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ORIGINAL ARTICLE

Comparative morpho-histological analysis on the vomeronasal organ and the accessory olfactory bulb in Balady dogs (*Canis familiaris*) and New Zealand rabbits (*Oryctolagus cuniculus*)

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ABSTRACT

Objective: This study investigated the comparative morphological analysis of the vomeronasal organ and the accessory olfactory bulb in dogs and rabbits.
Materials and Methods: A total of 15 heads obtained from each adult healthy Balady dog *(Canis familiaris)* and New Zealand rabbit (*Oryctolagus cuniculus*) of both sexes. The animals were sedated and anesthetized. Then, the heads were removed for computing topography, gross, and cross-sectional anatomy and histological techniques.
Results: The vomeronasal organ was blind bilateral tubes enclosed by J-shaped cartilage on each side of the nasal septum. In dogs, it extended from the level of the upper third premolar teeth to the third incisive teeth. While in rabbits, it had no relation with the upper teeth. In cross section, the vomeronasal organ was pear-shaped in dogs and oval in rabbits. The accessory olfactory bulb was a small oval-shaped in dogs, but larger and ovoid in rabbits with clear lamination in its structure. The vomeronasal epithelium in rabbits was higher in its thickness than that of the dog. The vomeronasal duct had medial sensory and lateral respiratory epithelium. The vomeronasal glands were voluminous and of serous type in rabbits other than were seromucous in dogs.
Conclusion: The most characteristic structural variations achieved in the vomeronasal organ and the accessory olfactory bulb of the dog and rabbit gave an indication that the organ was more functional in rabbits than in dogs. The detection and response to the pheromonal stimuli were referred to as the occurrence of olfactory epithelium in the vomeronasal organ.

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Accessory olfactory bulb; dog; rabbit; vomeronasal organ; vomeronasal nerve.

Introduction

The olfaction considered one of the most important senses for most mammals in our life; especially in animals whose babies born with immature visual or auditory functions to recognize their mother by their detection of certain pheromones [1,2]. It is indicated that olfactory receptors occur in three places in animals, namely, olfactory epithelium, nasal septum (NS), and vomeronasal organ [3]. Even, Grüneberg [4] illustrated Grueneberg ganglion is one of the olfactory sensory neurons that act as a chemo-detector of alarm pheromones [5] in some macrosmatic animals (mouse, marsupials, and rabbits) but failed to find in dogs [6].

The vomeronasal organ is a special compound construction, sending chemical signals (pheromones) to the central nervous system to manipulate mating, and social performance. The organ was lacking in birds, fish, crocodiles, chameleons, and the majority of marine mammals [7]. The vomeronasal duct (VD) has a sensory epithelium (SE) which their afferent axons connecting the duct with the accessory olfactory bulb [8]. The vomeronasal organ in most mammals is composed of different constituents; VD, seromucous glands, nerves, cartilage, and blood vessels (BV) [9]. In most animal species, the epithelium of the organ has two types, namely, non-sensory (respiratory) type and sensory type (olfactory) [3,10–12]. The vomeronasal SE makes the accessory olfactory system to be responsible for behavioral olfactory motivations [10].

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The strong olfactory ability of the dog makes them an essential constituent of law enforcement, exploration, salvage, and medical purposes. Rabbit is used as a studying model for the mammalian chemo-communication. However, there are few studies concerning the morphology of the vomeronasal organ of the dog [13–15] and rabbit [16–18]. Those research works mostly deal with the epithelial structure. Also, few research works are recorded about the anatomy of the accessory olfactory bulb in dogs [19,20] and rabbit [21,22]. The recent data focused mostly on the vomeronasal system function [23–25]. Still, there is a defect in our knowledge about the morphology of the vomeronasal system. To complete the knowledge gap of the dog and rabbit vomeronasal organ and the accessory olfactory bulb, comparative anatomical and histological study of these organs in two different animals category would be interesting.

Materials and Methods

Ethical approval

The animals were handled according to the strategy of the Institutional Animal Care and the Research Ethics Committee of the Zagazig University (ZU-IACUC/2/F/48/2019).

Animals

This study was carried out on 15 of each adult healthy Balady dogs (*Canis familiaris*) and New Zealand rabbits (*Oryctolagus cuniculus*) of both sexes. The control dogs of the experimental studies in the Clinical Departments of the Faculty of Veterinary Medicine, Zagazig University, Egypt, were used. Rabbits were obtained from the laboratory Farm Animals of Faculty of Agriculture, Zagazig University, Egypt. The dogs were sedated by intramuscular injection of xylazine (1 mg/kg) and then anesthetized by injection of ketamine (10 mg/kg). The rabbits were injected I.V with xylazine (3 mg/kg) followed by injection of ketamine (3 mg/kg) for anesthesia [26]. Then, all animal exsanguinations were performed via the common carotid artery in the dissecting room at the Department of Anatomy and Embryology in our faculty. The separated heads of both animals were examined for any signs of neurological diseases.

Computed tomography

Five heads of both species were used for computing topography (CT) (120 kV, 80 mA for dogs) (120 kV, 120 mA for rabbits) in AL-Bayan Center in Belbes, Sharkia Governorate, Egypt, and then returned for using in the anatomical examination.

Anatomical examination

For gross observations, seven heads (included the previous five CT heads) of the two species were fixed and preserved in a mixture of 10% formalin, 2% phenol, and 1% glycerin. These heads were dissected carefully to expose the brain, the vomeronasal nerves, the vomeronasal organ and its cartilage, and the nasopalatine duct in situ. Measurements were then made to the whole organ with a stainless Caliper. The average length and width of the VD were measured by the Image J program.

Cross sections of three frozen heads from both animal species were made for investigating the relation of the vomeronasal organ to the nasal concha and septum. The vomeronasal organ was crossed at its cranial, middle, and caudal parts, and then examined by using a stereomicroscope (Nikon SMZ-2T, Tokyo, Japan). The observations were recorded and photographed by using a digital camera (Sony DSC-W690, 16.1 MP). For the terminology, Nomina Anatomica Veterinaria [27] was used as if it is possible.

Histological examination

Pieces from the cranial, middle, and caudal parts of the vomeronasal organ, and parts of the brain containing the accessory olfactory bulb from the remaining five heads of the dogs and rabbits were fixed in 10% neutral buffered formalin for 48 h. The specimens of the vomeronasal organ were decalcified in 5% Ethylenediaminetetraacetic Acid (EDTA) for 48 h. All the specimens dried out in ascending grades of alcohol, cleared in three changes of xylene, embedded in paraffin wax, and 5-µm-thick sections were obtained. The sections were stained with Hematoxylin and Eosin (H&E), Crossmon's trichrome stain, Periodic Acid Schiff (PAS), and Alcian blue stains. The above-mentioned handing out technique and stains followed the guidelines of Bancroft and Gamble [28].

Results

Anatomical findings

Organum vomeronasale (vomeronasal organ) is a blind bilateral tube present underneath the dark nasal mucosa in dogs but a bright one in rabbits. The organ located in the rostroventral part of the nasal cavity on each side of the NS (Fig. 1A–C). It had a skeletopic relationship with vemor, maxilla, and incisive bones. In dogs, it is situated ventral to ductus nasolacrimale [the nasolacrimal duct (NLD)] and underneath the ventral turbinate crest by about 0.4 cm (Fig. 1C and D). The vomeronasal organ in both species was enclosed partially in cartilago vomeronasalis [vomeronasal cartilage (VC)] and located slightly ventromedially to Concha nasalis ventralis (the ventral nasal concha (VNC) (Fig. 2A–D). The organ in dogs extended caudally from the level of the upper third premolar teeth to the third incisive teeth. While in rabbits, it started 0.8 cm in front of the first upper premolar teeth and continued rostrally to about 0.4 cm posterior

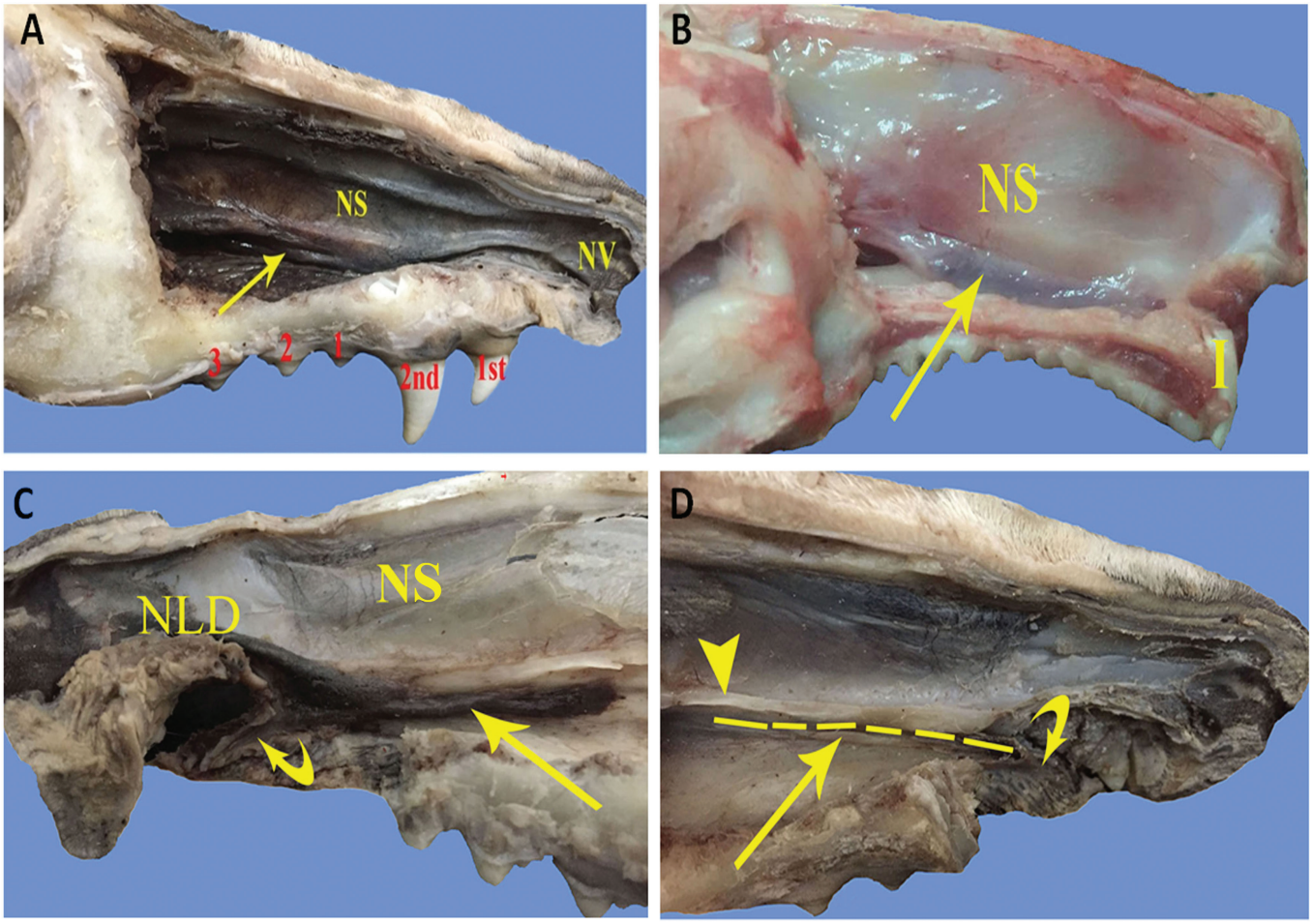

Figure 1. Right view of the nasal cavity of the dog (A) and rabbit (fresh head) (B) showing: the vomeronasal organ (arrow), NS, nasal vestibule (NV), incisor teeth (I), first canine (1st), second canine (2nd), first premolar (1), second premolar (2), and third premolar teeth (3rd). (C) Left view of the nasal cavity of the dog without mucosal covering and removal part of the incisive bone showing: the vomeronasal organ (arrow), opened ID (curved arrow), nasal septum (NS), and NLD. (D) Right view of the nasal cavity of the dog after removal of the vomeronasal organ (dotted line), showing: the VC (arrow), closed ID (curved arrow), and the ventral turbinate crest of the maxilla (arrowhead).

to the incisive teeth of the same side (Fig. 1A and B). The average length of the organ was about 4.16 and 1.95 cm in dogs and rabbit, respectively. It was thick and bulged caudally, thick in the middle, and thin in the cranial part by an average width of 0.3, 0.24, and 0.16 cm, respectively, in dogs. On the other hand, in rabbits, the organ had approximately the same width along its entire length by about 0.28 cm caudally, 0.27 cm in the middle, and 0.25 cm cranially.

In cross section, the vomeronasal organ was pear-shaped in dogs and oval in rabbits and its cartilage was a J-shaped; enclosed the organ except its dorsal aspect. Along the entire length of the organ, the shape and position of ductus vomeronasalis (VD), and arrangement of BV varied in caudal, middle, and cranial portions. The organ was highly vascularized resembling the cavernous tissue appearance (Fig. 2E and F). The duct of the organ has an average length of about 0.16, 0.16, and 0.18 cm in the caudal, middle, and cranial parts, but the mean width was 0.03, 0.03, and 0.02 cm, respectively, in the same parts. The duct of the rabbit had an average length of about 0.12, 0.36, and 0.34 cm in the caudal, middle, and cranial parts, respectively. However, the mean width was 0.02, 0.13, and 0.13 cm, respectively, in similar parts.

In dogs, the rostral end of the vomeronasal organ curved ventrally and united with ductus incisivus (the incisive or nasopalatine duct) of the same side, passed through the Palatine fissure to terminate on the oral cavity. While in rabbits, the organ was straight opened into the nasal cavity and indirectly connecting with the oral cavity by the nasopalatine duct. The nasal cavity connected with the oral cavity by the oral orifice of the incisive duct (ID), which lays on each side of the incisive papilla (IP). The

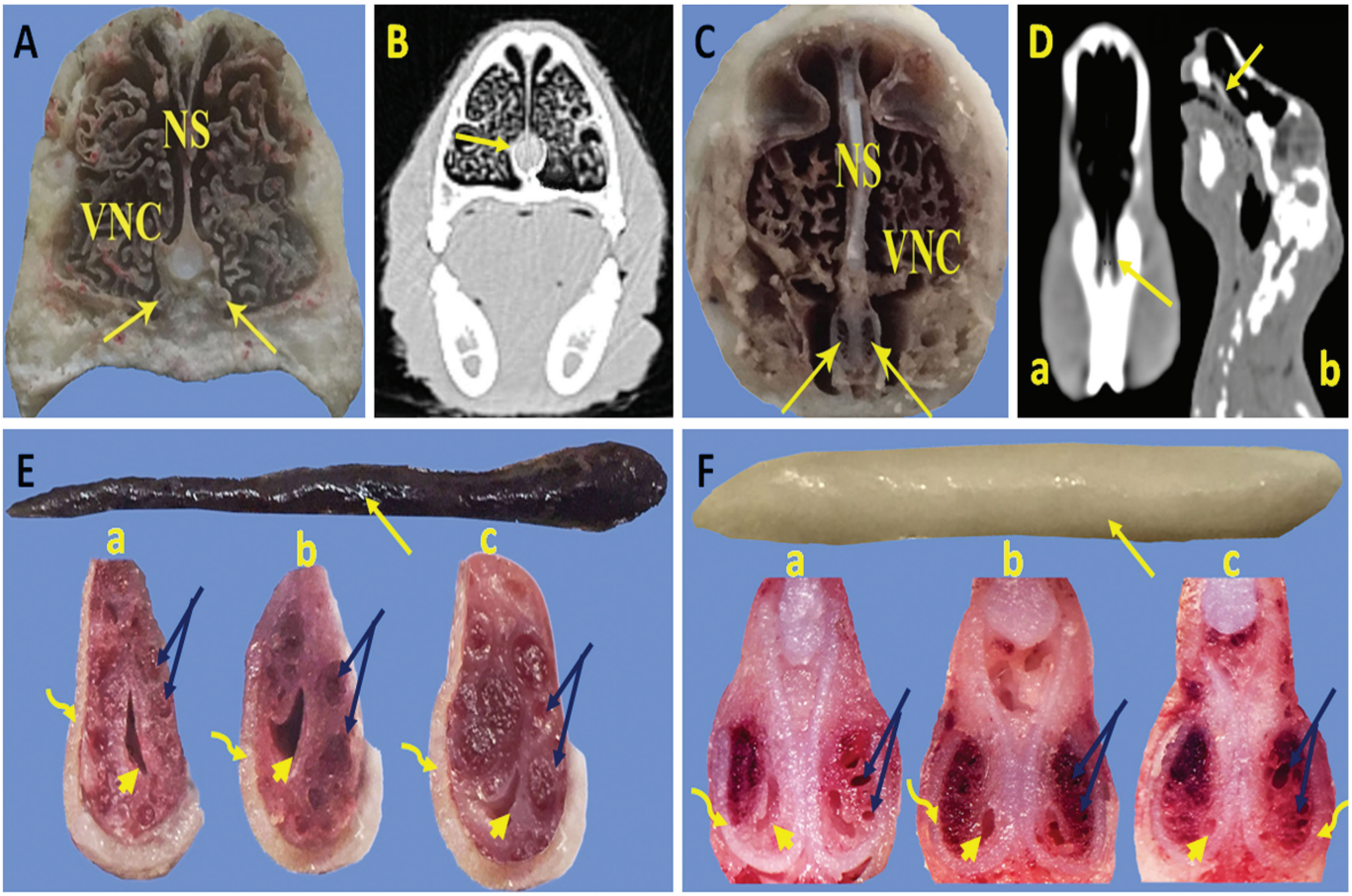

Figure 2. Frozen cross section in the middle of the head of the dog (A) CT image of the head of the dog (B) Frozen cross section in the middle of the head of the rabbit (C) CT image of the head of the rabbit (D) Cross section (a) Sagittal section (b) showing: the vomeronasal organ (arrow), the VNC, and the NS. Separated vomeronasal organ (yellow arrow) of the dog (E) and rabbit (F) crossed in the cranial (a), middle (b), and caudal (c) portions showing VD (arrowhead), VC (zigzag arrow), and BV (blue arrows).

nasopalatine duct in both species was a short tube and continued as a groove caudally in the floor of the nasal cavity; moreover, the duct of the dog was funnel-shaped (Figs. 1C and D, 3A–D). It was approximately 2 cm in length and 0.4 cm in width in dogs and approximately 1.2 cm in length and 0.2 cm in width in rabbits. The duct in both species had two openings, namely, nasal and palatine; the former opening was a wide and oval in dogs, and oblique elliptical in shape in rabbits (Fig. 3A and B). The nasal opening placed at the level of the second canine tooth, 3.2 cm, away from the nostril in dogs and about 1.9 cm in rabbits. The latter opening had 1 cm in length and 0.4 in width in the dog, but in rabbits with 0.2 cm in length and 0.1 in width. The oral (incisive) opening was an elongated slit in dogs and a C-shaped in rabbits (Fig. 3C and D). It had about 0.3 cm length and 0.1 cm width in dogs and 0.2 cm length and 0.09 cm width in rabbits. It is situated caudal to the upper incisor by about 0.4 and 0.3 cm in dogs and rabbits, respectively.

N. vomeronasalis (vomeronasal nerve) in the dogs and rabbits originated from Bulbus olfactorius accessories (accessory olfactory bulb). It left the cranium by one of the foramina of the cribriform plate. The nerve runs along the dorsal margin of the organ and divided into many small branches. Then, the branches were distributed on the medial surface of the vomeronasal organ. Also, the organ in dogs received an additional branch from the palatine nerve, which is distributed in its caudoventral part (Fig. 3E and F). The accessory olfactory bulb of dogs was a small oval in shape, but in rabbits was larger and ovoid. In dogs, it was placed in the posterior part of the main olfactory bulb (MOB), at its junction with the olfactory peduncle (OP). While in rabbits, it embedded in the caudodorsal aspect of the olfactory bulb. (Fig. 3G and H).

Histological findings

The VD was crescent-shaped in dogs and laterally situated with dorsal and ventral walls, while in rabbits, it was oval

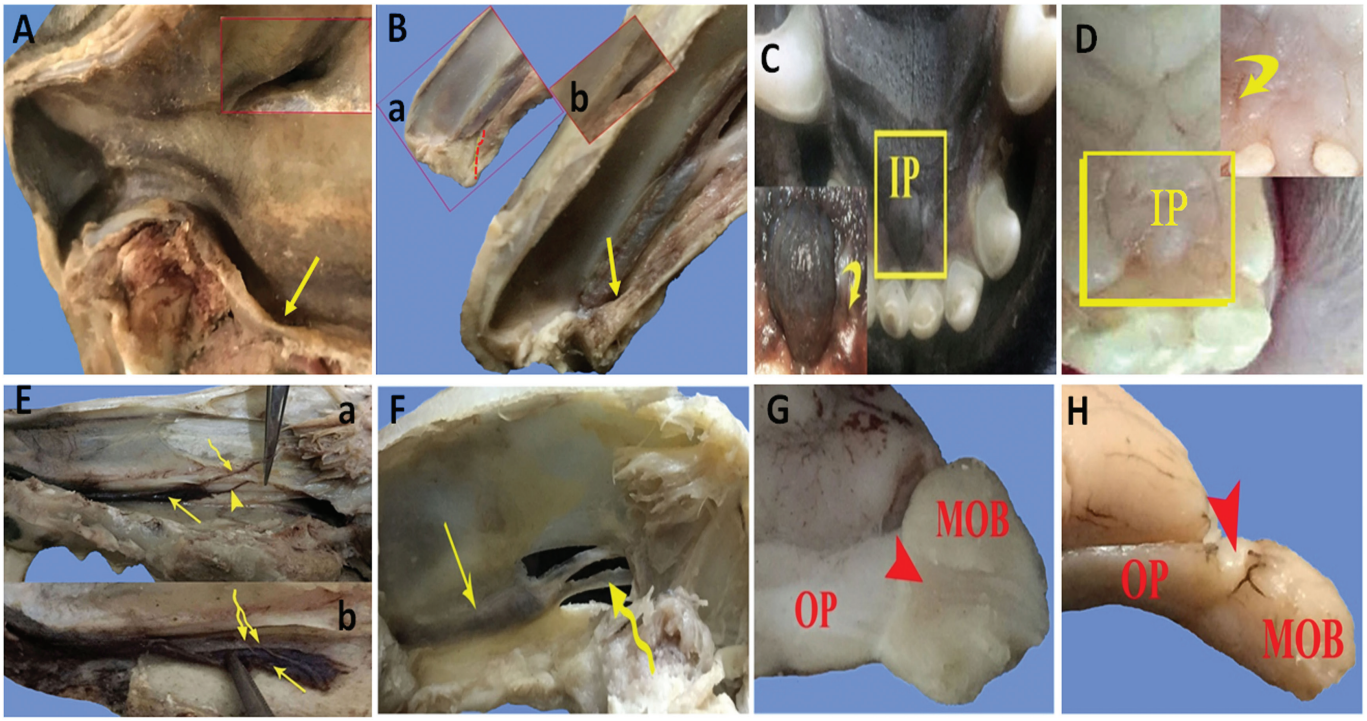

Figure 3. Left view of the nasal cavity of the dog (A) and rabbit (B) showing: the nasal opening of the nasopalatine duct (arrow) with inset (B a) of the nasopalatine duct (dotted red line) and inset (B b) higher magnification of the nasal opening of the nasopalatine duct. Hard palate of the dog (C) and rabbit (D) with IP and inset with stereomicroscope showing the oral opening of the ID (curved arrow). Left view of the nasal cavity of the dog (E a) and rabbit (F) showing: the vomeronasal organ (arrow), vomeronasal nerve (zig-zag arrow), branch from the palatine nerve (arrowhead) and (E b) Small branches of the vomeronasal nerve (double zigzag arrow) on the medial surface of the vomeronasal organ (reflected). Anterior part of the brain of the dog (G) and rabbit (H) showing: accessory olfactory bulb (arrowhead), MOB, and OP.

and medially positioned with lateral and medial walls. Hence, the two angles of duct inversely named in both species. The VD of dogs had a horizontal orientation in the caudal and middle parts and slightly vertical in the cranial one, but, in rabbits, it had a vertical orientation in the three parts of the organ. In dogs, the duct was relatively wide in the caudal part of the organ, although, in rabbits, it was wider in the middle part. The dorsal wall of VD in dogs was convex in caudal and middle parts while in the cranial one was straight as well as the ventral wall was concave in the three parts. In rabbits, the lateral wall of the duct was slightly straight, but the medial one was concave in the three parts of the organ. In dogs, the lateral end of the VD was elongated tapered in the caudal part, pointed in the middle part, and rounded in the cranial one. Moreover, the medial end of the duct was rounded, somewhat elongated tapered, and pointed in caudal, middle, and cranial parts, respectively. In rabbits, the dorsal angle of the duct was rounded in the caudal part and opened in the middle and cranial parts, but the ventral one was pointed in caudal part and rounded in the middle and cranial ones. Lamina propria-submucosa of the vomeronasal organ was composed of connective tissue, BV, nerves, and glands. The BV were numerous with different size and deficient laterally in dogs, and wide condensed laterally in rabbits. The vomeronasal glands in dogs were dorsally situated in the middle and the caudal parts of the organ but slightly fewer in numbers caudally, while it was scantly and laterally located in the cranial part. However, these glands were numerous and abundant dorsally in the three parts of the organ in rabbits. The VC in both species was a J-shaped and its two ends were apart from each other gradually from the caudal to the cranial part of the organ in dogs. In rabbits, its two ends were widely separated dorsally in cranial and middle parts but in caudal one, it was near to each other (Fig. 4A–E).

The vomeronasal epithelium in rabbits was higher in its thickness than that of the dog in the three parts of the organ (Fig. 4F and G). The cranial and middle parts were similar in both species; the lining epithelium of the dorsal (lateral) wall of the VD was (respiratory) pseudostratified columnar epithelium. In dogs, the epithelium contained ciliated and non-ciliated columnar cells with the presence of goblet cells. This epithelium in rabbits was decreased

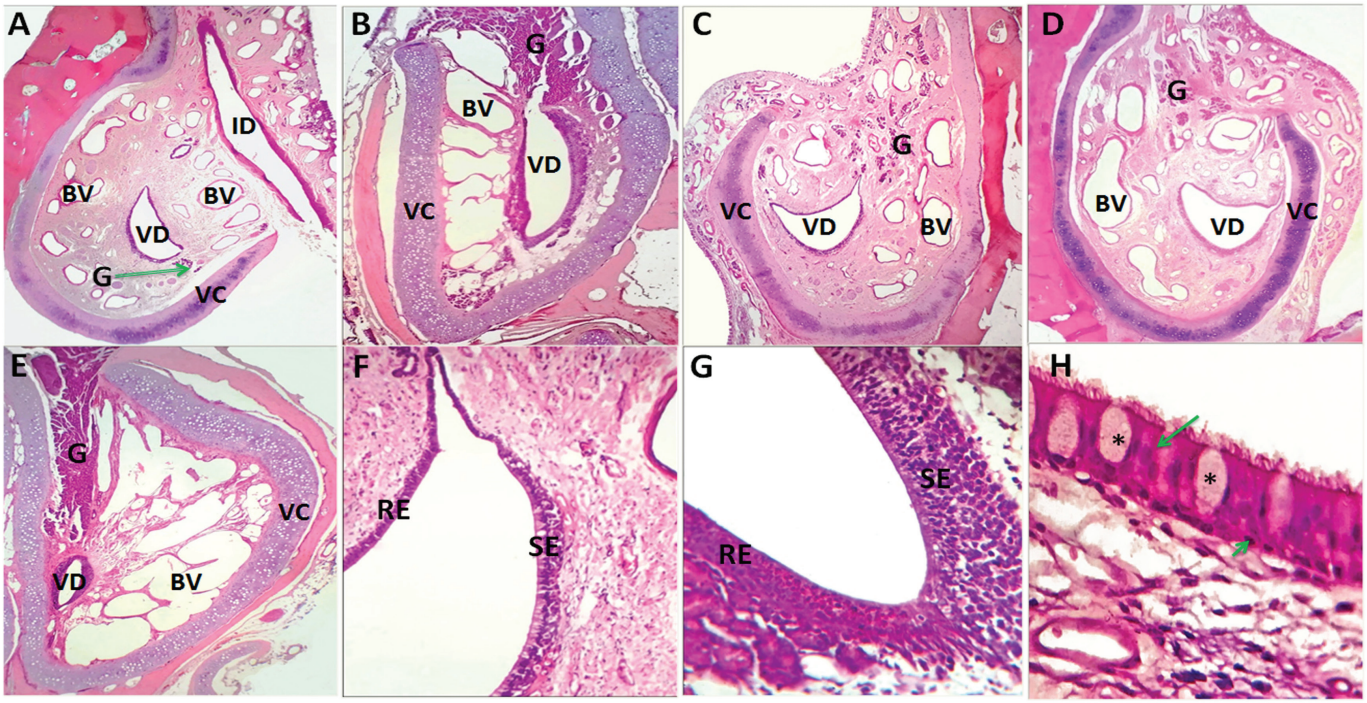

Figure 4. Photomicrographs showing: VC, VD, BV, glands (G), ID, the thin respiratory epithelium (RE), the thicker SE, ciliated columnar cell (arrow), basal cell (arrowhead), and goblet cell (asterisk). (A) Cranial part of the vomeronasal organ of the dog. (B) Middle part of the vomeronasal organ of the rabbit. (C) Middle part of the vomeronasal organ of the dog. (D) Caudal part of the vomeronasal organ of the dog. (E) Caudal part of the vomeronasal organ of the rabbit, H&E stain: Obj. × 4: Oc. × 10. (F) Lining epithelium of the VD of the dog, H&E stain: Obj. × 10: Oc. × 10. (G) Lining epithelium of the VD of the rabbit, H&E stain: Obj. × 40: Oc. × 10. (H) Pseudostratified columnar epithelium lining the dorsal wall of the VD of the dog, H&E stain: Obj. × 100: Oc. × 10.

gradually in thickness and became stratified squamous near the opened dorsal end. The columnar cells had an apical pale acidophilic brush border. Its nucleus was oval and centrally located. The epithelium was rich with flask-shaped ciliated goblet cells with pale acidophilic cytoplasm. The basal cells sited on the basement membrane (Figs. 4H, 5A and B). In both species, the sensory epithelium lining the ventral (medial) wall was composed of three types of cells; supporting, neuron, and basal. The supporting cells (SCs) were columnar with darkly stained, oval, and apical located nuclei. The neuron cells were characterized by pale large rounded nuclei with the clear nucleolus. The basal cells were few in number with the darkly stained and rounded nucleus. In dogs, the height of the epithelium was low and the neuron cells decreased in number with few goblet cells (Fig. 5C and D). The epithelium toward the medial and lateral angle decreased in its height. In the caudal part of the organ of the dog, the ventral and dorsal walls had only the SE with goblet cells but the dorsal one was low in height. This epithelium was rich in neuron cells with few SCs. In rabbits, the medial neural epithelial cells of the caudal part decreased in its number with clear apical microvilli and the basal cells increased in number. The vomeronasal epithelium showed positive reaction with PAS and not reacted with alcian blue, while the cartilage was positively reacted with the latter stain (Fig. 5E–H).

The loose connective tissue of lamina propria-submucosa was abundant with collagen fibers (CFs). It was higher in amount and equally distributed in dogs but in rabbits, it was fewer and condensed medially (Fig. 6A and B). The vomeronasal glands were seromucous in dogs and serous in rabbits. It lined with simple cuboidal with dark basophilic basally located nuclei. The cytoplasm was dark and filled with secretory granules and the lumen was narrow. The duct of the gland was lined with the cuboidal cell with a wide lumen (Fig. 6C and D). These glands gave a positive reaction with PAS and negative reaction with alcian blue stain (Fig. 6E and F).

The accessory olfactory bulb of the rabbit was well developed and differentiated from that of the dog. In rabbits, it comprised of the following layers: vomeronasal nerve layer (VNL) (nervous), glomerular, external plexiform, mitral cells, internal plexiform, and granular. The external plexiform, mitral cells, and internal plexiform layers (IPLs) were not easily distinguishable from each other. The dorsal olfactory tract (DOT) was located

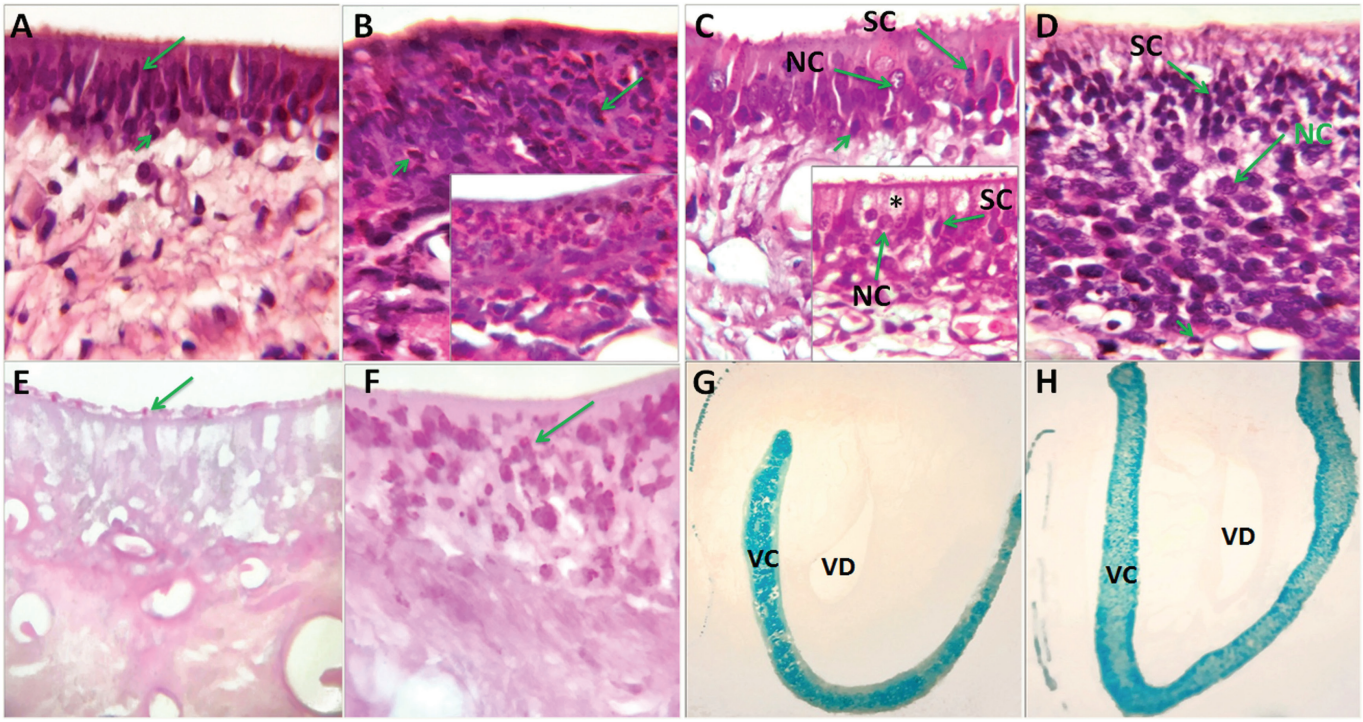

Figure 5. Photomicrographs showing: non-ciliated columnar cell (arrow), basal cell (arrowhead), SC, neuron cell (NC), goblet cell (asterisk), and the pseudostratified columnar epithelium lining the dorsal (lateral) wall of the VD of the dog (A) and rabbit (B) with inset showing stratified squamous epithelium, H&E stain: Obj. × 100: Oc. × 10. The SE lining the ventral (medial) wall of the VD of the dog (C) (with inset showing goblet cell in the SE) and rabbit (D), H&E stain: Obj. × 100: Oc. × 10. PAS positive reaction (arrow) in the SE of the dog (E) and rabbit (F), PAS stain: Obj. × 100: Oc. × 10. Alcian blue positive reaction in the VC and negative reaction in the lining epithelium of the VD of the dog (G) and rabbit (H), Alcian blue stain: Obj. × 4: Oc. × 10.

between the internal plexiform and the granular layers (GRLs). In dogs, the accessory olfactory bulb consisted of only three layers: nervous, glomerular, and mitral/ tufted. The plexiform and GRLs were not recognized in dogs (Fig. 6G and H).

Discussion

The vomeronasal organ of animals reveals certain morphological dissimilarities. It was confirmed that the situation of the organ in the two animals of the current study resembles that in other animals [29]. The well-developed vomeronasal organ in dogs and rabbits and its position accelerates the smelling of pheromones and the developed sniffing sensation.

According to the size of the animal, the vomeronasal organ of the dog extended from the level of the upper third premolar teeth to the third incisive teeth. On the other hand, in rabbits, it had no relation with the upper teeth. The organ terminated at different levels in other animals; second premolar tooth in Labrador retriever dog [15], goat [30], and Iraqi sheep [31] and close to the first and second premolar teeth in red fox [32].

The dimension of the vomeronasal organ varies between the animals, the length of the organ ranged from 2 to 22 cm [33]. In our study, the organ was longer in dogs than in rabbits and the thickest part of the organ was the caudal part in the two species. The average length of the vomeronasal organ of the dog and rabbit was 4.16 and 1.95 cm, respectively. Yilmaz et al. [15] in Labrador retriever dog recorded that it was 2.5 cm in length and the middle part of the organ was the thickest. It was 15 mm in cats [34]. However, sheep and goats had a bodyweight resembling that of dogs, but the length of the organ was 6–8 cm in goat [30], 6–9 cm in Iraqi sheep [31], and 10.6 mm in African giant rat [35]. The vomeronasal organ of rodents [16] and rabbit [36,37] opened in the nasal cavity and indirectly connecting with the oral cavity by the nasopalatine duct, whereas in carnivores [13,34], goat [30], and bears [38], the organ united with the nasopalatine duct and had a direct connection with the oral cavity.

Currently, the VC of the dog and rabbit was a J-shaped and deficient dorsally. Similar results in dogs were also previously noted [13]. In contrast, Yilmaz et al. [15] in Labrador retriever dog reported the cartilage was located only in the lateral portion of the organ. However, Dennis

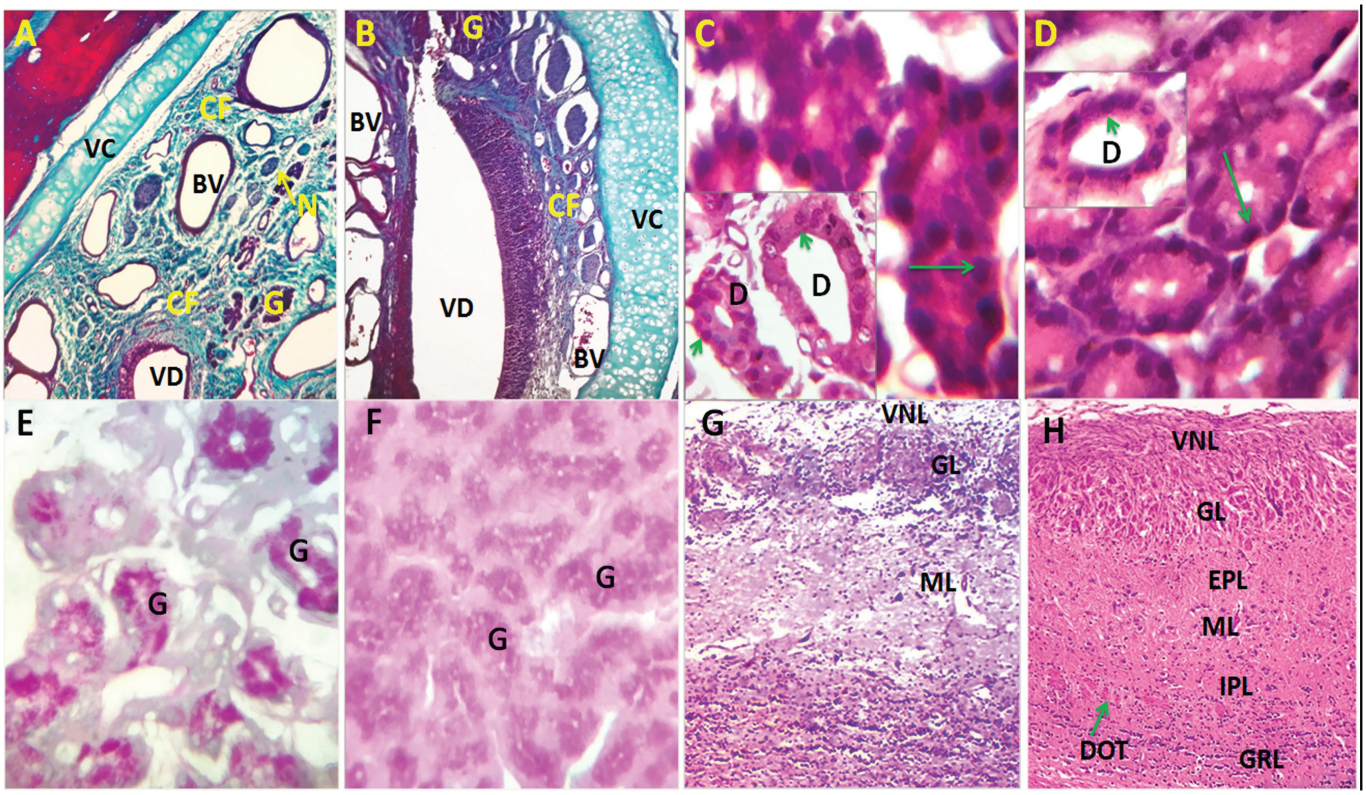

Figure 6. Photomicrographs showing: VC, VD, BV, glands (G), CFs, and nerves (N) in Lamina propria- submucosa of the dog (A) and rabbit (B) Crossmon's trichrome stain: Obj. × 10: Oc. × 10. Simple cuboidal cells (arrow) lining the glandular acini and cuboidal cells (arrowheads) lining the duct (D) of the seromucous glands of the dog (C) and serous glands of the rabbit (D). H&E stain: Obj. × 100: Oc. × 10. PAS positive reaction in the vomeronasal glands (G) of the dog (E) and rabbit (F). PAS stain: × 100 and × 40: Oc. × 10, respectively. VNL, glomerular layer (GL), external plexifom layer (EPL), mitral cell layer (ML), IPL, GRL, and DOT in the accessory olfactory bulb of the dog (G) and rabbit (H) H&E stain: Obj. × 10: Oc. × 10.

et al. [14] reported the organ of the dog was mostly enclosed by a cartilaginous capsule. While in red fox, the cartilage was C-shaped in the foremost parts and J-shaped caudally [32]. On the other hand, the cartilage in goat has completely encircled the duct, J-shaped, and C-shaped in the caudal, middle, and rostral parts, respectively [30]. In rat and mouse, the organ was encircled by a bony capsule [16]. While Mshiri and Tunsi [39] observed incomplete vomeronasal cartilaginous envelope in rodents (Ctenodactylus gundi). In rabbits, Villamayor et al. [36] found that the VC in the caudal part of the organ was ensheathed by a bony capsule.

The shape and position of the accessory olfactory bulb in rabbits confirm the result reported previously by Segovia et al. [22]. On the contrary, McCotter [40] described the accessory olfactory bulb of a rabbit as a small oval prominence. The latter author added that the vomeronasal nerves ranged from three to eight detached bundles and appeared as a single trunk only when passed through lamina cribrosa. Villamayor et al. [36], in rabbits, reported an additional branch from the nasal caudal nerve. Salazar et al. [20] described the accessory olfactory bulb of a dog as a small ovoid protrusion. Also, in dogs, McCotter [40] reported four bundles of the vomeronasal nerves, which conflicted with our results.

Our histological interpretations on the vomeronasal organ go away with the anatomical studies to exhibit their own association with the olfaction. Our observations were in accordance with Taniguchi and Mochizuki [16]; the cross section of the vomeronasal organ in rabbits was slightly elliptical or oval, while in rat and mouse, it was nearly rounded with a wide crescent duct. The present investigation revealed the VD was crescent-shaped and laterally situated in dogs, while in rabbits, it was oval and medially positioned. Comparable findings in dogs were reported by Adams and Wiekamp [13]. In cats, the duct was crescent-shaped in the middle part of the organ [34] and medially located in sheep [41].

There was a general agreement that lamina propria-submucosa of the vomeronasal organ of different animal species as well as the dog and rabbit of the current study formed from connective tissue, BV, nerves, and

glands [12], such organization supported the sexual odors olfaction. In the present study, lamina propria-submucosa of the organ was rich with vascular tissues that recommended changing the pressure within the tube and hence altering its liquid or air contents [41].

Concerning to the venous sinuses of the vomeronasal organ in rabbits, rat and mouse were abundant and located on the lateral wall of the duct, as well as these sinuses were enclosed by a smooth muscle layer in rat [16] and rabbit [36]. The vomeronasal glands were voluminous and of serous type in rabbits, other than were seromucous in dogs and mostly dorsally situated in the organ. These glands were of mucous type in rats, mice, and rabbits [16]. However, mixed mucous and serous vomeronasal glands were reported in the hamster [42]. On the contrary, Kratzing [41] in sheep reported that the glands were present only under the non- SE and of mucous type. However, in Gazella subgutturosa, there were myoepithelial cells surrounded the mucous glands, which lined with low columnar cells [43]. The vomeronasal glands gave a positive reaction with PAS but not reacted with alcian blue stain as mentioned in rabbits [36], cats [34], and goats [30]. Other animals shared the two reactions, such as bears [38] and cows [11].

Our work revealed that the VD of dogs and rabbits had medial sensory and lateral respiratory epithelium. Similar results were reported in dogs [13,14], rabbits [16,37], cats [44], sheep [41], and pigs [10]. Also, we found areas of stratified squamous near the dorsal end of the duct in rabbits and the caudal part of the organ of the dog had only the SE. In addition, in rats [35] and cats [34], four kinds of epithelium were recognized in the VD; the epithelium became simple columnar caudally and stratified squamous cranially. In contrast, the blind mole rats had only one type of epithelium; the sensory one [45].

The lamination of the accessory olfactory bulb of rabbits and rats was clearer than that of the dog [19,20–22,46]. The plexiform, mitral/tufted, and granular cell layers were indistinct in dogs [19,20]. However, the mitral/tufted cell layer was recorded in rat [47], it scantily distinct in ferret [48]. The structural findings of the vomeronasal organ in rabbits characterized by the thickening of the SE, the voluminous glands, and the larger well-developed accessory olfactory bulb. This result gave an indication that the organ of a rabbit was more functional than that of a dog in detection and response to the pheromonal stimuli which were very important in socio-sexual communication.

Conclusion

The most characteristic structural variations achieved in the vomeronasal organ and the accessory olfactory bulb of dogs and rabbits gave an indication that the organ was more functional in rabbits than in dogs. The SE of the vomeronasal organ indicated the detection and response to the pheromones which were very important in socio-sexual communication in the animals.

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Conflict of interests

The authors declare that they have no conflict of interest.

Authors' contribution

All authors performed all the anatomical and the histological techniques together in addition to writing the paper, reviewing, preparing, and approving the final manuscript.

References

[1] Teicher MH, Shaywitz BA, Lumia AR. Olfactory and vomeronasal system mediation of maternal recognition in the developing rat. Dev Brain Res 1984; 12(1):97–110; https://doi.org/10.1016/0165-3806(84)90179-2

[2] Halpern M. The organization and function of the vomeronasal system. Ann Rev Neurosci 1987; 10:325–62.

[3] Halpern M, Martinez-Marco A. Structure and function of the vomeronasal system: an update. Prog Neurobiol 2003; 70:245–318; https://doi.org/10.1016/s0301-0082(03)00103-5

[4] Grüneberg H. A ganglion probably belonging to the N. terminalis system in the nasal mucosa of the mouse. Z Anat Entwickl Gesch 1973; 140:39–52; https://doi.org/10.1007/bf00520716

[5] Brechbühl J, Klaey M, Broillet MC. Grueneberg ganglion cells mediate alarm pheromone detection in mice. Science 2008; 321(5892):1092–5; https://doi.org/10.1126/science.1160770

[6] Barrios WA, Sánchez-Quinteiro P, Salazar I. Dog and mouse: toward a balanced view of the mammalian olfactory system. Front Neuroanat 2014; 8:106; https://doi.org/10.3389/fnana.2014.00106

[7] Igbokwe ECO. The role of main olfactory and vomeronasal system in animal behavior and reproduction. Anim Res Int 2009; 6(3):1093–101; https://doi.org/10.4314/ari.v6i3.55994

[8] Karger S, Basel AG. Taste and smell: an update. In: Hummel T, Welge-Lüssen A (eds.). Advance oOtorhinolaryngology, vol. 63, pp 70–83, 2006.

[9] Bhatnagar KP, Meisami E. Vomeronasal organ in bats and primates: extremes of structural variability and its phylogenetic implications. Microsc Res Tech 1998; 43:465–75; https://doi.org/10.1002/(sici)1097-0029(19981215)43:6<465::aid-jemt1>3.0.co;2-1

[10] Salazar I, Sanchez-Quinteiro PS, Lombardero M, Cifuentes JM. Adescriptive and comparative lectin histochemical study of the vomeronasal system in pigs and sheep. J Anat 2000; 196(1):15–22; https://doi.org/10.1046/j.1469-7580.2000.19610015.x

[11] Salazar I, Sanchez-Quinteiro PS, Alemañ N, Prieto D. Anatomical, immnunohistochemical and physiological characteristics of the vomeronasal vessels in cows and their possible role in

vomeronasal reception. J Anat 2008; 212:686–96; https://doi.org/10.1111/j.1469-7580.2008.00889.x
[12] Kassab A, El-Shafey A. Light and Scanning microscopic study on the vomeronasal organ of the buffalo (*Bos bubalis*). Global Vet 2012; 8(5):491–7.
[13] Adams DR, Wiekamp M. The canine vomeronasal organ. J Anat 1984; 138:771–87.
[14] Dennis JC, Allgier JG, Desouza LS, Eward WC, Morrison EE. Immunohistochemistry of the canine vomeronasal organ. J Anat 2003; 202:515–24; https://doi.org/10.1046/j.1469-7580.2003.00190.x
[15] Yilmaz B, Yildiz H, Akkoc C, Arican I. Vomeronasal organ in labrador retriever dog (*Canis familiaris*). Bull Vet Inst Pulawy 2008; 52:185–8.
[16] Taniguchi k, Mochizuki k. Comparative morphological studies on the vomeronasal organ in rats, mice, and rabbits. Japanese Soc Vet Sci I983; 45(1):67–76; https://doi.org/10.1292/jvms1939.45.67
[17] Othman MA. Postnatal development of the female rabbit vomerosensory epithelium: a light and electron microscopic study. Egypt J Histol 2011; 34:69–79; https://doi.org/10.1097/01.ehx.0000394886.69777.b0
[18] Elgayar SA, Eltony SA, Othman MA. Morphology of nonsensory epithelium during postnatal development of the rabbit vomeronasal organ. Anat Histol Embryol (2014); 43:282–93; https://doi.org/10.1111/ahe.12073
[19] Salazar I, Cifuentes JM, Quinteiro PS, Caballero TG. Structural, morphometric, and lmmunohistological study of the accessory olfactory bulb in the dog. Anat Rec 1994; 240:277–85; https://doi.org/10.1002/ar.1092400216
[20] Salazar I, Cifuentes JM, Quinteiro PS. Morphological and immunohistochemical features of the vomeronasal system in dogs. Anat Rec 2013; 296:146–55; https://doi.org/10.1002/ar.22617
[21] Mori K, Imamura K, Fujita SC, Obata K. Projections of two subclasses of vomeronasal nerve fibers to the accessory olfactory bulb in the rabbit. Neurosci 1987; 20(I):259–18; https://doi.org/10.1016/0306-4522(87)90018-2
[22] Segovia S, Garcia-Falgueras A, Carrillo B, Collado P, Pinos H, Perez-Laso C, et al. Sexual dimorphism in the vomeronasal system of the rabbit. Brain Res 2006; 1102:52–62; https://doi.org/10.1016/j.brainres.2006.05.017
[23] Zufall F, Munger SD. From odor and pheromone transduction to the organization of the sense of smell. Trends Neurosci 2001; 24:191–3; https://doi.org/10.1016/s0166-2236(00)01765-3
[24] Chamero P, Marton TF, Logan DW, Flanagan K, Cruz JR, Saghatelian A, et al. Identification of protein pheromones that promote aggressive behavior. Nature 2007; 450:899–902; https://doi.org/10.1038/nature05997
[25] Leinders-Zufall T, Ishii T, Chamero P, Hendrix P, Oboti L, Schmid A, et al. A family of nonclassical class I HMC genes contributes to ultrasensitive chemodetection by mouse vomeronasal sensory neurons. J Neurosci 2014; 34:5121–33; https://doi.org/10.1523/jneurosci.0186-14.2014
[26] Hall LW, Clarke KW, Trim CM. Veterinary anesthesia. 10th edition, WB Saunders, Harcourt Publishers Limited, New York, pp 441–66, 2001.
[27] Nomina Anatomica Veterinaria. 5th edition, prepared by the International Committe on Veterinary Gross Anatomical Nomenclature (I.C.V.G.A.N.) and authorized by the General assembly of the World Association of Veterinary Anatomists (W.A.V.A.), konxville,T.N (USA). Editorial Committee, Hannover, Columbia, Ghent and Sapporo, p 55, 2012.
[28] Bancroft JD, Gamble M. Theory and practice of histological techniques, 6th edition, Churchill Livingstone, Edinburgh, London, UK, 2008.
[29] Kostov DL. Vomeronasal organ in domestic animals (a short survey). Bulgarian J Vet Med 2007; 10(1):53–7.
[30] Moawad UK, Awaad AS, Abedellaah BA. Morphological, histochemical and computed tomography on the vomeronasal organ (Jacobson's organ) of Egyptian native breeds of goats (*Capra hircus*). Beni-Suef Univ J Basic Appl Sci 2017; 6:174–83; https://doi.org/10.1016/j.bjbas.2017.03.003
[31] Abass TA, Al-Mayahi MS, Al-Hussany BF. Anatomical and histological investigate of vomeronasal organ (VNO) in Iraqi sheep Alawasi. Kufa J Vet Med Sci 2012; 3(1):98–112.
[32] Karimi H, Hassanzadeh B, Razmaraii N. Structure of vomeronasal organ (Jacobson) in the male red fox (*Vulpes vulpes*). Anat Sci 2016; 13(1):47–54.
[33] Takigami S, Mori Y, Ichikawa M. Projection pattern of vomeronasal neurons to the accessory olfactory bulb in goats. Chem. Senses 2000; 126:325–41; https://doi.org/10.1093/chemse/25.4.387
[34] Salazar I, Quinteiro PS, Cifuentes JM, Caballero TG. The vomeronasal organ of the cat. J Anat 1996; 188:445–54.
[35] Igbokwe CO, Nwaogu IC. Histological studies of the vomeronasal organ of African Giant rat (*Cricetomys gambianus*, waterhouse). Anim Res Int 2009; 6(2):1003–8; https://doi.org/10.4314/ari.v6i2.48132
[36] Villamayor PR, Cifuentes JM, Fdz.-de-Troconiz P, Sanchez-Quinteiro P. Morphological and immunohistochemical study of the rabbit vomeronasal organ. J Anat 2018; 233:814–27; https://doi.org/10.1111/joa.12884
[37] ALomaisi SAMA, El-Ghazali HM, Nosseur HM, Ahmed SA, Konsowa MM. Prenatal development of the vomeronasal organ in rabbit. Slov Vet Res 2019; 56:623–32; https://doi.org/10.26873/svr-801-2019
[38] Tomiyasu J, Kondoh D, Sakamoto H, Matsumoto N, Sasaki M, Kitamura N, et al. Morphological and histological features of the vomeronasal organ in the brown bear. J Anat 2017; 231:749–57; https://doi.org/10.1111/joa.12673
[39] Mshiri OA, Tunsi HM. Anatomical and histological study of the vomeronasal organ in (*Ctenodactylus gundi*). J Med Care Res Rev 2019; 2:190–5.
[40] McCotter RE. The connection of the vomeronasal nerves with the accessory olfactory bulb in the Opossum and other mammals. Anat Rec 1912; 6(8):299–318; https://doi.org/10.1002/ar.1090060802
[41] Kratzing JE. The structure of the vomeronasal organ in the sheep. J Anat 1971; 108:247–60.
[42] Taniguchi K, Mochizuki K. Morphological studies on the vomeronasal organ in the golden hamster. Nippon Juigaku Zasshi 1982; 44:419–26.
[43] Abood DA, Hussein ZM. Histological and histochemical features for Jacobson's glands in male gazella subgutturosa. Indian J Nat Sci 2018; (8):13107–14.
[44] Kogure N, Amemori T, Mizugushi S, Kimura J, Tsukise A, Okano M. Scanning electron microscopical study on the feline vomeronasal organ. Bull Coll Agric Vet Med, Nihon Univ 1989; 46:108–16.
[45] Zuri I, Fishelson L, Terkel J. Morphology and cytology of the nasal cavity and vomeronasal organ in juvenile and adult blind mole rats (*Spalax ehrenbergi*). Anat Rec 1998; 251:460–71; https://doi.org/10.1002/(sici)1097-0185(199808)251:4<460::aid-ar5>3.0.co;2-w
[46] Meisami E, Bhatnagar KP. Structure and diversity in mammalian accessory olfactory bulb. Microsc Res Tech 1998; 43:476–99; https://doi.org/10.1002/(sici)1097-0029(19981215)43:6<476::aid-jemt2>3.3.co;2-m
[47] Takami S, Graziadei PPC. Light microscopic Golgi study of mitral/tufted cells in the accessory olfactory bulb of the adult rat. J Comp Neurol 1991; 311:65–83; https://doi.org/10.1002/cne.903110106
[48] Kelliher K, Baum MJ, Meredith M. The ferret's vomeronasal organ and accessory olfactory bulb: effect of hormone manipulation in adult males and females. Anat Rec 2001; 263:280–8; https://doi.org/10.1002/ar.1097