

Anatomical and histological investigate of vomeronasal organ (VNO) in Iraqi sheep Alawasi

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Abstract:

This work was focused on the vomeronasal organ (VNO) anatomically and histologically in Iraqi sheep. Vomeronasal organ (VNO) plays essential role in the urinary sensation pheromones (sexual scent) which has not been studied extensively. In this study, six samples of the VNO for both sexes, adult were taken from the normal heads of Iraqi sheep Alawasi. The VNO was located in the anterior portion, just in the floor of the nasal septum (ventrolaterally), it is a tubular crescent shape, 6 cm in length and opens cranially in the incisive duct and blinded end caudally at the level of 2nd upper premolar tooth. Histological examination of the VNO was revealed three regions were divided into cranial, middle and caudal. It is encompassed inside a cartilaginous capsule which appeared as U- shape in the cranial region, while in the middle region was a free ends of the U-shape beside separation the narrow cleft dorsally, in the caudal region the cartilage became circular (ring shape) and surrounding the VNO completely, which separated the organ from the respiratory epithelium of nasal cavity. The lumen of VNO appeared in cranial and middle region as a comma shape, while, the caudal region was heart in shape, the border was irregular due to the folding of epithelial mucosa. The lumen of VNO was lined by a sensory medial wall showing the bipolar sensory neuron supporting and basal cells and non-sensory epithelium in the lateral wall was contained the basal cells, ciliated columnar and goblet cells.

دراسة تشريحية ونسجية للجهاز الميكعي الأنفي في الأغنام العراقية العواسي

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الخلاصة:

لقد ركزت هذه الدراسة على العضو الميكعي الأنفي للأغنام العراقية العواسي. إن هذا العضو يلعب دورا أساسيا في التحسس لمادة الفرمون المنفرزه في البول (كرائحه جنسيه) والتي لم تدرس بشكل مستفيض. في هذه الدراسة استخدمت 6 عينات لتحديد العضو الميكعي الأنفي من رؤوس الأغنام

العراقية العواسي الطبيعية لكلا الجنسين البالغة العمر، يقع العضو في الجزء الامامي (بطنياوحشيا) على ارضية الحاجز الانفي، وهو عضو انيوبي هلالى الشكل طوله 6 سم مفتوح اماميا عند القناة القاطعية وذو نهايه خلفيه مسدوده عند المستوى امام الطاحن العلوي الثاني. الفحص النسيجي للعضو اشير اليه بثلاث مناطق (اماميه، وسطى وخلفيه) والتي تكون محاطه بغضروف هلامي والذي يكون في المنطقة الامامية على شكل (U)، وعند المنطقة الوسطى تقترب كلا حافتي مع بعضهما تاركتا فراغا ضيقا ظهريا، اما خلفيا فالغضروف يكون محاط بشكل كامل للعضو (حلقي الشكل) منفصلا عن الظهارة للتجويف الانفي. اما تجويف العضو فيتميز بكونه يشبه الضمه في المنطقة الامامية و الواسطى بينما خلفيا يكون التجويف قلبي الشكل. ان التجويف خلال طوله يكون غير منتظم الحافات وذلك لوجود الطيات في الظهارة المخاطية للعضو. التجويف يبطن بظهاره حسيه عند الجدار الانسي محتويا على خلايا عاموديه مهدبه، عصيبه ثنائية القطب، سائده وخلايا قاعديه، اما الجدار الوحشي فيكون ذو ظهاره غير حسيه تتمثل بوجود خلايا عاموديه مهدبه، وقاعديه وخلايا مخاطيه.

Introduction:

Vomerolateral organ (VNO), or Jacobson's organ. It was discovered by Frederik Ruysch and later by Ludwig Jacobson in 1813 (1). It is an auxiliary olfactory sense organ, found in many animals. 1783-1843" was described an organ in mammalian nose (nasal cavity) which had not been already explained by other investigator. During these years most of the articles of VNO were mention to the reptiles and contain the mammals (mice, rats, dogs, cats, goat, sheep cattle, horse, pigs)(2,3,4,5,6,7,8) and human being. The numerous anatomical and histological comparative studies have been performed on the vomeronasal organ was using the techniques light microscopy. Dehya (2010) was study the vomeronasal organ anatomical and histological in more details of Iraqi cattle. The vomeronasal organ is mainly used to detect pheromones, chemical messengers that carry information between individuals of the same species, which play important role in social behaviors

and sexual reactions with functions in estrus detection in mammals (9,10,11,12,13,14,15,16) hence is sometimes referred to as the "sixth sense." Some mammals, particularly felids and ungulates, use a distinctive facial movement called the flehmen response to direct inhaled compounds to this organ (17,18,19). The animal will lift its head after finding the odorant, wrinkle its nose while lifting its lips, and cease to breathe momentarily. Flehmen behavior is associated with "anatomical specialization", and animals that present flehmen behavior have incisive papilla and ducts, which connect the oral cavity to the VNO, that are found behind their teeth. However, horses are the exception; they exhibit Flehmen response but do not have an incisive duct communication between the nasal and the oral cavity. Also in the mammalian sexual behavior of male and female changed via the stimulation of VNO through the perception of pheromones and detect the urine odor and vagina from the

male, in female VNO can smell the newly born and other females (11,20,21,22,23). In addition, were thought that the organ in domestic animal is determining the favors of food in mouth by olfaction, proved this function of the organ (16,24).

Many studies have been performed to find if there is an actual presence of a VNO in adult human beings. Trotier *et al* (25) estimated that around 92% of their subjects that had no septal surgery had at least one intact VNO. Kjaer and Fisher Hansen, on the other hand (26) stated that VNO structure disappeared during fetal development as it does for some primates (17). Thus, the human VNOs are histological identified as paired epithelial tubes structure located ventrolateral bilaterally along the nasal septum (27) and investigated shape, size and orientation of the VNO using magnetic resonance imaging (MRI) (14), also suggested that the VNO was tubular or pouch like mucosal invaginations of the anterior nasal septum (9,12,29,30,15,31) thus, they observed in the elephant by (32) in the horse(33,15,34), in bovine and buffalo (15,35,36,19,37) in pig (15,38) also in sheep and goat(19,18,38,22,37,18,23,27) dog (10,39,40,41) cat (42,43) mouse and rat (44) . The VNO is found at the base of the nasal cavity. It is split into two, being divided by the nasal septum, with both sides possessing an elongated C-shaped or crescent lumen. It is encompassed inside a

bony or cartilaginous capsule which opens into the base of the nasal cavity.

The lack of information and complete studies is mainly given the important of the Iraqi sheep (ALAWASI) in biomedical research and an increase of this species due to play important role in Iraqi economic source in meat, leathers and wool production. Therefore, our aim is to illustrate the morphology of VNO in this species and compare with other species, here was reported a study of the general morphology and histology of VNO in this species.

Material and method:

Six head of Iraqi sheep Alawasi were used from ALSHALA abattoir after slaughtering; the samples were fixed in 10% formaldehyde in phosphate-buffered saline (PBS) at pH 7.4 for 24 hours at room temperature. After fixation, the transverse section about eight levels 0.75cm in thicknesses thought its length was did, and then decalcified in formic acid solution 10% for 7 days. After decalcified, the processing [dehydration with an ascending grade of alcohol, clearing in xylene and impregnation (embedding) in paraffin wax] was done. Immediately after embedding, the samples were blocked with paraffin wax and then sectioned using standard histological techniques, exactly 7 μ m thick were prepared. The samples stained with hematoxylin and eosin (H&E) for

general histology, as well as PAS for demonstration of the glandular cells. The slides were then dipped in xylene and mounted with cover slip using DPX mounting medium. The slides were examined under light microscope to show the general morphology and histology of VNO in this species.

Result:

Anatomical:

Vomer nasal organ in Iraqi sheep Alawasia early morphogenesis was closed and consists of blind-ending tubes which are situated on each side of the nasal septum (Figure 1) and extended from the level of incisive papilla to the second upper cheek tooth (Figure 2). The bilateral vomer nasal duct was irregular crescent C-shaped lumen in cross section due to the folding lining, and this characteristic feature was only found in Iraqi sheep Alawasia (Figure 3), the vomer nasal cartilage is incomplete cartilaginous capsule which located beneath the nasal cartilaginous septum on the floor of the nasal cavity. The cross examination was divided the area into three regions (cranial, middle and caudal region) the cranial region was narrow duct opened into the lateral surface of the incisive duct and extended to the 5th palatin ridge, the middle region was wide lumen and it is extended to the 6-11th palatine ridge. While, the caudal region is blind duct extended from 12th palatine ridge to the third upper premolar tooth (Figure 4).

Histological:

Histological sections revealed that VNO of Alawasi Iraqi sheep connected with oral cavity by incisive duct which extended from incisive papillae to 2nd premolar tooth. However, these duct beginning from the incisive papillae which located caudal to dental pad of upper jaw. While, the histological examination showed that this duct lining with pseudostratified keratinized epithelium and changing to non keratinized at the junction like T-shape with VNO (Figure 5). The lumen was encompassed inside a cartilaginous capsule which showed three different regions cranial, middle and caudal. The cross sections of the lumen VNO revealed that the side of the epithelium lumen was convex medially and concave laterally, in addition, the epithelium was appear in folds (Figure 6), this feature and phenomena was found only in Alawasi Iraqi sheep. Therefore, the diameter of the VNO lumen was wider than the cartilage that encompassed the organ in the normal case when an animal is alive.

The lateral side of the lumen (convex surface) was lined by pseudostratified columnar epithelium (non-sensory epithelium) and consist of the basal cells, ciliated and non-ciliated columnar cells (depend on the region), and the goblet cells (Figure 7). While, the medial side (concave surface) of the lumen was lined by pseudostratified columnar epithelium (neuro epithelium) which consist of the

bipolar sensory cells (neuron), basal cells and supporting cells. Supporting cells also named are sustentacular cells, these cells like columnar cells which extending from the basal membrane to apical surface. Bipolar sensory cells have bipolar extension i.e. the dendrite and axon extend to the lamina propria, the nuclei was central location and contain the microvilli in apical surface (Figure 8), in this region the cell haven't Nissle body like all the neurons, thus, all researchers mention that these cells must be sensory cell of VNO instead of bipolar neuron.

Lamina propria

The lamina propria contained the dense irregular collagen connective tissue, this collagen bundle play an important role to support the glandular tissue and blood supply during the ensuring the resistance force, stretch and tension occurs through pumping mechanism. Lamina propria has a great number of nerve plexuses, glands, and several blood vessels in both epithelium types. The glands in Alawasi sheep was only mucus type and filled most of the proeria in both lateral and medial side of VNO, this glands its named also Jacobson glands, these glands were tubule acinary type which positive PAS stain, which characterized by high cuboidal or pyramidal cells with darkly nucleus, and contain narrow

lumen (Figure 9). The glands have excretory duct open between epithelium to secret crypts of mucosal glands in medial side. About vascular element in this present study show wide vein along all serial section and play important role in pumping action through the outflow and inflow of blood stream during seasonal breeding by push mucus and helping to mixing with pheromone by felmen phenomena. Also the submucosa is filled by vascular loose connective tissue, and the VNO was largely surrounded by vomeronasal hyaline cartilage (VNO). The perichondrium of the VNO consisted of chondroblasts numerous chondrocytes present in lacunae were surrounded by extracellular matrix; there were also many isogenous groups, the shape of cartilage in the Alawasi sheep have the main characerstic feature in its shape, in cranial portion the cartilage was U-shape and its lumen was comma shape narrow dorsally and wide ventrally (Figure 10), while, in the middle region its dorsal free ends of U-shape nearing together, with its lumen appear crypts of mucosal glands in medial side. The caudal region showed the cartilage was completely envelope and take ring shape with crescent to heart shape lumen, all regions above have somewhat irregular border due to the mucosa folds.



Figure 1. Photograph show the cross section of the nasal cavity Alawasia Iraqi sheep. Note that the vomeronasal organ on both side of nasal cavity surrounded by (blue circle).

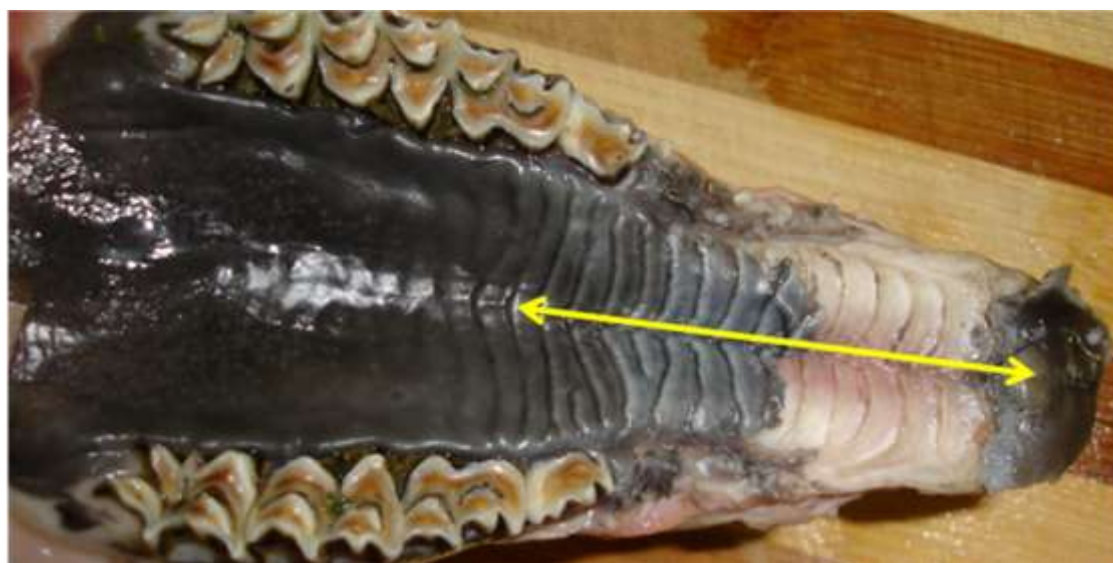


Figure 2. Photograph of the palatine ridge show the area of the vomeronasal organ extended from the incisive papilla to the second upper cheek tooth (yellow arrow).

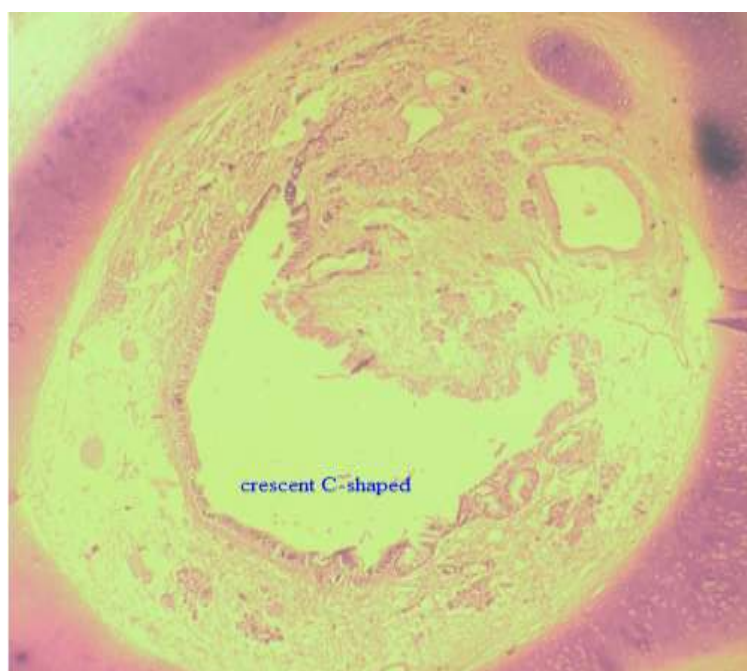


Figure 3. Microphotograph of the vomeronasal duct showing an irregular crescent C-shaped lumen due to the folding lining.

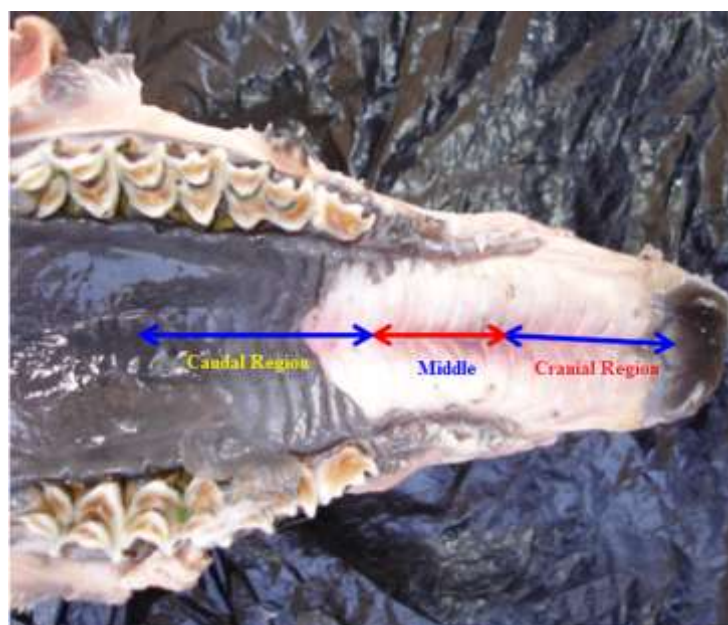


Figure 4. Photograph of the palatine ridge detecting the cranial, middle and caudal region of the vomeronasal organ extension (blue and red arrows).

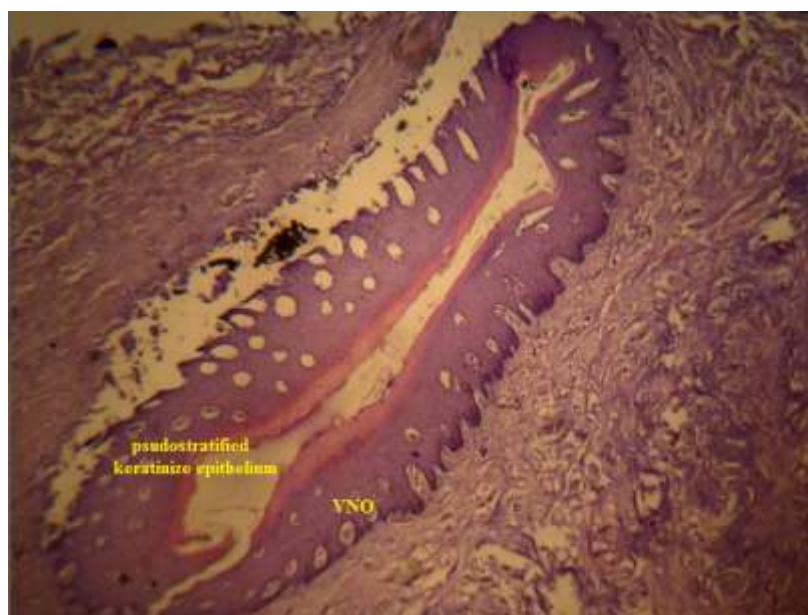


Figure 5. Microphotograph show the incisive papilla vomeronasal organ epithelium was pseudostratified keratinized epithelium.



Figure 6. Microphotograph shows the lumen of the vomeronasal organ. A, concave laterally, and (B) was convex medially.

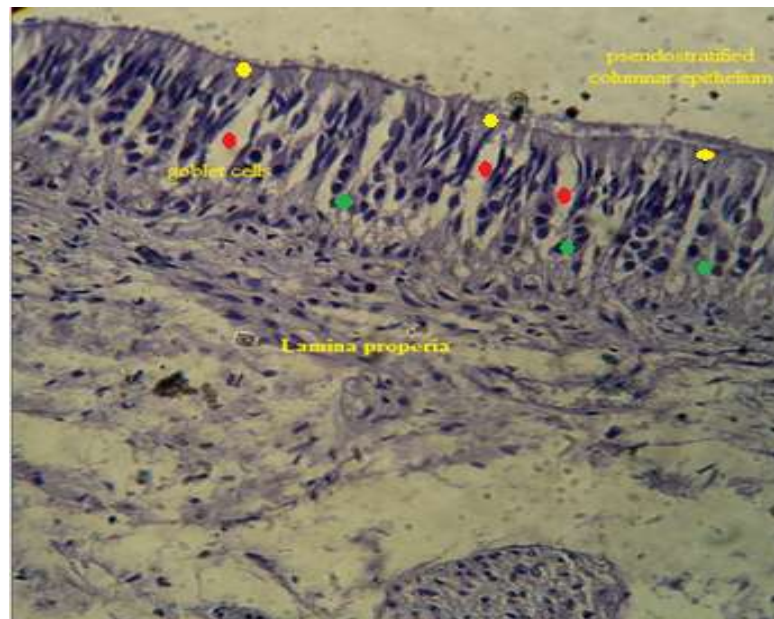


Figure 7. Microphotograph show the convex surface was lined by pseudostratified columnar epithelium (non-sensory epithelium), basal cells (green point), ciliated and non-ciliated columnar cells (yellow point) and the goblet cells (red point).

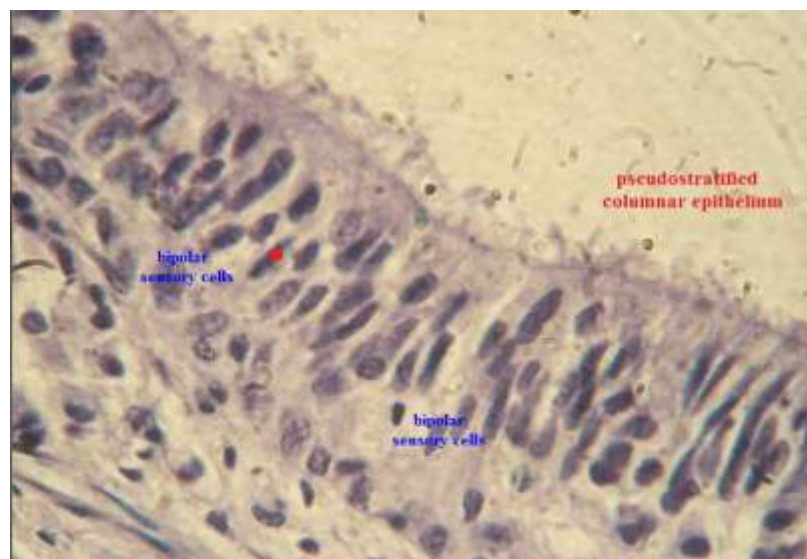


Figure 8. Microphotograph show the pseudostratified columnar epithelium (neuro epithelium) on the concave surface which consist of the bipolar sensory cells (red point), basal cells and supporting cells.

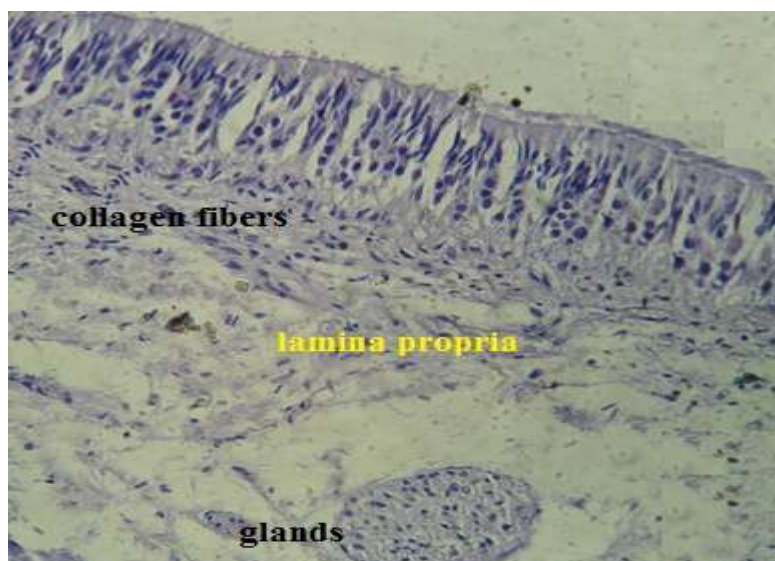
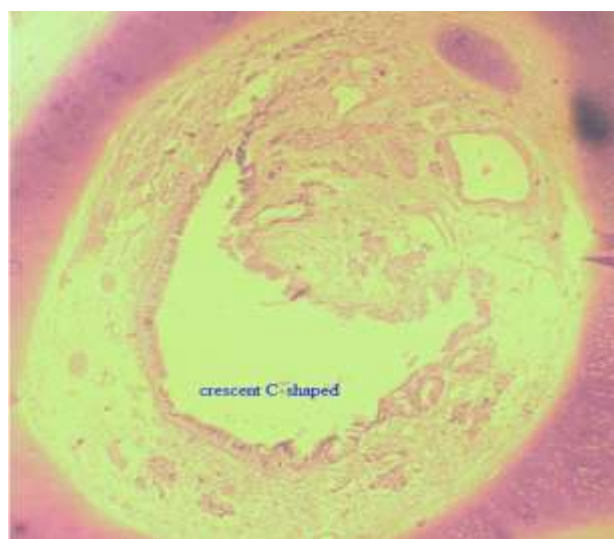
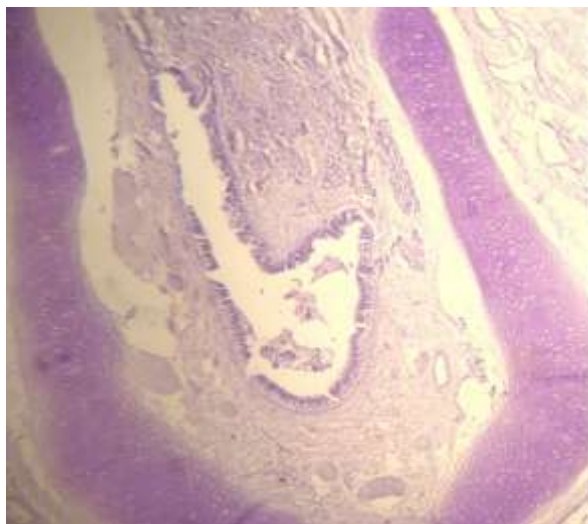


Figure 9. Microphotograph show, (A) the glands in the lamina propria and, (B) dense irregular collagen connective tissue

A



B



C

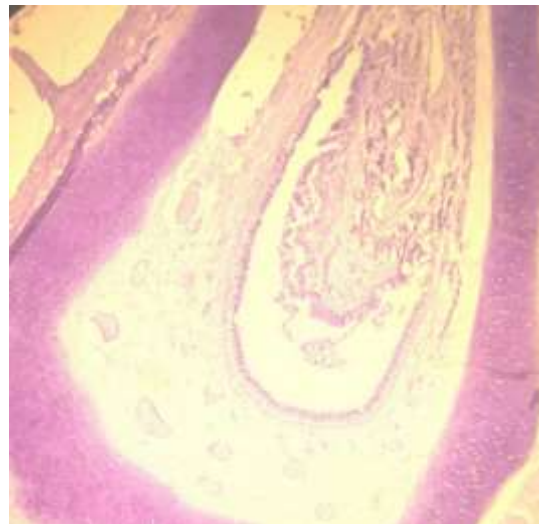


Figure 10.

Microphotograph shows the different regions of the cartilage, (A) cranial region the cartilage was U-shape, (B) middle region the free ends of U-shape nearing together, and (C) caudal region the cartilage was completely envelope and take ring shape.

Discussion:

The previous studies of VNO were not mention to the all components of this organ. VNO is a tubular crescent shape and split into two pairs, separated by the nasal septum which lies on the base of nasal cavity (9,12,15,27,29,30,31,40, 45,46). Thus, this study indicates that VNO communicate with oral cavity via the incisive opening which located on both sides of incisive tubercle which mention by (32,47). The length of organ from point of incisive opening to its caudal end at 2nd upper premolar tooth was 6 cm while in dog 5-6 cm (10), 15-20 in cow (35,48), horse 12-15, buffalo 18.9 cm (19,21,23,38) in Iraqi cattle 16 cm (16). Currently study showed that the VNO of Iraqi sheep length is 9-6 cm in average, while Alawsi Iraqi sheep not exceeded 6cm, and shorter in length in human was 7 mm (27).

The VNO of Alawsi Iraqi sheep was lining with two type of epithelium, the respiratory epithelium was pseudostratified columnar ciliated, while other animals have non ciliated columnar like (19,27,32,33,39,49) in the medial side was lining with non-ciliated pseudostratified columnar epithelium and this were agreement with all researcher except (50) who mention in (animal) opposite lining.

The main characteristic feature found in Alawasia Iraqi sheep not observed in other animals are;

(1) the folds of epithelial mucosa in both side on the length of VNO duct this point play important role to explain the function of the VNO during the breeding season which increase the amount of mucus secretion by glands, and this was gave high chance to mixing with pheromone to induce meeting (sexual behavior) and also explains the high sensation of epithelium of VNO to external environment like chemical pheromone to produce more mucus to dissolved this substance and stimulation bipolar neuron in medial side to induce flemen phenomena, this stimulate Iraqi workers on hormonal influence to elevate twins lambs born.

(2) The lumen of VNO was irregular borders along its length due to it is folds of epithelial mucosa and takes in cranial and caudal region comma shape and heart shape in caudal region.

(3) The changes of the cartilage from the U-shape to another shape depend on the regions was important for help to absorbed the tension force during extension diameter of VNO during its function.

These are three items looking only in this study was not mention before

from the researches in all animals including human.

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