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Light and Scanning Microscopic Study on the Vomeronasal Organ of the Buffalo *(Bos bubalis)*

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Abstract: The aim of the present study was to describe the structural features of the vomeronasal organ (VNO) in the buffalo (*Bos bubalis*). The vomeronasal organ of 12 adult buffaloes of different ages and sexes were studied macroscopically, by light microscope and by scanning electron microscope. The results of the present study revealed that the vomeronasal organ of the buffalo consists of paired tubes situated on each side of the base of the nasal septum. The lumen of the VNO is lined by medial sensory and lateral non-sensory epithelium. The sensory epithelium is a pseudostratified epithelium containing three morphologically identical cell layers: supporting cells, vomeronasal receptor neurons and basal cells. The respiratory (nonsensory) epithelium is a pseudostratified ciliated columnar epithelium with three cell types: ciliated cells, nonciliated secretory cells and basal cells. The vomeronasal glands are compound branched tubuloacinar glands mainly located in the submucosa. The present study gives a detail anatomical description of the VNO of buffaloes that may help in understanding more about its function especially flehmen behavior.

Key words: Buffalo · Scanning Electron Microscope · Vomeronasal Organ

INTRODUCTION

The mammalian vomeronasal organ (VNO), lying on either side of the base of the nasal septum, is thought to elicit reproductive behavioral responses through the perception of pheromones [1-7]. The pheromones are chemical substances that are released by a member of a species to communicate with other members [8]. The adult male buffalo also displays a flehmen response to pheromones [9].The vomeronasal organ has received much attention in recent years. The structure of the VNO in mammals has been the subject of several reports in sheep [10], horse and cattle [11,12], calves [13], pigs and sheep [14], goats [15], dogs [1,16], cats [17,18] and elephant [19]. However, little or no information is available on the normal morphology of the vomeronasal organ in buffaloes.

The lumen of the VNO is lined by two kinds of epithelia, sensory and nonsensory, in most species [13,20]. The lateral-respiratory and medial-receptor epithelial pattern occurs only in a particular segment of the vomeronasal duct [11,21]. Communication either with the nasal or the oral cavities or both may be found [22]. To our knowledge, there is no previous study on the buffalo vomeronasal organ using scanning electron microscope.

Therefore, The aim of the present study was to describe the structural features of the vomeronasal organ in the buffalo (*Bos bubalis*) by light and scanning electron microscopic studies.

MATERIALS AND METHODS

Animals and Light Microscopy: Specimens for light and electron microscopy from 12 adult buffaloes (*Bos bubalis*) of different ages (3-12) and sexes (7 males and 5 females) were used for this study. They were obtained from the slaughter house in Egypt (Benha). The vomeronasal organ was removed along with the cartilaginous capsule immediately after slaughter. Then it was sliced in cross-sections of 1-3 mm^3 and was immersed in 10% formalin phosphate buffer (pH 7.4) for fixation. After 48 h the samples were rinsed in tap water, decalcified in 5% EDTA over 48 h at 35° C, dehydrated in alcohol, embedded in paraffin wax and transversely serially sectioned at 5-6 µm thickness were prepared.

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The sections were stained following standard protocols described by Bancroft [23]. Harris' Haematoxylin and Eosin (H and E) [24], Masson's trichrome stain [25]. Crossmon's stains [26] and Periodic Acid Schiff (PAS) were used.

Scanning Electron Microscopy: For SEM, the samples were rinsed with physiological saline (0.9% NaCl) and then cut into small-fragments which were placed on a metal grid and washed in cold physiological saline for one hour. Washed samples were fixed in 10% buffered formaldehyde. The fixation time depended on the thickness of the samples and varied between 24 and 48 hours. After fixation the samples were washed four times in saline solution (15 minutes each change) and dehydrated in a series of alcohol: 10, 30, 50, 70 and 90% and 2 times in absolute ethanol (15 minutes in each change). After drying, samples were sputter coated (Polaron Range S.C. 7620Sputter Coater) with 30 nm layer of gold-palladium (Au/Pd) and examined using JEOL 5500 LV SEM scanning electron microscope at an accelerating voltage of 15 kV in the Faculty of Agriculture, Alazhar University.

RESULTS

Macroscopic Anatomical Results: The vomeronasal organ (VNO) of the buffalo is a paired tubes situated on each side of the base of the nasal septum. The tube ends blindly caudally and joins the incisive duct to communicates with the nasal and oral cavities rostrally. It was enveloped by incomplete ring of cartilage. The cartilage encloses the vomeronasal duct, glands, connective tissue, blood vessels and nerves (Fig. 1).

Light and Scanning Microscopic Results: The lumen of the VNO is lined by sensory and non-sensory epithelium (Fig. 2, 3, 4, 5. 6 and 7). The thickness of the epithelium in the medial wall is thicker than its lateral wall. At the apposition of the two epithelial types, there is a transitional zone, the sensory epithelium gives way abruptly to respiratory (non-sensory) epithelium (Fig. 2). There is an epithelial fold that increases gradually where the lumen is wider.

Medial Sensory Epithelium: The SE is a pseudostratified epithelium containing 3 morphologically identicable cell layers: supporting cells, vomeronasal receptor neurons and basal cells. *The supporting cells* were columnar and extends from the basal membrane to the epithelial surface. The nucleus is elongated and situated apically. The apical surface contains numerous microvilli (Fig. 4).

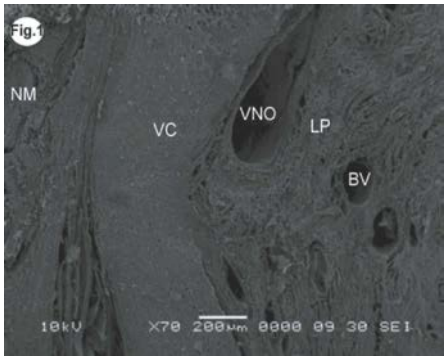

Fig. 1: Scanning electron micrograph of frontal section through the vomeronasal organ in the buffalo. the vomeronasal organ (VNO) and the lamina propria (LP) contain numerous glands and blood vessels (BV) and is surrounded by vomeronasal cartilage (VC). There is also the mucosa of the ventral nasal meatus (NM).

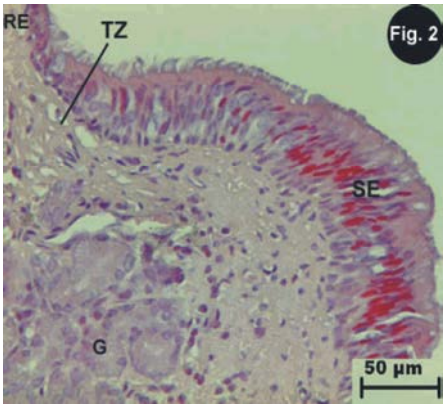

Fig. 2: Photomicrograph of the VNO showing the receptor (sensory) epithelium (SE) with three distinct layers and the respiratory epithelium (RE). Group of glands (G) present in the lamina propria.TZ, transition zone.

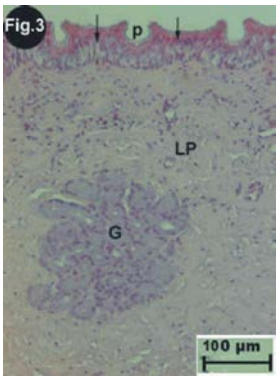

Fig. 3: Photomicrograph of the VNO showing the respiratory (nonsensory) epithelium (arrows) and the lamina propria (LP) is filled with glandular tissue (G). pocket-like evagination (p) appear.

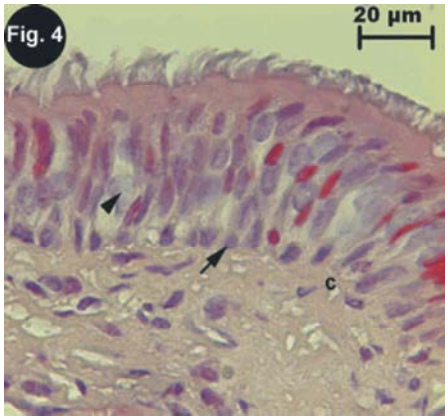

Fig. 4: Photomicrograph of the VNO showing the receptor (sensory) epithelium with three distinct layers and abundant cilia on the surface. The SE contains sensory neurones (arrowhead). The neurons are immediately apical to basal cells (arrow). Numerous subepithelial capillaries (c) are also seen.

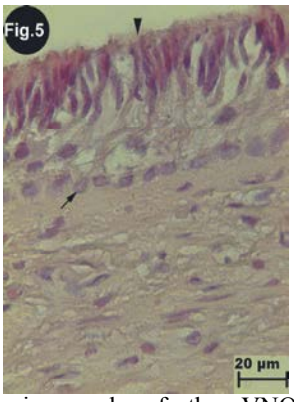

Fig. 5: Photomicrograph of the VNO showing the respiratory (nonsensory) epithelium. Ciliated cells (arrow heads) and basal cells (arrows) can be distinguished.

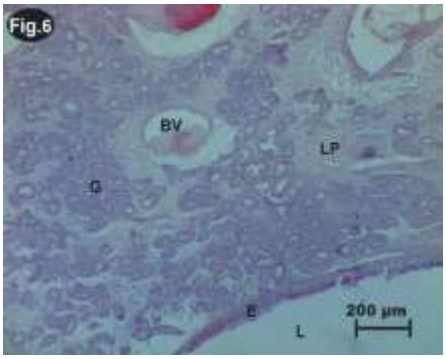

Fig. 6: Low-magnification photomicrograph of transverse section of the buffalo vomeronasal organ showing the epithelium (E) and the lamina propria (LP) containing numerous glands (G) and blood vessels (BV). The glands open into the lumen (L).

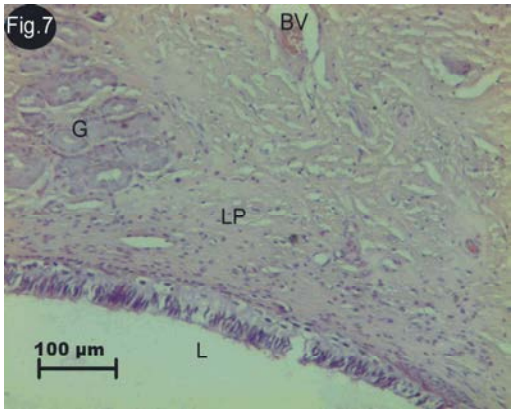

Fig. 7: Medium power photomicrograph showing the lamina propria (LP) of non-sensory mucosa containing glands (G) and blood vessels (BV). The glands open into the lumen (L).

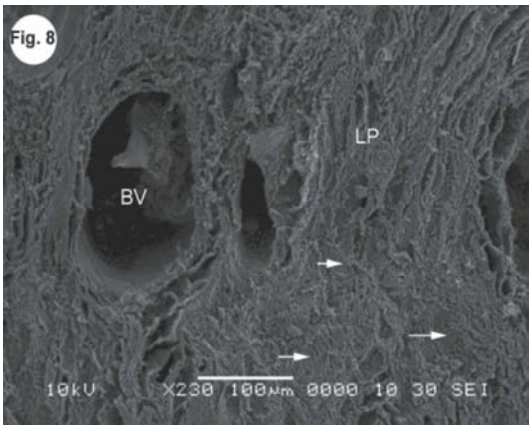

Fig. 8: High magnification scanning electron micrograph showing the entire lamina propria (LP) that contain numerous glands (arrows) and blood vessels (BV).

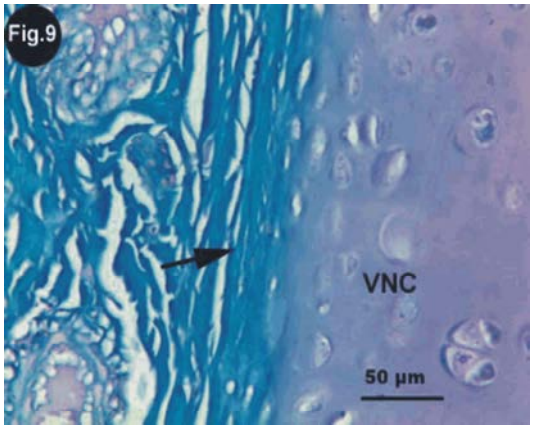

Fig. 9: Photomicrograph showing the vomeronasal cartilage (VNC) and the collagen fibers (arrows) adjacent to it. Crossman stain.

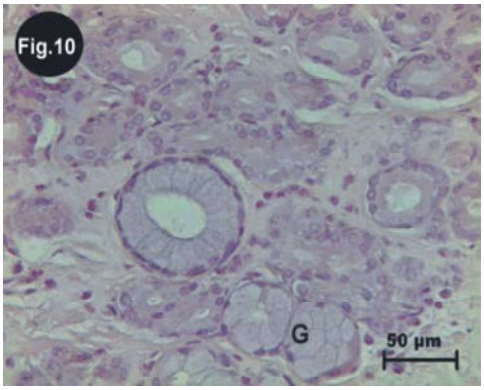

Fig. 10: High magnification photomicrograph of the lamina propria of the buffalo vomeronasal organ showing the glands (G).

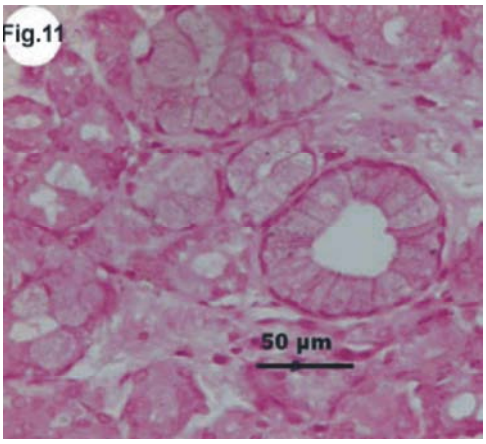

Fig. 11: Photomicrograph of the glands of the vomeronasal organ show positive reaction with PAS stain.

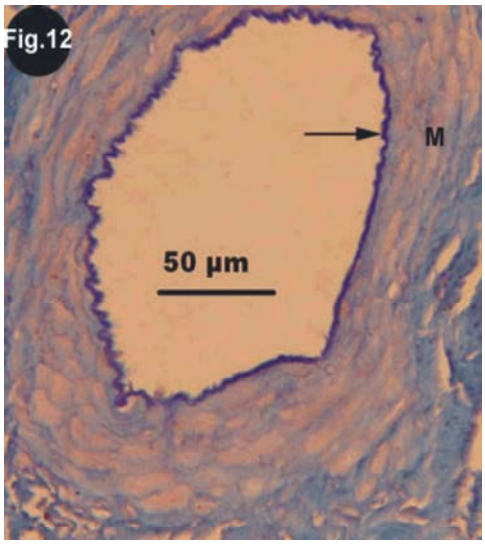

Fig. 12: Photomicrograph showing an artery in the VNO with the tunica intema (arrow) and the tunica media (M)

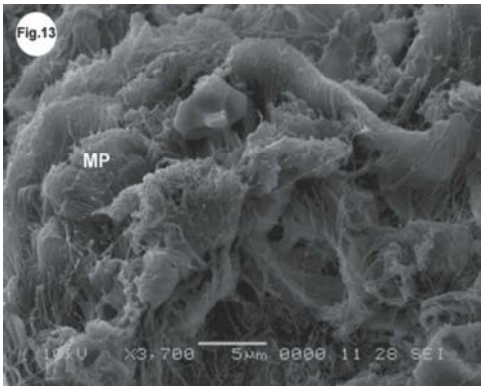

Fig. 13: Scanning electron micrograph of the fractured surface of the epithelium showing protrusion of the microprocesses.

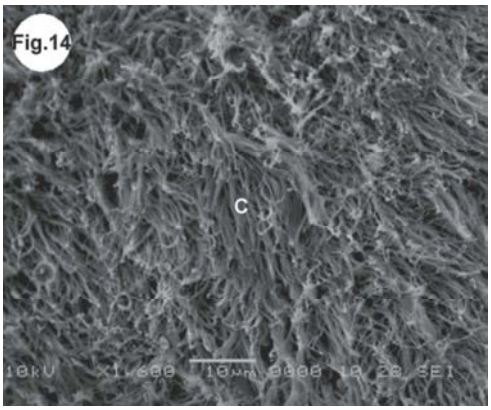

Fig. 14: Scanning electron micrograph showing the surface features of the respiratory epithelium in the buffalo. The cilia (C) are numerous.

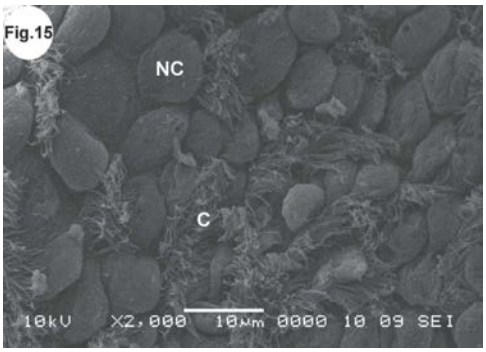

Fig. 15: Scanning electron micrograph of a portion of the vomeronasal organ showing ciliated (C) and non-ciliated cells (NC) in the epithelium.

The Vomeronasal Receptor Neurons Are Bipolar Cells: The nucleus is round and situated usually in the middle below the level of the supporting cells (Fig. 4). It has two processes. A peripheral dendritic process extends from the cell body towards the epithelial surface, whereas a central axon goes towards the basal lamina.

The dendrite runs between the nuclei of the supporting cells before expanding into a region that ends in a rounded ending carrying numerous microvilli. The basal cells are located adjacent to the basement membrane and their nuclei are round or oval (Fig. 4). There is pocket-like invagination observed in the sensory epithelium (Fig. 3).

Lateral Respiratory (Nonsensory) Epithelium: The NSE is a pseudostratified ciliated columnar epithelium 70-80 ì in height with 3 cell types: ciliated cells, nonciliated secretory cells and basal cells (Fig. 5).

Numerous cilia and microvilli present on the apical surface of both sensory and non sensory epithelia (Fig. 2, 3, 4, 5. 13, 14 and 15).

The Lamina Propria: Consists of loose connective tissue with a great number of collagen and elastic fibers that occupied the space between the glands and blood vessels. Blood capillaries and sinuses were distinguished (Fig. 6, 7 and 8). Nerve fibers were also seen to form the vomeronasal nerve. The glands were distributed in the lamina propria around the vomeronasal duct (Fig. 6 and 7).

Vomeronasal Glands: The VG are compound branched tubuloacinar glands mainly located in the submucosa along the long axis of the dorsal aspect of the VNO (Figs. 6, 7, 11 and 12). Their secretions are released into the lumen of the VNO through ducts ending in the dorsal junctional area. The lumen is circular or ovoid in cross section. The glands are of serous acinar type, with the nucleus in the basal part of the cell and the secretory granules in the apical part; they are PAS positive (Fig. 11).

DISCUSSION

There is wide agreement that the vomeronasal duct is lined by two different types of epithelium, respiratory and receptor [27]. However, neither Seifert [6] nor Kogure *et al.* [28] in the cat mention the fact that the VNd is additionally lined with another two types of epithelium: simple columnar in the caudal part and stratified squamous in the rostral part. Vaccarezza *et al.* [21] and Salazar *et al.* [18] found 4 types of epithelium in the vomeronasal duct of rat and cat.

Our result revealed that the buffalo vomeronasal organ shows SE in the medial wall and NSE in the lateral wall as in most species like sheep [10], horse and cattle [12], pigs and sheep [14], goats [15], dogs [1,16] and elephant [19]. In contrast, the NSE is absent in the common marmoset [29], man [30] and blind mole rats [31].

The respiratory epithelium linning the lateral wall of the lumen of the VNO is generally accepted to be ciliated, similar to mentioned in many mammalian species such as horse and cattle [12]; sheep [10]; cat [32]. Since the RE is devoid of structural features that suggest olfactory function, it is assumed to perform only such normal functions as warming and humidification of the inspired air, secretion, removal of debris and general sensation [12,33].

In the present study, the highly developed vascular network and abundant glands in the VG are noteworthy. The highly vascular tissues underlying the epithelium have been suggested as a means of altering pressure inside the tube and thus changing its content of fluid or air [10].

The vomeronasal gland found in the submucosa of buffaloes was similar to that described in other mammals [13]. Only serous acini were observed in the buffalo. Serous acini have also been described in prosimian primates [34], rabbits, guinea pigs, cats, dogs [7], marsupials [35] and armadillo [36]. However, mixed mucous and serous vomeronasal glands were described in the hamster [37] and calves [13].

The present study revealed that the glands are abundant distributed everywhere under the epithelium, in contrast to that recorded by Kratzing [10] in the sheep about the absence of any glands below the sensory epithelium and the glands present only under the non-sensory epithelium and of mucous type.

The pocket-like invagination observed in the sensory epithelium are morphologically similar to the “glandular crypts” in the sensory epithelium of the adult sheep VNO described by Kratzing [10], may be one of the initial “folds” that distinguish the lumenal border of the Asian elephant VNO [19].

The functional mechanism of the VNO has been discussed by several authors [12]. As the VNO of buffalo communicates with the incisive duct, flehmen [22,38] is thought to be involved in the function of the VNO. Because odorants don’t automatically enter the VNO, but must be actively introduced by flehmen, they are not thought to enter the lumen of the lumen of VNO frequently, which may also indicate reduced importance or a restricted role of the VNO in the olfactory system [12]. The VNO has been suggested to have roles in detection of volatile odoriferous compounds in urine [20,22] and in sexual behavior [39,40].

Detailed investigation has indicated that flehmen behavior is associated with anatomical specialization. Thus, ruminants, including buffaloes have an incisive papilla and incisive ducts located on the hard palate just behind the dental pad [41].

CONCLUSION

In conclusion, this study gives some light and scanning electron microscopy description of the VNO of buffaloes (*Bos bubalis*) that may help in understanding more about its function especially flehmen behavior.

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