**RESEARCH ARTICLE**
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.2.432-439

# Histomorphological studies on the vomeronasal organ in domestic pig (*Sus scrofa domesticus*) #

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Citation: Nikhila, K.P.S.N., Raja, K., Lakshmi, M.S., Kumar, R.V.S., Damini, V., Rao, T.S.C., Supriya, B. and Chakravarthy, M.K. Histomorphological studies on the vomeronasal organ in domestic pig (*Sus scrofa domesticus*). *J. Vet. Anim. Sci.* **55**(2):432-439
DOI: https://doi.org/10.51966/jvas.2024.55.2.432-439

*Received: 05.01.2024* *Accepted: 30.03.2024* *Published: 30.06.2024*

**Abstract**

*The present study was undertaken on vomeronasal organ in SVVU T-17 breed of domestic pig at different levels i.e., papilla incisiva, first palatine rugae, third palatine rugae and fifth palatine rugae. The vomeronasal organ consisted of an internal epithelial duct, middle propria submucosa and cartilage. At the level of papilla incisiva, it was lined by stratified squamous non-keratinized epithelium. At the level of first palatine rugae, stratified squamous non-keratinized epithelium to respiratory epithelium was seen. At the level of third palatine rugae, lateral and medial walls were lined by respiratory and olfactory epithelium, respectively. At the level of fifth palatine rugae, both the walls were lined by olfactory epithelium. The propria submucosa had dense connective tissue at the level of papilla incisiva whereas, loose connective tissue was observed at remaining levels with collagen, elastic and reticular fibres. Nerve bundles were observed at the level of third and fifth palatine rugae levels. The cartilage was incomplete dorsolaterally at papilla incisiva and first rugae level whereas, incomplete dorsally at remaining levels.*

**Key words**: *Vomeronasal organ, vomeronasal duct, domestic pig*

*# Part of MVSc thesis submitted to Sri Venkateswara Veterinary University Tirupati, Andhra Pradesh*

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The nasal cavity is the first segment of the respiratory tract and plays an important role in filtering, warming and moistening inspired air. Additionally, it contributes to the sense of olfaction. The vomeronasal organ enhances olfactory function, aiding in the discrimination of odours (Kumar, 1991). The vomeronasal organ in pig is a paired tubular structure that is situated on the ventral aspect of the median nasal septum on either side of the vomer bone. The organ is extended from the level of papilla incisiva to the level of fifth palatine rugae (Kumar *et al.,* 2016). The vomeronasal duct is an epithelial tissue forming a hollow tube with sensory and non-sensory epithelium on the walls of the duct. This intricate structure plays a crucial role in detecting chemical signals and enhancing olfactory capabilities in domestic animals (ICVGAN, 1994). The olfaction holds a significant importance for most mammals, particularly for those whose young were born with underdeveloped visual and auditory abilities. In such cases, the detection of specific pheromones became crucial for the offspring to identify their mothers (Teicher *et al*., 1984 and Halpern, 1987). The "Flehmen response" is a distinct facial movement observed in various mammals, particularly felids and ungulates aimed at directing inhaled substances to the vomeronasal organ (Abbasi and Khosravinia, 2002, Besoluk *et al*., 2001 and Kostov, 2007). The literature available on vomeronasal organ in domestic pig is very scanty. Hence the present study is undertaken to elucidate the histomorphological studies on vomeronasal organ in domestic pigs.

**Materials and methods**

For histomorphological studies, the vomeronasal organ was collected from domestic pigs after slaughter in AICRP on pigs, SVVU, Tirupati, at the level of papilla incisiva and first, third and fifth palatine rugae of hard palate. The specimens were washed in normal saline and were fixed in 10% Neutral Buffered Formalin. Further, the tissue samples were subjected to routine tissue processing techniques and embedded in paraffin wax (58-60 °C). The sections of 5-6 µm thickness were obtained on clean adhesive smeared glass slides with the help of Leica semi-automatic microtome (Leica RM2125RTS) and were subjected to Haematoxylin and Eosin method for routine histological examination, Masson's trichrome for collagen fibres, Verhoeff's method for elastic fibres, Gomori's method for reticular fibres, Bielschoswky method for nerve fibres (Singh and Sulochana, 1997, Bancroft and Gamble, 2008). Micrometrical studies on the mean thickness of the epithelium, propria submucosa (medial and lateral wall) and cartilage of the vomeronasal organ was measured statistically and analysed by using the one-way Anova (Snedecor and Cochran, 1994).

**Results and discussion**

At the level of papilla incisiva, the vomeronasal duct had a narrow elliptical lumen rostrally which became wider caudally. The medial and lateral walls of the duct were lined by stratified squamous non-keratinized epithelium (Fig. 1). Similar observations were reported by Vaccarezza *et al.* (1981) in rats. However, Salazar *et al.* (1997) in pigs, cows and horses observed stratified cuboidal or columnar epithelium. The epithelial height of the medial wall (58.5 ± 5.45 µm) was greater than the lateral wall (47.4 ± 2.08 µm). The stratified squamous non-keratinized epithelium contained three layers namely stratum basale, stratum spinosum and stratum granulosum arranged from outside to the luminal side (Fig. 2). The propria submucosa of the vomeronasal organ had dense irregular connective tissue with collagen, elastic and reticular fibres as reported by Ganganaik *et al*. (2006) in sheep. Lymphocytic infiltration was also observed in the sub-epithelial connective tissue around the opening of glandular ducts. The vomeronasal glands were seromucous in nature and appeared more at the dorsal aspect of the duct which opened into the lumen of the vomeronasal duct on both walls. Apparently, the thickness of the medial propria submucosa (1047.79 ± 76.89 µm) was more than the lateral propria submucosa (398.51 ± 70.05 µm) (Fig. 1). The propria submucosa of both sides was continuous with each other towards the dorsal and ventral sides of the vomeronasal organ. The cartilage was C-shaped as it was incomplete in the dorsolateral region as reported by Moawad *et al*. (2017) in goats. Contrary to present study, the cartilage was U-shaped in sheep (Abass *et*

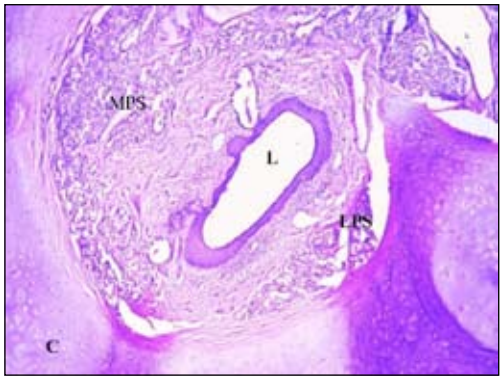


**Fig. 1.** Photomicrograph of VNO in pig at papilla incisiva level showing Lumen (L), Medial propria submucosa (MPS) and lateral propria submucosa. H&E X 40

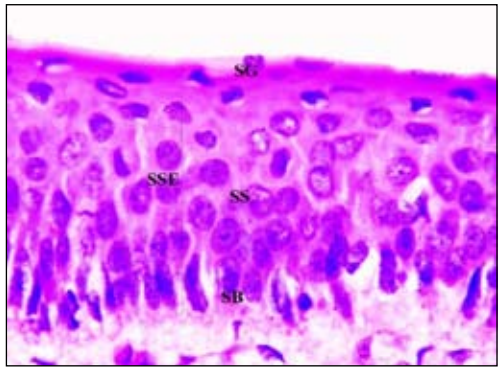


**Fig. 2**. Photomicrograph showing stratified squamous non-keratinised epithelium (SSE) with stratum granulosum (SG), stratum spinosum (SS) and stratum basale (SB) at the level of papilla incisiva in pig. H&E X 100

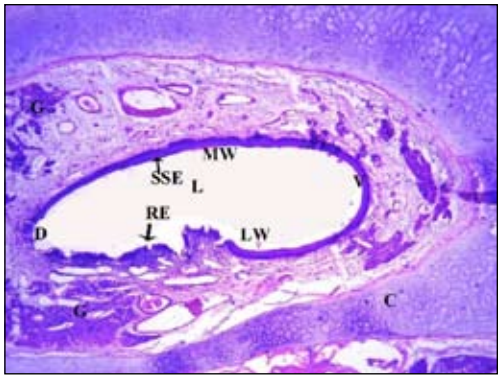


**Fig. 3.** Photomicrograph of VNO in pigs at the level of first rugae showing the transition of stratified squamous non- keratinised (SSE) of medial wall (MW) to respiratory epithelium (RE) of lateral wall (LW).D-Dorsal, V-Ventral, L-Lumen, G-Glands, C-Cartilage. H&E X 40

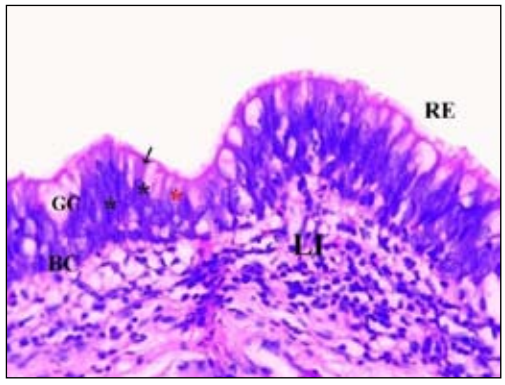


**Fig. 4.** Photomicrograph showing the respiratory epithelium (RE) with basal cells (BC), ciliated columnar cells (*), non-ciliated columnar cells (*), goblet cells (GC) and cilia (Arrow). LI-Lymphocytic infiltration. H&E X 400

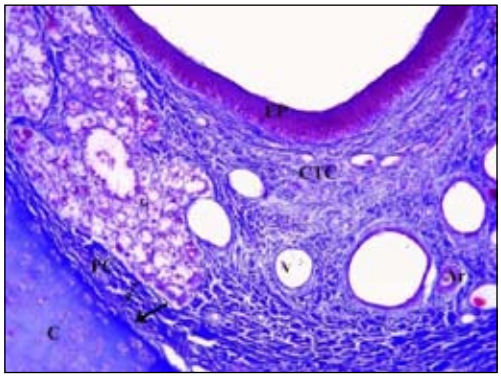


**Fig. 5**. Photomicrograph of VNO in pig at first rugae showing the distribution of collagen fibres (CF) in the subepithelial connective tissue around glands (G), veins (V) and near to the perichondrium(PC) of cartilage (C). EP-Epithelium, C-Cartilage.
Masson's Trichrome method X 100

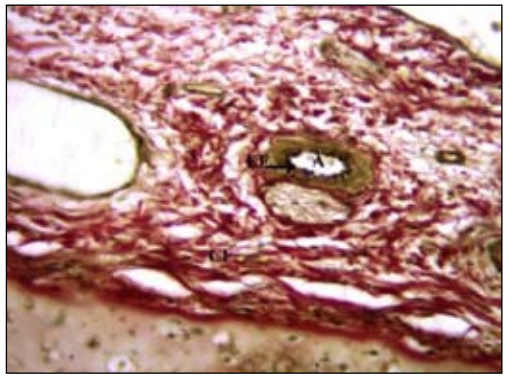


**Fig. 6**. Photomicrograph of VNO in pig at first rugae showing elastic fibres (EF) in the tunica intima of artery (A). CF-Collagen fibres.
Verhoeff's method X 400

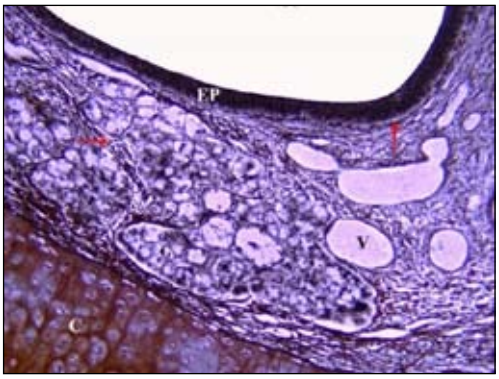


**Fig. 7.** Photomicrograph of VNO in pig at the level of first rugae showing the distribution of reticular fibres (Red Arrow) surrounding the basement membrane of epithelium and glands. EP-Epithelium, V-Vein. Gomori's Reticulum method X 100

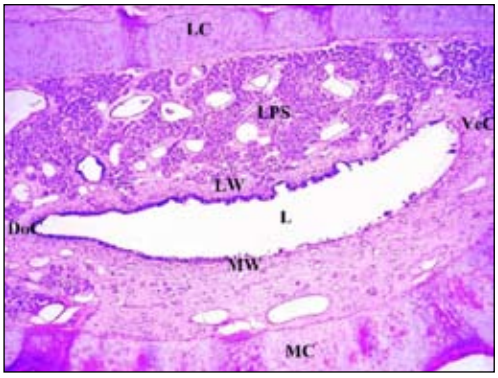


**Fig. 8.** Photomicrograph of VNO in pig at the level of third rugae showing lateral wall (LW) and medial (MW) wall lined by respiratory and olfactory epithelium.L-Lumen, LPS-Lateral propria submucosa, DoC-Dorsal commissure, VeC- Ventral commissure, LC-Lateral cartilage, MC-Medial cartilage. H&E X 40

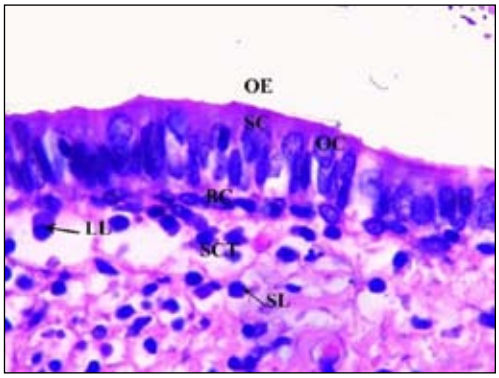


**Fig. 9.** Photomicrograph of VNO in pig at the level of third rugae showing the olfactory epithelium (OE) with basal cells (BC), olfactory cells (OC) and supporting cells (SC). LL-Large lymphocyte, SL-Small lymphocyte. H&E X 1000

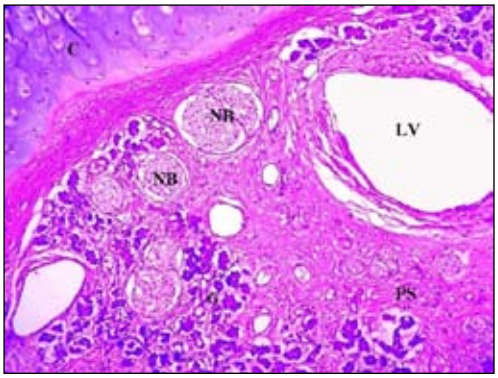


**Fig. 10**. Photomicrograph of VNO in pig at the level of third rugae showing the distribution of nerve bundles (NB) in the deeper portion of propria submucosa (PS). LV-Large vein, C-Cartilage. H&E X 100

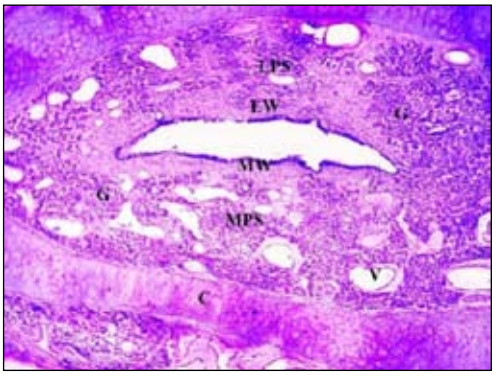


**Fig. 11.** Photomicrograph of VNO in pig at the level of fifth rugae showing its lateral (LW) and medial walls (MW) lined by olfactory epithelium. LPS-Lateral propria submucosa, MPS-Medial propria submucosa, V-Vein, G- Gland, C- Cartilage. H& E X 40

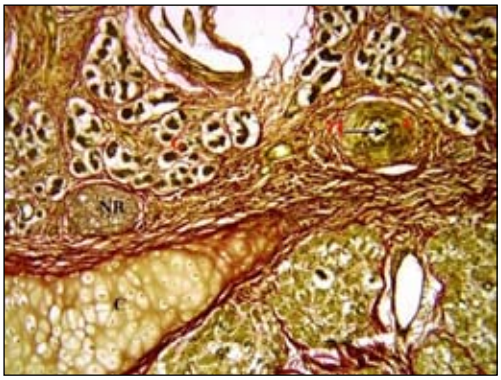


**Fig. 12** Photomicrograph of VNO in pig at the level of fifth rugae showing elastic fibres (EF) in the tunica intima of artery (A). NB-Nerve bundle, C- Cartilage. Verhoeff's method X 400

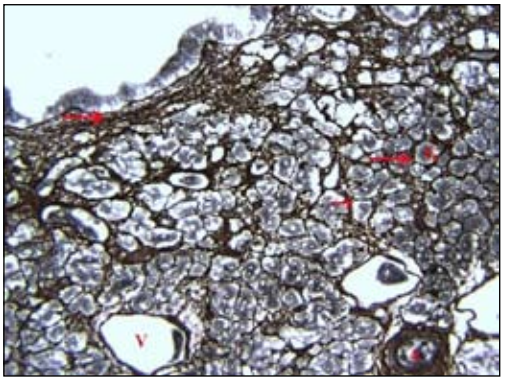


**Fig. 13.** Photomicrograph showing the reticular fibres (Red Arrow) in the stroma of glands (G) and around the basement membrane of epithelium at the level of fifth palatine rugae in pig. V-Vein. Gomori's Reticulum method X 100

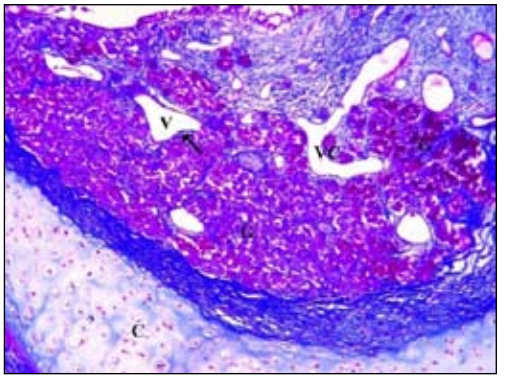


**Fig. 14.** Photomicrograph showing the distribution of dense collagen fibres (Arrow) near to the cartilage (C). V-Vein, VC-Venous caverns. Masson's Trichrome method X 100

*al*., 2012) and cartilage was absent in camel (Karimi *et al*., 2014).

At the level of first palatine rugae, the entire medial and ventral part of the lateral walls were lined by stratified squamous non-keratinized epithelium. Occasionally, the dorsal half of the lateral wall was lined by pseudostratified ciliated columnar epithelium with goblet cells (Fig.3) as reported by Kumar *et al*. (2016) in young pigs. However, Ganganaik *et al*. (2006) in sheep reported that both the walls were lined with pseudostratified ciliated columnar epithelium with goblet cells. The lateral and medial walls of the vomeronasal duct were lined by respiratory and olfactory epithelia, respectively according to Kassab and El-Shafey (2012) in buffalo, Besoluk *et al.* (2001) in Angora goats and Abbasi and Khosravania (2002) in Lori sheep. The pseudostratified ciliated columnar epithelial lining contained four different cells namely basal cells, ciliated columnar cells, non-ciliated columnar cells and goblet cells (Fig. 4). The epithelium lining the lateral wall of the vomeronasal duct showed an undulating appearance and contained short crypts along its length, while the medial wall epithelial lining was continuous in a linear fashion without any crypts as reported by Abbasi (2007) in buffalo and Abbasi and Khosravinia (2002) in Lori sheep. The medial wall epithelial lining dipped down at several places to form crypts lined by mucous-producing cells in sheep (Kratzing, 1971). The epithelial height of lateral wall (57.27 ± 12.12 µm) was comparatively greater than that of medial wall (56.66 ± 8.511 µm). Diffused lymphocytic infiltration was noticed in the underlying connective tissue. The propria submucosa of vomeronasal organ was made up of loose connective tissue composed of connective tissue fibres *viz*., collagen (Fig. 5), a few elastic (Fig. 6) and reticular fibres (Fig.7). On contrary to this, Kumar *et al*. (2016) in young pigs and Ganganaik *et al*. (2006) in sheep reported that the propria submucosa was made of dense irregular connective tissue. The collagen fibres were densely packed towards the cartilage and they were wavy, arranged parallel nearer to the outer surface of the glands (Fig.5).The medial propria submucosa (787.26 ± 87.94 µm) was comparatively larger than the lateral propria submucosa (499.65 ± 31.882 µm) with more seromucous glands in addition to different sizes of blood vessels and venous caverns.The C-shaped hyaline cartilage surrounded the vomeronasal organ except at the dorsolateral region which was in accordance with the observations reported by Kratzing (1971) in sheep, Samuelson (2007) in domestic animals and Siraj *et al*. (2020)in dogs. However, cartilage was absent in camel (Karimi *et al*., 2014). The connective tissue fibres especially elastic and collagen fibres were abundant closer to the perichondrium of the hyaline cartilage.

At the level of third palatine rugae, the vomeronasal duct showed a narrow vertical lumen with epithelial lining of two different types. The medial wall was lined by olfactory

epithelium while the lateral wall was lined by respiratory epithelium (Fig. 8). Three different cells namely basal cells, olfactory cells and supporting cells lined the olfactory epithelium (Fig.9). These observations were concurrent with the findings of Kratzing (1971) in sheep, Hare (1975) in dogs and pigs, Besoluk *et al.* (2001) in Angora goats, Abbasi and Khosravinia (2002) in Lori sheep and Abass *et al*. (2012) in Alawasi Iraqi sheep. Contrary to the present study, both the walls were lined by respiratory epithelium in sheep (Ganganaik *et al.,* 2006) and camel (Karimi *et al*., 2014) whereas, both the walls were lined by olfactory epithelium in young pigs (Kumar *et al*., 2016). The epithelial height was more towards the lateral wall (56.66 ± 4.91 µm) than the medial wall (51.93 ± 13.13 µm). The lateral wall showed an undulated appearance and presented several short and long crypts at which the ducts of the glands were opened. The propria submucosa of the vomeronasal organ had loose connective tissue as reported by Kumar *et al*. (2016) in young pigs whereas Ganganaik *et al.* (2006) in sheep and Abass *et al*. (2012) in Alawasi Iraqi sheep stated that it had dense irregular connective tissue. The medial propria submucosa (536.93 ± 66.91 µm) was comparatively thinner than the lateral propria submucosa (875.93 ± 8.95 µm). Lymphocytes were found in varied sizes and shapes. Numerous nerve bundles with unmyelinated axons were observed in the deeper portions of medial propria submucosa (Fig.10) as reported by Salazar *et al*. (1996) in cats, Kumar *et al*. (2016) in young pigs and Kratzing (1971) in sheep. The medial propria submucosa contained fewer glands compared with the lateral propria submucosa. Few glands and glandular ducts were found to be open into the lateral wall. The vomeronasal organ was completely lined by the cartilage except in the dorsal aspect which had a split-like opening. The shape of the cartilage was round to oval. Similar observations were reported by Kumar *et al*. (2016) in young pigs. Contrary to this, Ganganaik *et al*. (2006) in sheep stated that C-shaped cartilage enclosed the vomeronasal organ which was incomplete dorsomedially. However, pear-shaped cartilage enclosed the vomeronasal organ in buffalo (Abbasi, 2007). Karimi *et al*. (2014) in camel and Moawad *et al*. (2017) in goats found that J-shaped cartilage enclosed the vomeronasal organ. The medial limb (446.39 ± 24.33 µm) of the cartilage was thicker than the lateral limb (358.38 ± 13.02 µm) and extended more dorsally whereas, the lateral limb terminated at the lower portion leaving a small space between the two limbs as reported by Kumar *et al*. (2016) in young pigs.

At the level of fifth palatine rugae, both walls of the vomeronasal duct were lined by olfactory epithelium (Fig. 11) which was in accordance with the observations reported by Kumar *et al.* (2016) in young pigs and Karimi *et al.* (2014) in camel, whereas both the walls were lined by respiratory epithelium in sheep (Ganganaik *et al*., 2006). Vaccarezza *et al.* (1981) in rats and Salazar *et al.* (1996) in cats stated that columnar epithelium lined both the walls of the vomeronasal organ. The epithelial height of the medial wall (25.68 ± 1.45 µm) was comparatively greater than the lateral wall (24.59 ± 0.667 µm). The epithelial crypts were pocket-like and they were noticed in the walls of both sides (Fig. 11). The propria submucosa of vomeronasal organ at the lateral wall (857.85 ± 21.10 µm) was comparatively thicker than the medial wall (684.04 ± 16.89 µm) and had loose irregular connective tissue in the sub-epithelial portion as reported by Kumar *et al*. (2016) in young pigs. The collagen, a few elastic (Fig. 12) and reticular fibres (Fig. 13) were present along with the connective tissue cells and microvessels. The deeper portions contained a greater number of mucous acini along with blood vessels of different sizes and venous caverns as reported by Kumar *et al*. (2016) in young pigs. Numerous nerve bundles with unmyelinated axons were seen across the medial propria submucosa and also in the dorsalwall of the vomeronasal duct. The connective tissue fibres especially collagen fibres became denser close to the cartilage (Fig. 14). The vomeronasal organ was completely surrounded by the cartilage except at the dorsal opening. The shape of the cartilage was oval or elliptical which was in accordance with findings of Kumar (1991) in goats and Kumar *et al*. (2016) in young pigs. The medial limb was thicker at the extremities (502.49 ± 45.24 µm), thinner in the middle (386.45 ± 12.38 µm) and it was projected beyond the level of lateral limb. On contrary, Kumar (1991) in goats reported

that the medial limb was thicker in the middle than at the extremities.

**Conclusion**

The vomeronasal organ plays an important role in olfaction and maintenance of sexual behaviour by detecting the pheromones. The cilia in the epithelium help to facilitate the mixing of mucous fluid along the lumen of vomeronasal duct and improve good contact between the molecule of pheromones and the olfactory cells. The undulating feature of the vomeronasal duct serve in the distension of lumen that help in drawing more mucous fluids containing pheromones from incisive canal into the lumen of vomeronasal duct that acts as a part of mechanical pumping system.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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