Structure of Vomeronasal Organ (Jacobson) in the Male Red Fox (Vulpes Vulpes)

Hamid Karimi[1*], Belal Hassanzadeh[2], Nasser Razmaraii[3]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
2. Central Lab, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
3. Parasitology Laboratory, Razi Vaccine and Serum Research Institute, Northwest Branch, Marand, Iran.

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Dr. Hamid Karimi is the associated professor in Veterinary anatomy. He got DVM degree from Shiraz University in 1994; Then he got DVSc in veterinary anatomy from Urmia University in 1998. He is working on animal anatomy, ART in animals and comparative histology in university of Tabriz. His reserch interests are electron microscopy, descriptive animals' osteology, animals cloning & Basic Histology.

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ABSTRACT

Introduction: Most mammalians possess an accessory olfactory system, which its first part is called vomeronasal organ (VNO). In this research, we studied the structure of this organ in Azerbaijani red fox.

Methods: Heads of 10 healthy male fox carcasses were collected from areas around Tabriz and transferred to the laboratory in frozen form or in fixative solution. Biometrical experiments were done, then the maxillary bones cut into 5 pieces, and the pieces decalcified and embedded in paraffin. Then, 7-µm tissue sections were stained with H&E, PAS, and Masson's trichrome methods and explored under light microscope.

Results: Two ducts of VNO start at the roof of mouth, about 3.17±0.28 mm behind the central incisor teeth, extend back into 2 sides of nasal septum and end near the first or second premolar teeth. This organ is surrounded by a hyaline cartilage, which is C-shaped in the first pieces and transform to "J" shape structure toward the back. The lining epithelium of lumen changes from nonkeratinized stratified squamous epithelium near the valve to pseudostratified columnar in the posterior portions. Presence of bipolar neurons in epithelium of medial wall shows VNO sensory function of smelling. Lamina propria-tunica submucosa in most portions have many serous and mucous secretory units and composed of a loose connective tissue with numerous blood vessels, which secretes pheromone. Also, this is an erectile tissue that can function in association with flehmen reaction to push toward the sensory epithelium of VNO.

Conclusion: Orifice of VNO of Azerbaijani red fox same more mammalian open in oral cavity. It has 2 type epithelial tissues in end region. Sensory epithelium indicates important role of this organ for received of Pheromones.

*** Corresponding Author:**
Hamid Karimi, DVSc
Address: *Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.*
Tel: *+98 (914) 3135810*
E-mail: *karimi@tabrizu.ac.ir*

1. Introduction

Among the vertebrates, birds and fish have no vomeronasal organ, but in other classes like amphibian (frogs) and reptiles (snakes), the presence of this organ has been reported. In this regard, numerous studies have been focused on the presence of VNO in different mammalian species. Accordingly, the presence of this organ has been confirmed in most mammals except human (functional in adults), some primates, and marine mammals [1].

VNO is one of 4 anatomical regions for sensing odors, especially sexual pheromones. Sexual organs of female animals excrete pheromones in oestrus period, perceived by male animals as a sign of appropriate time for copulation [1-3]. Stimulation of this organ causes a strange reaction in male animals called the flehmen response. This reaction is reported in many mammals like horses, cattle, sheep, goat, dogs, cats, elephants, rats, some monkeys and some non-mamalian species like snakes, frogs and salamanders [1-9].

The first experiment that showed VNO involvement in mammals' reproduction was done by Planel [10]. He showed that destruction of this organ can disable copulation in male guinea pigs and reduce interest to males in females with falling in pregnancy rate. Bruce observed that 4 days after presence of a stranger male in the cage of female mice, embryo implantation in uterus stopped [3]. He stated that the odors activated by the VNO function causes that.

In 1988, Clancy, based on his experiments, explained how injection of gonadotropin-releasing hormone (GnRH) induced reproductive behavior in animals with damaged VNO [7]. Thus, he concluded that in normal animal, stimulation of VNO releases GnRH and subsequently increases luteinizing hormone in blood and also induction of reproductive behaviors [7].

Beside numerous studies on physiological changes in this organ and subsequent behavior for better understanding of function and structure of VNO, many works have done on exploring its location and histological structure. Many researchers have focused on this organ in different animals such as snakes, salamanders, felidae, ungulates, frogs, dogs, moose, and others rodents [11-15]. In 1958, Witten for the first time stated the physiological functions of VNO. However, its anatomical structure had been already explained by Jacobson in 1813, thus, this organ has been known as Jacobson organ [16].

VNO in the studied animals comprised two bottom closed tube located bilaterally in two sides of nasal septum and in most cases surrounded by a hyaline cartilage. The orifice of VNO opens in incisive duct and its closed bottom extends towards soft palate and pharynx and in different species and genders its length differs depending on the length of muzzle. In these animals, the incisive duct opens in both mouth and nose except snakes, horses, and camels. Also in most of these animals, there is a mucosal valve in the opening of VNO. In snakes, it opens only at the roof of mouth; however, in horses and camels it opens only in the nose[1,17, 18]. In rodents, VNO has a cigar-like structure which is located close to the lower portion of nasal septum [3, 11, 19].

Most of the anatomical studies on VNO have accompanied by morphometric studies, and this type of information has constituted a large portion of results in these studies. For instance, connection between this organ and incisive duct has reported as 13-17 mm behind of its orifice in mouth, while Barone has reported 40 mm length for VNO of dogs [6, 20]. The average (SD) length of VNO has reported 189 mm in buffalo, 90.5 mm in Lori sheep, 78 mm in male goats, and 156.1 mm in one humped camel, which extends from incisive orifice at the roof of the mouth towards back and end near the second premolar teeth [5-9]. Maximum diameter of the organ in goats (about 18mm) and camels has reported in midway of its length [9, 18].

Anatomical studies on this organ usually accompany with microscopic and histological studies which indicates its special organization for smelling. In terrestrial vertebrates, VNO has 2 different epitheliums, one is sensory and another respiratory and non-sensory. The sensory epithelium has basal cells, receptor neurons, and supportive cells which are superficial to neurons. Also, basal cells are close to the basement membrane [3, 4].

In snakes, the lumen of the organ is mostly occupied by fungi form bodies, which are covered by ciliated epithelium, besides the sensory epithelium of medial wall is usually thicker and more complicated than that of mammals [7]. In rodents, the olfactory epithelium of medial wall is pseudostratified columnar and contains basal cells, supportive cells, and bipolar receptor neurons with an axon and a dendrite [9, 21]. Regarding the mentioned studies, the main objectives of researchers in this area are the type of epithelium and its spread in different parts of the organ, type of secretory units in lamina propria-tunica submucosa, as well as characteristics of surrounding cartilage.

Among the carnivores, dog is the most studied animal and its VNO physiology, anatomy, and histology have

been explained completely [4, 22, 23]. Besides, some works have been done on Felidae family, but there is no study like ours in the case of fox. Fox is a predator animal and usually uses olfactory sense to snoop and detect its preys, but it is not clear how much the accessory olfactory system and VNO are involved in this job [6]. Anatomical and histological knowledge about structure of fox VNO can be useful in understanding its function and involvement in snooping and copulation. Because of geographical spread of these animals in Azerbaijan, every day we can find some of them killed by cars in roads and highways. During cold season (4-5 months), people who are plying on the roads of this area can find some frozen fox carcasses or dying foxes. If these people could be trained to take and handle specimens properly, the specimens could be used in anatomical and histological studies. Having this in mind, we tried to study gross and microscopic anatomy of vomeronasal organ in the male red fox (Vulpes vulpes), known as grey fox among the native people.

2. Materials and Methods

In this research, heads of 10 male fox (killed by car accident or villagers) collected from different areas of East Azerbaijan. The heads were transferred in frozen form to the laboratory. To reduce postmortem changes, this work was done in cold seasons (late autumn and winter); however, finding a good sample with uncrushed head was too difficult. Another problem was defrosting frozen samples, which destroyed mucosal epithelium during tissue processing. Two different studies were done on the samples; anatomical-biometric study and histological-microscopic study.

In anatomical study, 5 heads were transferred to the laboratory in frozen condition, then after defrosting their mandible were removed. At first, their palatine folds were counted and images taken from the opening of VNO. Then, the length of organ was measured by a 0.2-mm venoject and red latex was injected into the organ and the whole maxilla was cut on sagittal lines to detect the form and openings of VNO. In the rest of samples and after removing the mandible, the length of muzzle was divided in 5 segments with the mean length of 7 mm and location of cut lines were as follows: the first line behind the incisor teeth, second line behind the canine teeth, third line behind first premolar teeth, fourth line behind the second premolar teeth, and fifth line behind the third premolar teeth. After trimming, these segments were fixed in 3.7% buffered formalin with one change 24 hours later, then specimens was decalcified one week in 10% nitric acid solution and dehydrated by ethanol and cleared with xylene and embedded in paraffin. Then, 7-µm sections stained by 3 methods; H&E, PAS, and Masson's trichrome and explored by light microscope (Olympus BX60-Japan) and photographed by digital camera (Olympus DP12, Japan).

3. Results

Anatomical study showed that opening of VNO in the fox is located on the hard palate (roof of mouth), 3.17±0.28 mm behind the incisor teeth and next to the first palatine fold (Figure1-a, b) and there is 4.83±0.29 mm distance between the right and left openings. The end of VNO ducts in both left and right sides are located in the vicinity of the first and second premolar teeth in upper jaw. A semilunar valve was observed in the orifice of VNO, which was made by the mucosal epithelium of hard palate (Figure 1-a, b). The length of VNO was 17.46±0.33 mm and its diameter was 0.2-0.25 mm and the number of palatine folds was counted in each sample (Table 1).

Histological study showed that VNO lumen in the beginning (in segment 1 and 2) was narrower and toward the end (in segment 3 and 4) became somewhat wider (Figure 2). The maximum diameter of organ was measured and recorded in the second half of segment 3 (Figure 2). The organ lumen in segments 1 and 2 was surrounded by a C-shaped hyaline cartilage while its continuing side was located towards the nasal septum (Figure 2). The lining epithelium of lumen in segment 1 and beginning of segment 2 was the extension of

Table 1. Characteristics of palatine folds in 5 foxes.

No	Length of VNO (mm)	Number of palatine folds	Distance between two entries of VNO (mm)
1	17	11	4.9
2	17.6	11	4.8
3	17.9	11	4.5
4	17.3	11	5
5	17.5	11	5

ANATOMICAL SCIENCES

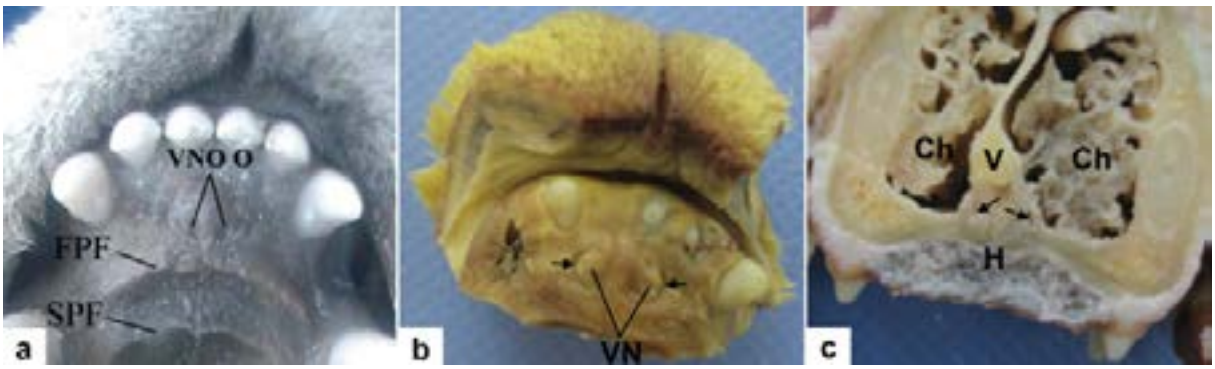


Figure 1. a) Hard palate, upper lip, incisive, and canine teeth before fixation. Abbreviations: VNOO: Orifice of vomeronasal Organ; FPF: First Palatine Fold; SPF: Second Palatine Fold.

b) First segment of upper jaw after fixation. Abbreviation: VN: Mucosal Valve of Vomeronasal Organ. Arrows: Openings of Vomeronasal organ.

c) Second segment of upper jaw after fixation. Abbreviations: Ch: Ventral nasal conchae; H: Hard palate; V: Nasal septum. Arrows: Lumen of vomeronasal organ.

mouth epithelium (nonkeratinized stratified squamous) and under the epithelium there was a continuous lamina propria-submucosa consisting of loose connective tissue with many blood vessels somewhat like a cavernous erectile tissue. Few seromucous secretory units with majority of mucous were found in some sections of these segments (Figure 3).

According to these observations, from the second half of the segment 2, diameter of lumen begins to increase and reach to the maximum size in segment 3, the surrounding cartilage turns into J–shaped and saves it until the end of organ in the middle of segment 4 (Figure 2). In all cases, the segment 5 was devoid of any structures like VNO. From the beginning towards the end and until the midway of the organ, blood vessels of lamina propria-submucosa increased in number and size and gave it a more cavernous shape, also the number of secretory units increased in this cavernous tissue (Figure 4). From the end of segment 2, the lining epithelium began to change into a ciliated pseudostratified columnar epithelium without goblet cells in medial wall. Basal cells are close to the basement membrane, supportive cells with long vertical nuclei and neuroepithelial cells which are ciliated were seen, too (Figure 4). However in small portion of lateral wall and floor of the lumen in segments 2 and 3, nonkeratinized stratified squamous epithelium was observed, too.

4. Discussion

The important function of vomeronasal organ and its involvement in flehmen response has been known and recently compared among mammals and to this end many anatomical studies have been conducted on this organ. In these studies like our study, VNO has been described as 2 bottom closed tubes located bilaterally in 2 sides of nasal septum. In most mammals, except horses and camels, the duct of Jacobson organ connects to the incisive duct in the vicinity of its oral opening [5, 7-9, 14, 18, 19]. In the goat, VNO duct opens directly to the nose while in Bovidae family like buffalo, moose, and sheep there is an accessory opening of VNO to the nose allows odors from nose to VNO. Also, absence of flehmen response has reported in some of Bovidae family like African antelope which do not have incisive ducts [9, 12, 24].

Our experiment showed that entrance of incisive duct on the hard palate have 2 semilunar valves, which are similar to dogs and some ruminants like cow, sheep, and helical horn goat [2, 4, 9]. With regard to other reports, results of this study indicate that although VNO closed bottom is located in the vicinity of the second and third premolar teeth, but the length of VNO in mammals directly depends on the length of muzzle, i.e. the distance between premolar and incisive teeth.

Two main types of epithelium floor the lumen of VNO; one has the ability to receive and transfer the chemical stimuli and the other without receptor duties. It differs from nonkeratinized stratified squamous epithelium and contains pseudostratified columnar one with goblet cells. Study of Veidin and colleagues on the structure of VNO in Scandinavian moose showed that the sensory portion of the organ, which is located on the lateral wall of its posterior half has pseudostratified columnar epithelium with goblet cells [15]. In red fox, the lining epithelium of lumen starts with nonkeratinized stratified squamous epithelium at the entrance of organ and transforms to ciliated pseudostratified columnar towards back, like other mammals, which is sensory in medial wall and non-sensory in lateral wall [1, 14]. The neuroepithelial

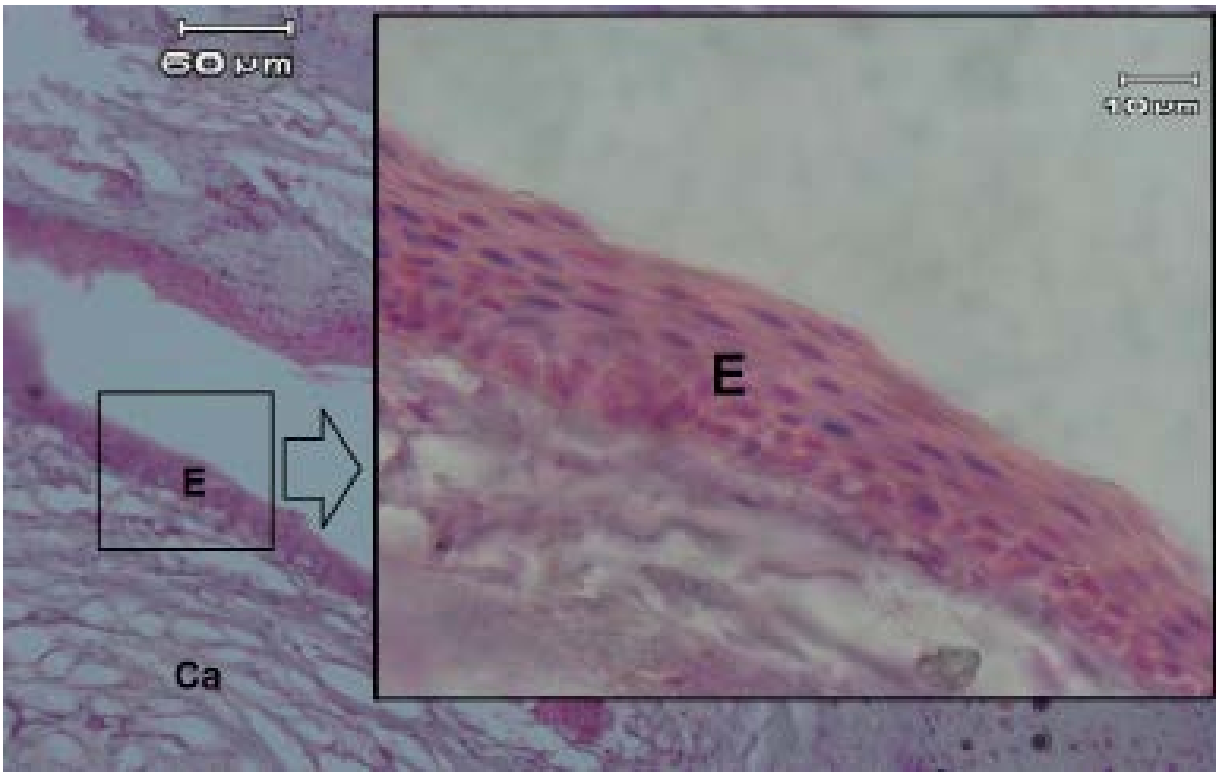


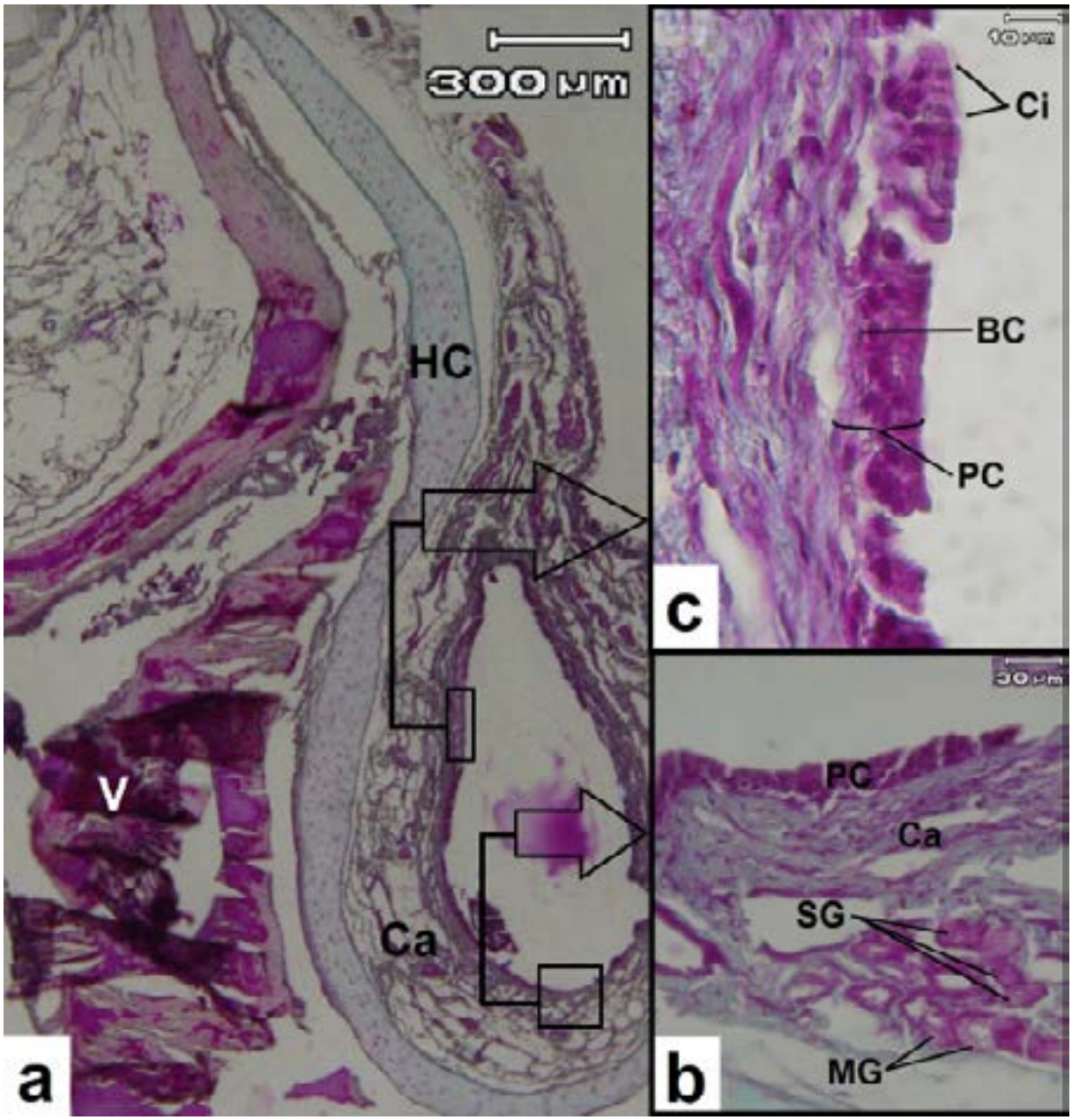
Figure 3. Cross section of vomeronasal organ H&E staining, central lumen coated by nonkeratinized stratified squamous epithelium, conjunct lamina propria-tunica submucosa contain numerous blood vessels and form an erectile cavernous tissue. Abbreviations: Ca: Cavernous tissue; E: nonkeratinized stratified squamous epithelium.

cells in the epithelium of medial wall usually have a euchromatic big nucleus and cilia and their nuclei are often located at a lower level relative to cylindrical nuclei of supportive cells. Basement membrane is present like other epithelium layers and round euchromatic nuclei of basal cells are located closed to that. All of these characteristics resemble the olfactory epithelium of nasal cavity and confirm sensory functions of VNO [25]. Small portions of nonkeratinized stratified squamous epithelium extend to segments two and three, which we took it as an important finding that shows change of epithelium does not occur simultaneously in a distinct line. These radial extensions of stratified squamous epithelium are only found between pseudostratified columnar epithelium in the floor and lateral wall of the lumen.

The connective tissue of lamina propria-tunica submucosa in all studied mammals like red fox was full of blood vessels with some secretory units [1, 14, 18]. In Scandinavian moose there are many small and large blood vessels in lamina propria-submucosa along the VNO. Also, from the middle of the duct to its end, there are some exocrine compound secretory units which most of them are located in the floor and roof of the lumen [15]. Presence of fibroelastic connective tissue with numerous blood vessels along with erectile tissue and seromucous glands with majority of serous ones in camel, loose connective tissue with branched tubuloacinar serous and in low rate compound glands in buffalo, and seromucous with majority of serous glands in goat have been reported, while in sheep most of the seromucous glands in lamina propria-tunica submucosa are mucous ones [4, 9, 19, 26].

In the fox, loose connective tissue of lamina propria-tunica submucosa has numerous blood vessels which form an organized erectile tissue to facilitate entry of chemicals and passing them toward the sensory epithelium of VNO (performing pumping reaction). Dissolving the chemicals in the serous secretion of tubuloalveolar glands can give a boost to pass them towards the neuroepithelial cells and be in direct contact with their cilia producing action potential in these cells. Studies on the function and number of receptor neurons in different animals show that the neuroepithelium of VNO, structure of secretory units, and shape of surrounding cartilage in carnivores are similar to Artiodactyla order (even-toed ungulates), and differ from rodents [27].

Another important aspect of VNO histology relates to its surrounding cartilage, which is a hyaline cartilage in



ANATOMICAL SCIENCES

Figure 4. Cross section of vomeronasal organ in the segment 3 shows sensory epithelium and cavernous lamina propria-tunica submucosa contain serous and mucous gland units, 2 portions of image a are magnified in images b and c, Green Masson's trichrome staining. Abbreviations: BC: Basal Cell nucleus; Ca: Cavernous tissue; Ci: Cilia; MG: Mucous Gland units; PC: Pseudostratified Columnar epithelium; SG: Serous Gland units.

almost all mammals like fox [1, 14]. This cartilage is C-shaped in the beginning and became like an upside down wand (J shape) towards the back. As regards other mammals, similar forms could be found in camel and helical horn goat. The important point is the location of sensory epithelium relative to this cartilage, which in all these animals, is located in the medial wall, and completely supported by continuous part of the cartilage [18]. The shape of this cartilage, which is like a continuous capsule in buffalo and moose are different from the fox ones. Thus, it is clear that diverse anatomical shapes and histological characteristics of VNO directly associate with its function despite the shape of adjacent tissues and organs [2]. Despite the important role of sensory epithelium, we cannot disregard the role of surrounding cartilage, seromucous glands, and cavernous lamina propria-tunica submucosa in the function of VNO, including the flehmen reaction.

Finally, we propose that in similar studies (which need the animal samples from the wildlife, especially in cold regions), taking specimens by this method and using naturally or accidently killed animals not only helps wildlife preservation but also produces valuable biological information and will advance tissue processing methods for using similar specimens in biological studies. The important point in these studies is cooperation with common people who have access to these carcasses and they should be taught to take the right specimens, handle them properly, and then transfer them to the laboratory as soon as possible.

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Conflict of Interest

The authors of this study declared no conflict of interests.

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