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Comparative Anatomy of the Vomeronasal Organ (VNO) in Sheep (*Ovis aries*) and Dogs (*Canis familiaris*) with Simple Reference to its Histological Structure and Vasculature Supply

Anatomía Comparada del Órgano Vomeronasal (OVNO) en Ovejas (*Ovis aries*) y Perros (*Canis familiaris*) con Simple Referencia a su Estructura Histológica e Irrigación Vascular

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SUMMARY: The vomeronasal organ (VNO) is located in the anteroinferior part of the nose and the accessory olfactory organ in mammals which is responsible of sense of smell. This study aims to compare the macro and microanatomical structure of the VNO between sheep and dogs. In the current study, we used ten adult slaughtered sheep and ten adult synchronized dogs with different sexes ages 1-2 years. The head of both animals were preserved in 10 % formalin for one week. This study shows in both animals, the VNO occupies the same position in the cavity of the vomer bone and the same relationship in the cranial part of the nasal cavity. Furthermore, the VNO is divided into three parts based on shape that are the rostral, central, and caudal part. The results show the VNO in sheep has a (U) shape and is opened dorsolaterally. It has a small and narrow cavity. It is long 6 cm long, and it has different diameters on its course. In comparison, the vomeronasal organ in dogs is very developed and has a (J) shape. It has a large and long cavity and ends at the fourth molar. Its length is about 10 cm, and it has one diameter on its course. The VNO receives the blood supply from the sphenopalatine and caudal palatine arteries. The present study shows main differences between sheep and dogs VNO in which the structure of vomeronasal bone between the sheep and dog is completely different. The finding will illustrate fundamental differences and provide specific structural differences between the two species.

KEY WORDS: Vomer-nasal; Morphological study; Sheep; Dog.

INTRODUCTION

The vomeronasal organ (VNO) is a structural organ that is involved with olfactory system. The VNO is responsible for the sense of smell is which plays an important role in attracting males to estrous females through pheromone reception (Tiwary & Solanki, 2020). It has been shown that sense of smell is much more complex in dog comparing to other domesticated species (Buzek *et al*., 2022). The VNO is adapted to animal's need and lifestyle based on food smelling, sexual pheromones smelling and hunting (Jasso del Toro & Nekaris, 2022). This is especially true for animals whose newborns lack developed optic or auditory systems and must detect specific pheromones to identify their mother (Torres *et al*., 2023). The VNO is linked to sexual behavior as a result of its location which is linked to the olfactory nerves. This can be explained when pheromones are detected by the mucous membrane of the olfactory nerves in the nasal cavity which communicate with the olfactory bulb in the brain (Bakker *et al*., 2022).

The anatomical structure of the VNO significantly differs between mammalian species (Xi *et al*., 2023). In sheep, May (1970) described the nasal VNO as being test-tube-like narrow and elongated 6.5 cm long and 1 cm wide at the center which is attached to the vomeronasal bone. The cavity of the organ is small in diameter and flattened transversely. Another point of the anatomical structure of the VNO, it consists of two tubes with a solid end located in the base on both sides of the nasal septum. Each tube is surrounded by a cartilage in the form of an incomplete C shape dorsally and laterally (Alsafy *et al*., 2021). The VNO in sheep is a cartilaginous canal located in the ventral part of the nasal cavity that is connected directly with the oral and nasal cavities through the incisive canal (May, 1970).

In dogs, the VNO is a pair of solid mucous membranous tubes which it is found in both sides of the nasal septum and are attached to the palatal cols of the

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incisive and vomeronasal bones and covered by a thin cartilaginous plate (Buzek *et al*., 2022). The VNO is a complex and highly developed structure that takes a crescent shape in the transverse section and is partially closed through the nasolabial cartilage. It has the shape of the letter ((J), but it lacks the cartilages on the lateral and dorsal sides (Adams & Wiekamp, 1984). The VNO has a large cavity-shaped bilateral organ located in two rows along the anterior ventral surface of the nasal septum and associated with many glands and blood vessels. Also, the VNO is a relatively slender organ in dogs with short nerves emerging from it (Young & Trask, 2007).

The VNO has cavernous tissues and condensed by blood vessels. The blood supply comes from the sphenoid palatine artery (Døving & Trotier, 1998). Comparisons between sheep and dog blood supply of the VNO was not reported yet.

Macro and microanatomical comparison between sheep and dogs are in great interest because it highlights the major anatomical differences between the two species. Furthermore, such a comparison will show more details regarding the blood supply between the two species. In this study, we hypothesis that the macro, microanatomical structures and blood supply of VNO will highlight the major differences of the VNO and explain the differences of sense of smell between the two species.

MATERIAL AND METHOD

Animals. We used twenty heads; ten freshly slaughtered adult sheep and ten adult dogs of different sexes. The sheep heads were obtained from a typical Buraydah slaughterhouse, age (8-12 months) Qassim Region, KSA, while the dogs of local breeds are obtained from areas of wilderness far from habitation (12-24 months). The dogs were anesthetized using 10 % (1 mg/ kg) of xylazine to euthanasia of animals by intermuscular injection (IM). Subsequently, mercy killing of the dogs was applied by opening the common carotid artery to remove blood from the animals. After that, 10 % formalin was injected to the same artery in order of fixation.

Fixation and sample preparation: All heads were fixed in a 10 % formalin solution for 7 days. Subsequently, accusive amount of formalin was removed by distal water in order to process the samples. The samples were divided into five groups. The first and second group: a median and transverse section at several levels were made along the rostral-caudal axis in the head region on 3 heads of each species. The third group 3 heads of each species were used for radiographs in different positions to view the region of the VNO. The fourth group: to describe the VNO and samples were injected with colored latex to study its blood supply on 2 heads of each species. The fifth group 2 heads of each species were used for histological examination.

Anatomical examination: A total of 8 heads from each species were used for the anatomical examination. A dissection was carefully preformed in the median plane and different parameters of transverse plane to visualize bone structure, cartilage and blood supply of the VNO in both species. Results were recorded in notes and photographs were captured during the analysis using a digital camera (Sony DSC-W750, 32 MP). We used Nominia Anatomica Veterinaria 5th edition (2012) to describe terminological terms of the VNO (International Committee on Veterinary Gross Anatomical Nomenclature, 2012).

Histological preparation and examination: For histological examination specimens of the VNO were decalcified in 5 % Ethylenediaminetetraacetic Acid (EDTA) for 48 h. Subsequently, samples were dehydrated in ascending grades of alcohol. After that, samples were cleared in three changes of xylene, and then embedded in paraffin wax. Once the wax dried, 5-mm-thick sections were cut using microtome. The sections were attached to new clean prewarmed slides. All slides were stained with Hematoxylin and Eosin (H&E). The complete protocol was derived from (Bancroft & Gamble, 2008). Slides were analyzed and pictures were captured using OMAX 40X-2500X LED with USB Camera and Mechanical Stage (OMAX Microscope.com).

RESULTS

Anatomical structure of bone and cartilage in sheep: The VNO is a bilateral structure that is represented by two tubes that are situated in the nasal cavity on both side in the rostral part of the nasal septum (Figs. 1 to 5). It occurs in the area formed by the vomer bone medially, the nasal septum mucous membrane, dorsally, and the hard palate ventrally (Fig. 2). Each of the nasal VNO two tubes measures around 6-8 cm in length along its course (Figs. 3 and 4, Table I). The VNO in sheep starts in the nasolabial duct and extends caudally to the third premolars. Each tube is approximately 6-8 cm in length with irregular and varying diameters along the course (Fig. 5, Table I).

Due to the difference in diameter along its course, the VNO can be divided to three parts (cranial, middle, and caudal part). First, the rostral part is a narrow tube which has a simple dimension of 0.2 cm that extended from incisive papilla to the cranial part of Palatine rugae, in which it is open to the lateral surface of the incisive papillae (Fig. 1). Second, the middle or central part is the largest parts and

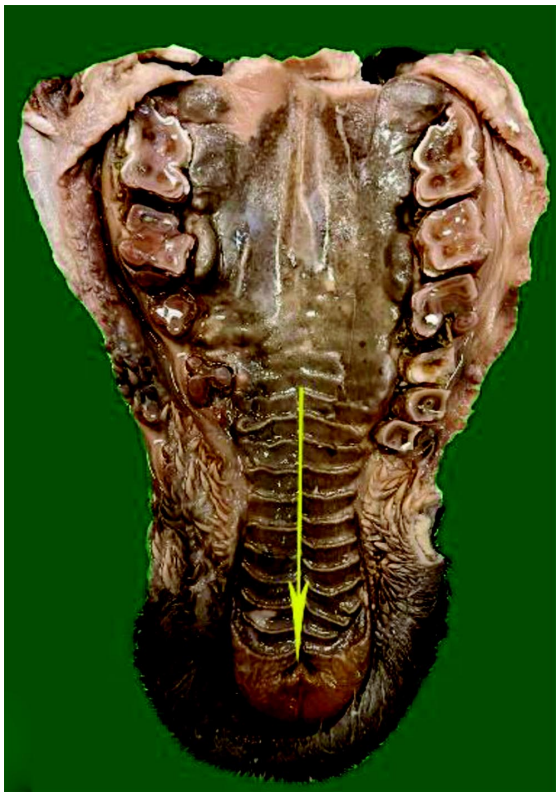

Fig. 1. A photograph of the palatine ridge shows the region of the VNO extending from the level of the upper second molar to the incisal papilla in sheep (yellow arrow).

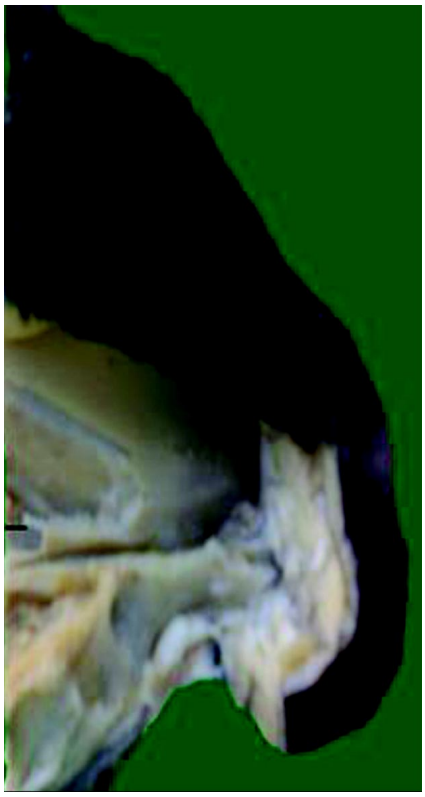

Fig. 2. A photograph demonstrates the sagittal section showing the position of the VNO in sheep laterally.

Fig. 3. A photograph shows the shape of the VNO in sheep after removing the hard palate. It demonstrates the ventral surface showing the shape and position of the VNO in sheep.

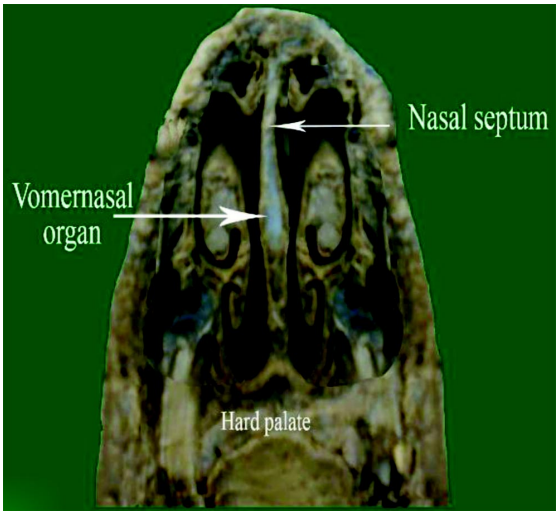

Fig. 4. A Transverse section of the head region demonstrates the location of the VNO in sheep.

Fig. 5. A photograph shows attachment the VNO to the nasal septum in the floor of the nasal septum in sheep.

clearer with a wide lumen and the diameter of this part increased becomes a bigger diameter of 5 mm. Finally, the caudal part is extended within the mucous membrane lining of caudal portion of the nasal septum which is situated within the rostral part of the olfactory bulb in the olfactory fossa within the cribriform bone (Table I).

Histological structure in sheep: The VNO in sheep has a U shape and a semilunar form in the sagittal section within the nasolabial canal. Its lumen has a comma-shape narrow dorsally and wide ventrally (Fig. 7, Table I). It is surrounded by incomplete cartilage dorsolaterally along its length within the nasolabial duct. The tube of the VNO contains many glands, nerves, and blood vessels, particularly on its lateral wall (Table I). The VON contains dense irregular connective tissue. This connective tissue is consisted of many nerve plexuses, glands, and blood vessels. Furthermore, it shows a wide vein along all serial sections. Additionally, vascular loosened connective tissue fills the sub-mucosa. The VNO was mostly encircled by vomeronasal hyaline cartilage. (Fig. 6).

Table I. comparative anatomical and histological of the main differences between the VNO in sheep and dogs.

	Sheep	Dogs
Location and relations	It is located in the anterior part of the nasal cavity, on both sides of the nasal septum	
The degree of development	Normal	Very developed
Shape	U open dorsolaterally	J open laterally
Length	Normal, 6 ± 2 cm	Long, 10 ± 2 cm
The diameter	It has different diameter along its length	It has one diameter
The cavity	Small, narrow	Wide and large
The parts	Undivided three parts	Divided parts: three clear parts
The beginning of the organ	The nasolabial incisive aperture sharply	The nasolabial aperture in a conical shape
The end of the organ	At the level of the second molar	At the level of the fourth molar
The cross section	C-Crescent Shape	J- Shape
The structure	Contained dense irregular connective tissue, and it has many nerve plexuses, glands, and blood vessels.	Contained dense irregular connective tissue. Blood vessels are numerous and varied in size. There is a lack of blood vessels laterally.
The glands	The glands are only mucus type.	The glands are only mucus type. They are dorsally situated in the middle and the caudal parts of the organ.
Cartilage type	The VNO is surrounded on all sides by vomeronasal hyaline cartilage.	The VNO is surrounded by vomeronasal hyaline cartilage laterally.
Blood supply	The sphenoid palatine artery and the great palatine artery	

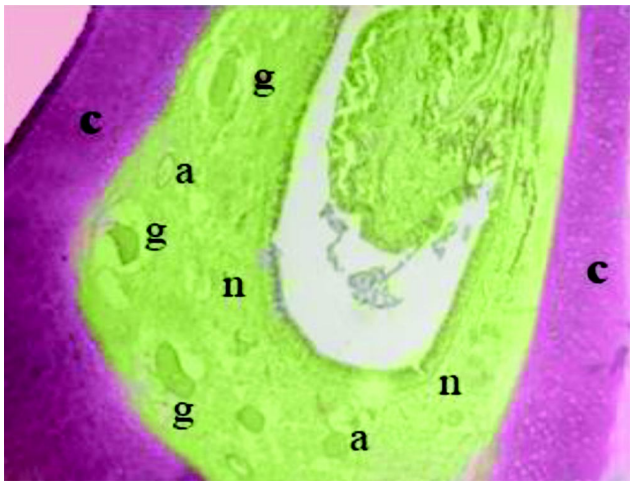

Fig. 6. Microphotograph showing the lumen of the VNO which is crescent-shaped; artery (a) nerve (n) gland (g) VNO cartilaginous (c).

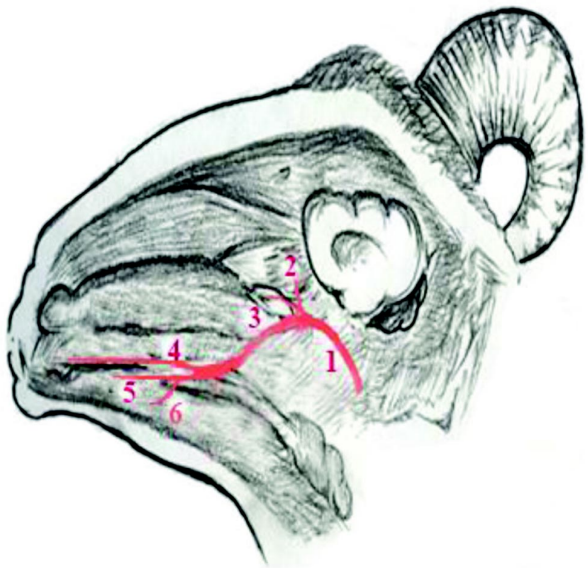

Fig. 7. An illustration showing the blood supply to the VNO in sheep: 1- Maxillary artery. 2- Infraorbital artery. 3- Descending palatine. Artery. 4- Sphenopalatine artery. 5- Greater palatine artery. 6- Lesser palatine artery.

Anatomical structure of blood supply in sheep: The VNO organ in sheep has a larger blood supply which is supplied primarily by the sphenoid palatine and the great palatine arteries. The sphenoid-palatine artery enters the sphenoid-palatine foramen and gives several branches inside the nasal cavity. The major one is the caudal palatine artery, which continues its course in the caudal part of the VNO and provides blood to the caudal part of the organ. A greater palatine artery continues directly into the palatine groove and provides branches to the cranial and middle of the VNO (Fig. 7).

Anatomical structure of bone and cartilage in dogs: The VNO is highly developed in terms of length and size. It begins from the level of the fourth tooth in the proper nasal cavity to the incisive papilla (Fig. 8, Table I). It situates on the dorsal surface of the hard palate and extends on both sides of the nasal septum laterally (Figs. 9 and 10). The VNO is represented by two long, transparent tubes, each measuring about 10 ± 1.25 cm in length and having the same diameter of its entire course (Table I).

The OVN divides into three parts within the vomeronasal duct: the rostral part extends from the nasal vestibule at the nasolabial papilla to the level of the canine, the middle part extends from the level of the canine to the fourth molar, which is the longest part of the organ (Table I).

On the other hand, the caudal part is the shortest of the three parts and extends behind the level of the third molar for a short distance and takes the truncated conical shape. At this part, the NVO communicates with the olfactory bulb by means of the mucous membrane lining the nasal cavity and extending along the nasal septum within the nasolabial duct until the olfactory bulb in the olfactory fossa within the cribriform bone.

Histological structure in dog: The VNO is shaped like (J)

Fig. 8. A photograph of the palatine ridge demonstrates the region of the VNO externally, extended from the level of the fourth upper cheek tooth to the incisive papilla (yellow arrow) in dogs.

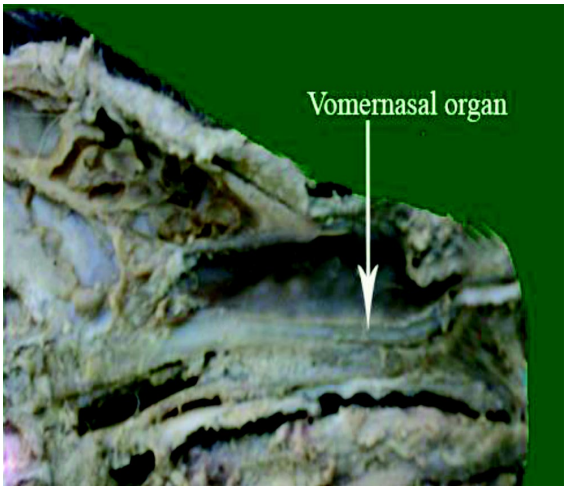

Fig. 9. Median section demonstrates the position and shape of the VNO in dogs.

with a big transparent chamber that was open laterally within the nasolabial canal (Fig 11). The rostral part of the VNO folds ventrally and connects to the incisive duct, which is on the same side. The incisive duct passes through a fissure known as the Palatine and terminates at the beginning of the oral cavity. The VNO is completely surrounded by the cartilage (nano-vomeronasal) of the nasal cavity. It has poor gland function and poor blood vessel function (Fig. 11, Table I). The VON comprises irregular connective tissue and blood vessels, nerves and glands. Blood vessels are numerous with varying sizes and lacked laterally in dogs. Vomeronasal glands are located dorsally in the center and caudal part of the organ.

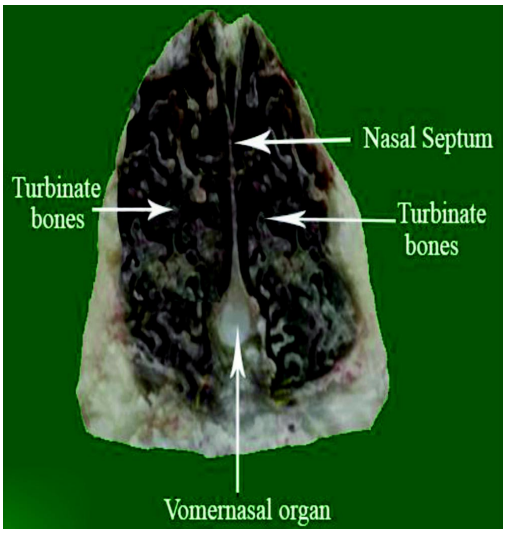

Fig. 10. A Transverse section of the head region demonstrates the location of the VNO in dogs.

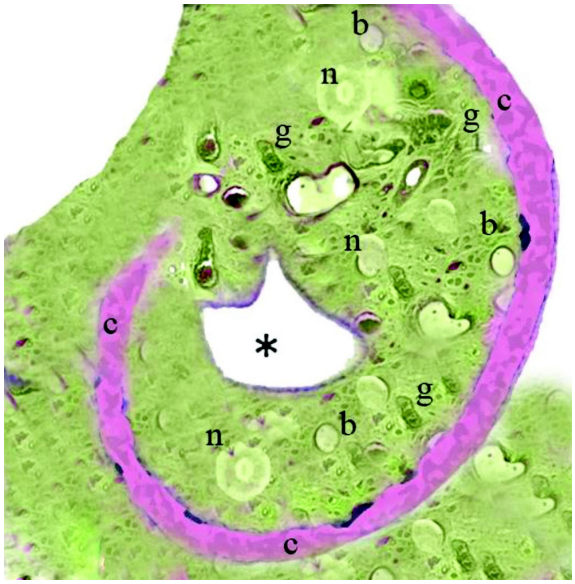

Fig. 11. Microphotograph showing the lumen of the vomeronasal organ which is J-shaped; artery (a) nerve (n) gland (g) VNO cartilaginous (c).

Anatomical structure of blood supply in dog: The VNO blood supply in dogs is similar to sheep. It receives blood supply from the sphenoid palatine artery and the great palatine artery, both branches from the descending palatine artery.

The sphenoid palatine artery supplies the caudal part of the VNO through the caudal branch of the sphenoid palatine artery. On the other hand, the rostral and middle parts of the organ receive the blood supply from the great palatine artery. The palatine artery runs through the greater palatine canal. After that, it emerges from the greater palatine

foramen near the caudal margin of the hard palate. Subsequently, it continues to the rostral and middle parts of the VNO (Fig. 12, Table I).

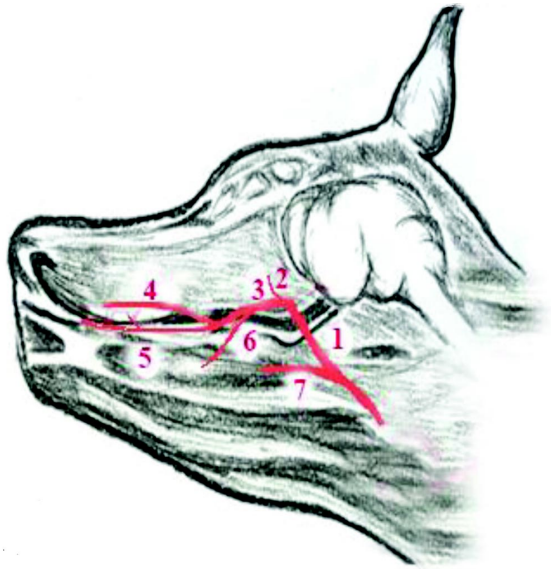

Fig. 12. An illustration showing the blood supply to the VNO in dog: 1- Maxillary artery. 2- Infraorbital artery. 3- Descending palatine artery. 4- Sphenopalatine artery. 5- Greater palatine artery. 6- Lesser palatine artery. 7- Linguofacial artery

DISCUSSION

The results of this study shows that the VNO in sheep and dogs is located in the cranial part from the ventral side and on both sides of the nasal septum. It is connected ventrally to the hard palate, medially to the vomeronasal bone, and laterally to the mucous membrane lining the nasal cavity. These results are in consistent with what was previously discovered in both species (Stoyanov *et al*., 2021). The caudal part of the VNO ends at the level of the second molars in sheep, while the caudal part ends at the level of 4th into 5th transverse palatine ridges in dogs (Al-Kafagy & Reshag, 2020). The VNO in sheep and dogs is a bilateral organ located at the base of the anterior nasal septum represented by an epithelial tube. It has additional structures that include cartilage, the nasopalatine canal, glands, nerves, blood vessels, and the accessory olfactory bulb. These structures form the nasal tract complex. These findings agree with the general anatomical structure of VNO in mammals (Dyce *et al*., 2017).

Significant differences are recorded between the diameter and length of the VNO in sheep and dogs. The study shows that the VNO 8 cm length in average in sheep. It starts from the point where it opens at the caudal end of the 2ed upper premolar teeth. On the other hand, it has an average of 10 cm in length in the dogs from the level of the incisive papilla to its caudal end at the 4th upper premolar. Our results are different than what was reported previously according to the length of the VNO which was 6 cm in sheep and 5-6 cm in dogs (Adams & Wiekamp, 1984; Abass *et al*., 2012).

The shape of the VNO differs between the two animal species. In sheep, it has a (U) shape and is surrounded by a thin cartilaginous plate. Whereas, in dogs, it has (J) shape and is surrounded by a very thick, solid cartilaginous plate. Our findings are in concordance with previously reported studies (Kratzing, 1971; Getty, 1979).

The shape of the VNO was not clearly classified previously. Our results reveal that the VNO can be divided into three parts: the front, middle, and caudal in both species. Also, it extends from the nasal vestibule to the level of the third molar. In contrast, May (1970) descripted the VNO shape as transversely flattened in sheep. Also, Adams & Wiekamp (1984) described the VNO as partially closed through the vomeronasal cartilage, which is absent on the dorsolateral side. On the other hand, Getty (1979) descripted the VNO as a pair of solid mucous membranous tubes. Whereas, Dennis *et al*. (2003) stated that the VNO covered by a thin cartilaginous plate on the ventral aspect of the nasal septum.

In other words, the VNO can be divided into three different distinct parts the rostral, central, and caudal portions. These three parts extend from the nasal vestibule to the level of the third molar in sheep and fifth molar in dogs. The results agree with Abass *et al*. (2012) in Alawasi Iraqi sheep. Our results do not agree with Al-Kafagy & Reshag (2020) in which they recorded the VON divided into two parts, the conductive part, and the olfactory part.

The VNO in our study is composed of various components, including vomer duct, nerves, glands, cartilage and blood vessels confirming the result reported previously by Kratzing (1971) in sheep, Bhatnagar & Meisami (1998) in animals and Mahdy *et al*. (2019) in dog and rabbit. Our results confirm that the VNO receives blood supply from the sphenoid palatine artery and the great palatine artery. These branches are from the descending palatine artery in both dogs and sheep. Confiding agrees with Besoluk & Bahar (2006) in goat and Salazar *et al*. (1995) in dogs. The sphenopalatine artery and a greater palatine artery branch supply to the arterial blood of the VNO (Besoluk & Bahar, 2006). Also the base of the rostral section of the ventral nasal meatus and the major palatine artery anastomosed with the branches of the sphenopalatine artery. Finally, the VNO exclusively receives blood from the sphenopalatine palatine artery without mentioning the greater palatine artery.

In conclusion, the findings demonstrated that whereas the vomeronasal organ is located in the same place in dogs and sheep, its extensions differ. It reaches the level of the fourth molar in dogs and the second molar in sheep. The VON organ differs in length in dogs and sheep, measuring approximately 6 cm in sheep and 10 cm in dogs. Their forms are different in the two animals; in sheep, it takes the form of a crescent in sheep, and J in dogs. The two animals in our study, have the same blood supply. The tissue structure has been generally researched. There are cartilage structures, blood arteries, and glands in both animals.

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ALLOUCH, G. M. & ALSHANBARI, F. A. Anatomía comparada del órgano vomeronasal (OVNO) en ovejas (*Ovis aries*) y perros (*Canis familiaris*) con simple referencia a su estructura histológica e irrigación vascular. *Int. J. Morphol., 42(2)*:374-381, 2024.

RESUMEN: El órgano vomeronasal (OVN) se encuentra en la parte anteroinferior de la nariz y el órgano olfativo accesorio en los mamíferos es responsable del sentido del olfato. Este estudio tuvo como objetivo comparar la estructura macro y microanatómica del OVN entre ovejas y perros. En el estudio utilizamos diez ovejas adultas y diez perros adultos de diferentes sexos con edades de 1 a 2 años. Las cabezas de ambos animales se conservaron en formol al 10 % durante una semana. Este estudio mostró que en ambos animales, el OVN ocupa la misma posición en la cavidad del hueso vómer y la misma relación en la parte craneal de la cavidad nasal. Según su forma el OVN se divide en tres partes: rostral, central y caudal. Los resultados mostraron que el OVN en las ovejas tiene forma de (U) y está abierto dorsolateralmente. Presenta una cavidad pequeña y estrecha. Además, tiene una longitud de 6 cm y tiene diferentes diámetros en su recorrido. En comparación, el órgano vomeronasal en los perros está muy desarrollado y tiene forma de (J). Presenta una cavidad grande y larga y termina en el cuarto molar. Su longitud es de unos 10 cm y tiene un diámetro distinto en su recorrido. El OVN recibe el suministro de sangre de las arterias esfenopalatina y palatina caudal. El presente estudio muestra las principales diferencias entre el OVN de ovejas y perros en el que la estructura del hueso vomeronasal entre estos dos animales es completamente diferente. Además, los hallazgos ilustran diferencias fundamentales y determinan diferencias estructurales específicas entre las dos especies.

PALABRAS CLAVE: Vómer-nasal; Estudio morfológico; Oveja; Perro.

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