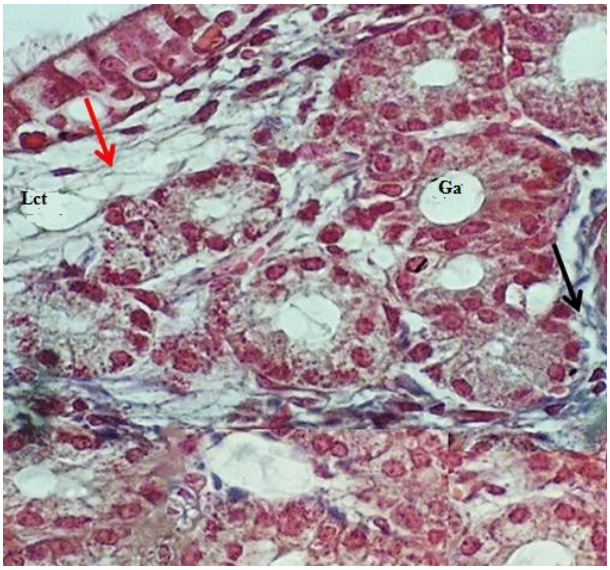

Fig 13: Magnified section of lamina propria under respiratory epithelium (Rostral portion) shows: loose connective tissue (Lct), collagen bundles (black arrow) reticular fiber (red arrow), alveolus of Jacobson's gland (Ga), Masson trichrom stain.400x

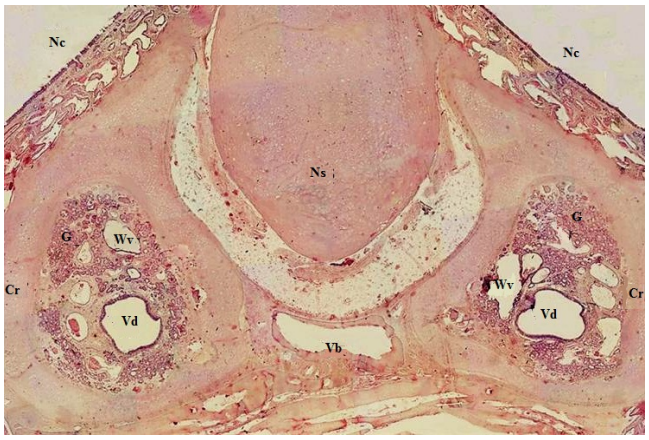

Fig 14: Transverse section at middle portion of VNO shows: vomer nasal duct (Vd), cartilage indicates complete status at lateral aspect (Cr), nasal cavity (Nc), nasal septum (Ns), vomer bone (Vb), glandular tissue (G) & wide veins (Wv). H&E stain.40x

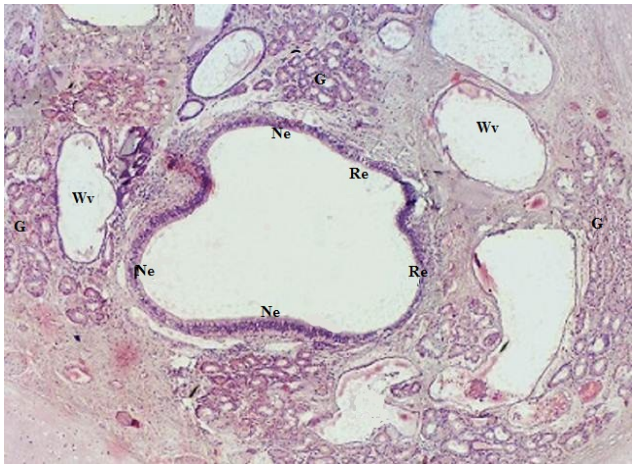

Fig 15: Section of Vomer nasal duct (Middle portion) shows: neuro-epithelium (Ne), respiratory epithelium (Re), Glandular tissue (G), wide vein (Wv). H&E stain. 100x

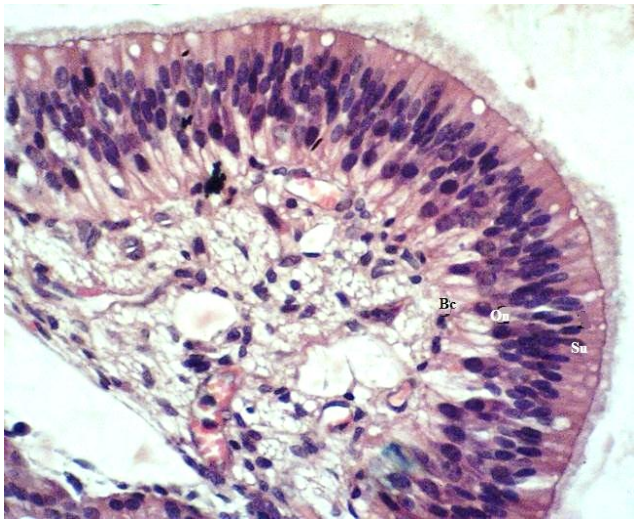

Fig 16: Magnified section of neuro epithelium (Middle portion) shows: thick layer of sustantecular cells (Su), nuclei of bipolar neurons layer (On), nuclei of basal cells (Bc). H&E stain. 400x

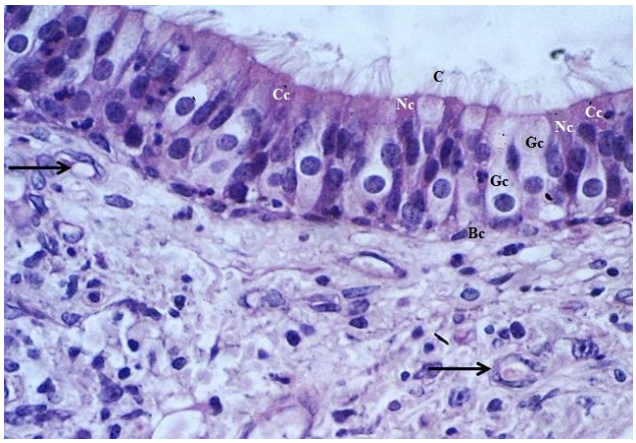

Fig 17: Section of Vomer nasal duct (Middle portion) shows: cilia (C), ciliated cells (Cc), non- ciliated cells (Nc), goblet cells (Gc) basal cells (Bc) & capillary (arrow).H&E stain.400x

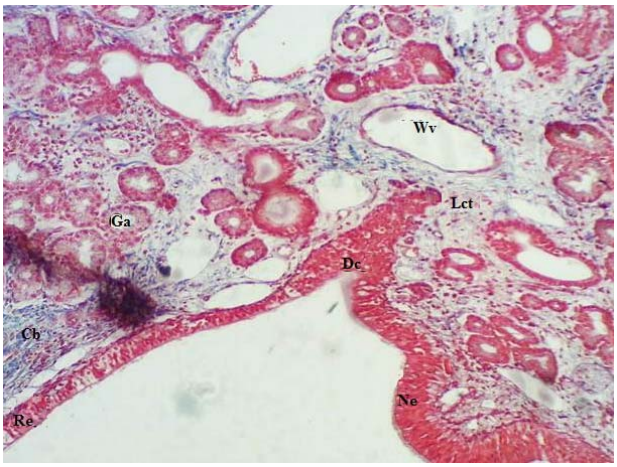

Fig 18: Section of Vomer nasal duct (Middle portion) shows: neuro epithelium (Ne), respiratory epithelium (Re), Glandular alveolus (Ga), wide vein (Wv) & loose connective tissue (Lct), dorsal commissure (Dc)& collagen bundles (Cb).Massons trichrom stain.100x

Conclusion

The present study has concluded that, in general, the histological features of vomer nasal organ of indigenous Gazelle were almost similar those of small and large ruminants except that the incisive duct had completely lined with respiratory epithelium. Significant diameter of the duct of the vomer nasal organ was recorded at the incisive duct and at rostral portion of vomernasal duct compared with other portions. Significant epithelial height was recoded at the rostral & middle portions of vomernasal duct in compared with those in other portions. The vomer nasal duct showed feature of undulating respiratory epithelium so, the distension of vomernasal duct depends on the distension in epithelium of lateral wall. Both neuro-epithelium and respiratory epithelium have been extending into the caudal portion of vomer nasal organ, this is an important feature. Finally in indigenous Gazelle the Jacobson glands are mucous-tubuloalveolar type.

Acknowledgement

The authors would like to acknowledge all technical staff and assistances in department Of Anatomy, Histology &Embryology-College of veterinary Medicine at University Of Baghdad.

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