Slov Vet Res 2019; 56 (Suppl 22): 623–32
DOI 10.26873/SVR-801-2019

Original Research Article

PRENATAL DEVELOPMENT OF THE VOMERONASAL ORGAN IN RABBIT

Saleh A. M. A. ALomaisi[1,2]*, Hanaa M. El-Ghazali[2], Hamed M. Nosseur[2], Salah El-dein A. Ahmed[2], Mervat M. Konsowa[2]

[1]Departments of Animal Research Regional Research Station, Agriculture Research and Extension Authority, 87148 Thamar Yemen, [2]Departments of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt. Paper extracted from PH.D. thesis of Saleh Ahmed Mohammed Ali Al-Omaisi

*Corresponding author: elemesy18@vet.zu.edu.eg, alomisy78@yahoo.com

Abstract: The vomeronasal organ (VNO) is the peripheral receptors, with the ability to detect pheromones, and so it has a role in social behavior, sexual reactions and reproduction. The aim of this study was to designing, categorize, define and demonstrate the normal explaining of the onset time of first appearance and origin as well as developmental changes of vomeronasal organ of the rabbit and its allied structures. This study was carried out on 116 rabbit embryos and fetuses of both sexes. The specimens were obtained, from 14 normal and apparently healthy adult female rabbits. At days (D) 9-11 of pregnancy the nasal placode was invaginated to form the nasal (olfactory) pits. The primordia of the VNO was formed at the ventromedial area of this nasal pit. At D13, the VNO appeared as bilateral un-differentiated epithelial thickenings of the rostroventral part of nasal septum. At D14, the dorsomedial part of the epithelium was about a twice as thick as its ventrolateral part. At D16the VNO opened into the nasal cavity the miniature VNO nerve fascicles were appeared. At D18, VNO immature glands appeared in the dorsolateral part of the VNO the chondral plate was differentiated into chondroblasts. The lumen of the VNO was extremely increased in wide and closed to be oval lumen. At D20, the rostral opening of VNO duct opened directly into the floor of nasal cavity. The medial wall was thicker and had about 8-10 layers of stratified columnar and the lateral one consisted of 3-4 pseudostratified columnar cell layer. At D22, the of acini of the VNO glands determined at the dorsal commissure of the VNO duct. At D28, the lumen was lined by a thick medial mucosa with stratified olfactory like epithelium and thin ventrolateral respiratory epithelium.

Key words fetus; vomeronasal organ; nerve; glands; olfactory epithelium; respiratory epithelium

Introduction

The mammalian vomeronasal organ (VNO) is the site for peripheral receptors of the accessory olfactory system. By detecting pheromones, the VNO has an important role in social behaviour and reproductive or sexual reactions. In mammals, the latter occurs due to its

Received: January 2019
Accepted for publication: February 2019

ability to detect pheromones, and so VNO has an important role in many social and sexual behaviour. The olfactory system is well developed in rabbit (1). The vomeronasal complex (vomeronasal organ, cartilage, vessels, glands and nerves) is the most outstanding of the peripheral sensory structures found in the nasal septum of mammals. Although recent findings suggest it could be involved in pheromone-mediated behavior (2), VNO subserved basic chemosensory functions in rodents, mainly related to sexual behavior (3). It also plays a major role in the perception of stimuli related to social and/or reproductive behavior in many species of vertebrates (4). The vomeronasal system (VNS), an olfactory neural network that participates in the control of reproductive physiology and behavior, is sexually dimorphic (5).

The real function of VNO is still controversial, a further developmental studies on this may give some more useful information to explain its function. However, there have been so far published only a little reports and details of morphogenesis remain unknown (6). There were several papers discussed the development of the VNO in rabbit (7, 8), rat (9), mouse (10), hamster (11), pig (12-14), mammals (15), primates (16), human (17-19), bovine (20), goat (21), camel (22), and domestic animals (23). The aim of this study was to categorize, define and demonstrate the normal explaining of the onset time of first appearance and origin as well as developmental changes of VNO of the rabbit and its allied structures.

Materials and methods

This study was carried out on 116 rabbit embryos and fetuses of both sexes. The specimens were obtained from 14 normal and apparently healthy adult female rabbit. The rabbits were obtained from the rabbit farm of the Faculty of Agriculture, Zagazig University. They were housed for one week before experiment for acclimatization standard pellet ration (El-Nasr Chemical Company, Cairo, Egypt) and were given free accesses to water *ad libitum*. All animals managed according to Animal Ethical Committee of Faculty of Veterinary Medicine, Zagazig University approval number ZU-IACU/2/F/109 /2018.

The female were served by natural mating and each female housed individually in stainless-steel cages in environmentally controlled rooms and each maternal rabbit was given about100g-day of certified rabbit nitration free access to water. The pregnant rabbit were tested at age 9-28th days of pregnancy. The age of embryo was estimated by the pregnancy records and age of pregnancy depended on the time of mating. Just after slaughtering, evisceration and evacuation of their uteri.

The obtained embryos and fetuses were classified into two group representing the all ages of pregnancy. Group (A) were immersed as a whole in 10% neutral buffered formalin and the other group (B) were immersed as a whole in Bouin's solution for 3-24 hours and then washed carefully with distilled water and transferred to 70% ethyl alcohol. Then the specimens were subjected to the following techniques:

Histological technique

The heads of fetuses over 20 days were immersed in EDTA 5.5% buffered to 7.0 PH with sodium hydroxid and neutralized in 5% sodium sulphate. The time taken for decalcification depended on the age of fetuses according to (24). After all specimens assembled for normal histological technique, all specimens dehydrated in ascending grades of alcohols, cleared in three changes of benzene and embedded in paraffin wax. Paraffin sections of 5-7μ thickness were obtained and stained by different histological stains such as: Hematoxylin and Eosin (H&E) stain for general histological demonstration and silver impregnation (24, 25). The slides were examined by using both light and stereo (Zeiss, Germany) microscopes and the observations were recorded.

Scanning electron microscope

The specimens were delivered at hourly post conception. Specimens were trimmed and fixed in glutaraldehyde for 12-24 hour and then post fixed in 1 % osmium tetroxide for 90-120 min (26). The palates were dehydrated

through an ascending concentration of ethyl alcohol followed by 2.5 % buffered glutaraldehyde + 2 % paraformaldehyde, in 0.1 M sodium phosphate buffer pH 7.4. The specimens were washed 3 x 15 min in 0.1 M sodium phosphate buffer + 0.1 M sucrose and refixed in 2 % sodium phosphate buffered osmium tetroxide pH 7.4 for 90 min. Following washing and dehydration, the specimens were incubated overnight in 70 % acetone + 0.5 % uranyl acetate + 1 % phosphotungstic acid (at 4° C for 15 min), 80 % ethanol (2 x 15 min), 90 % ethanol (2 x 15 min), 96 % ethanol (3 x 20 min), and 100 % ethanol. The specimens were coated with gold-palladium membranes and observed in a Jeol JSM-6510 L.V SEM, The microscope was operated at 30 KV at EM Unit, Mansoura University, Egypt.

The nomenclature used in this manuscript was adopted by Nomina Anatomica Veterinaria (27), Nomina Embryologica Veterinaria (28) and Nomina Histologica Veterinaria (29).

Results

Rabbit embryo of 11 days old

The primordia of VNO performed at the medial aspect of the nasal pit (olfactory pits) as a thickening of the epithelium. The epithelium lined the pit was similar to that of the placode, and merged steadily with the general ectoderm contiguous the outside opening, which migrate towards the mesenchyme and causes a slight recess on the olfactory purse. Later on, cellular bud grew dorsally, caudally, and to the midline on both side and formed the primordia of the vomeronasal groove (Figs. 1A-C).

Rabbit embryo of 13 days old

The first appearance of VNO was in the arrangement of bilateral undifferentiating epithelial thickenings on rostroventral region of the nasal septum. The organ was enclosed via the immature vomeronasal cartilage. The ventral part of the primitive nasal cavity had an invagination of the epithelial covering the ventral part of the nasal septum giving rise to the future vomeronasal duct (Fig.1D).

Rabbit embryo of 14 days old

The primordia of VNO appeared as narrow luminal tube at the base of nasal septum (Fig. 2A). The primordial vomeronasal tube was lined laterally by a layer of stratified columnar epithelium with darkly stained and basely located elongated nucleus. The medial lining epithelium was thicker than the lateral one (Fig. 2B).

Rabbit embryo of 16 days old

The duct of future VNO was in contact with the nasal cavity (Fig. 2C). The rostral part of the future vomeronasal duct was lined by stratified cuboidal to stratified columnar epithelium (Fig. 2D). The lumen of the middle part of the vomeronasal duct was slit like. The dorsomedial wall of this duct was thicker than the ventrolateral one. Vomeronasal cartilage appeared more condensed than in the previous stage (Fig. 3A).

Rabbit fetus of 18 days old

The lining epithelium of vomeronasal duct was differentiated into thick olfactory like epithelium and thin respiratory epithelium. The C-cartilage of the vomeronasal organ was built up of chondroblastic cellular aggregation (Fig. 3B).

Rabbit fetus of 20 days old

The rostral opening of vomeronasal organ opened directly into the floor of nasal cavity (Fig. 3C). While at the middle region, the future vomeronasal duct had slit-like lumen and its medial wall was thicker and had about 8-10 layers of stratified columnar and the lateral one consisted of 3-4 cell layer (Fig. 3D)

Rabbit fetus of 22 days old

The vomeronasal duct showed two types of epithelium, the medial one resembled the olfactory form, while the lateral one appeared to be formed of respiratory type. The Primordia of acini of the vomeronasal glands were determined at the dorsal commissure of the vomeronasal duct (Fig. 4A).

Rabbit fetus of 28 days old

VNO opened rostraly in the nasal vestibule (Fig. 4B). The mucous membrane was of stratified columnar, aggregations of the acini of vomeronasal glands were determined at the dorsal commissure, some of them were luminized and others were still obliterated (Figs. 4C and D).

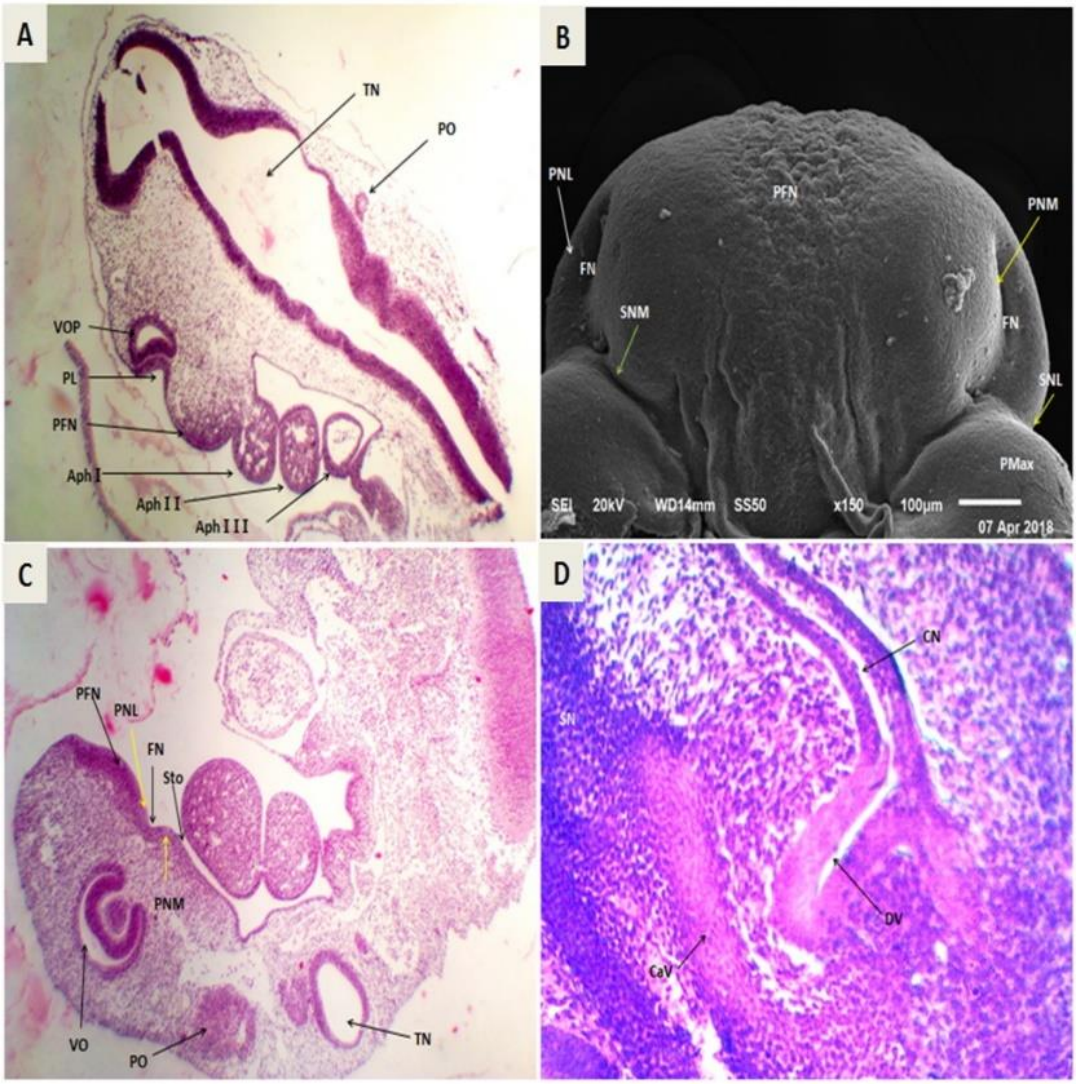

Figure 1: (A) A photomicrograph of L. S. of the rabbit Embryo of 9 days old showing; Prominentia frontonasalis (PFN), Placoda lentis (PL), Vesicula optica (VOP), Placoda otica (PO), Tubus neuralis (TN) with existence of the Arcus pharyngei [branchiales] I- I I I (APh I, APh I I, APh I I I). (H.E. stain 40X).**(B)** A photomicrograph of scanning electron micrograph (SEM) of the head of rabbit Embryo of 11 days old showing; the nasal placodes as a Fovea nasal is (FN), Prominentia nasalis lateralis (PNL), Prominentia nasalis medialis (PNM), Prominentia frontonasalis (PFN), Processus maxillaris (PMax), Sulcus nasolacrimalis (SNL) and Sulcus nasomaxillaris (SNM). **(C)** A photomicrograph of L. S. of the rabbit Embryo of 11 days old showing; Fovea nasalis (FN), stomodaeum (Sto), Prominentia frontonasalis (PFN), Placoda otica (OP), Prominentia nasalis lateralis (PNL), Prominentia nasalis medialis (PNM), Tubus neuralis (TN), Vesicula optica (VO) (H.E. Stain 40X). **(D)** A photomicrograph of C. S. of the head of the rabbit Embryo of 13 days old showing; the primordia of Ductus vomeronaslis (DV), primordia Cartilago vomeronaslis (CaV), Cavum nasi (CN) and Septum nasi (SN). (H.E. stain 100X)

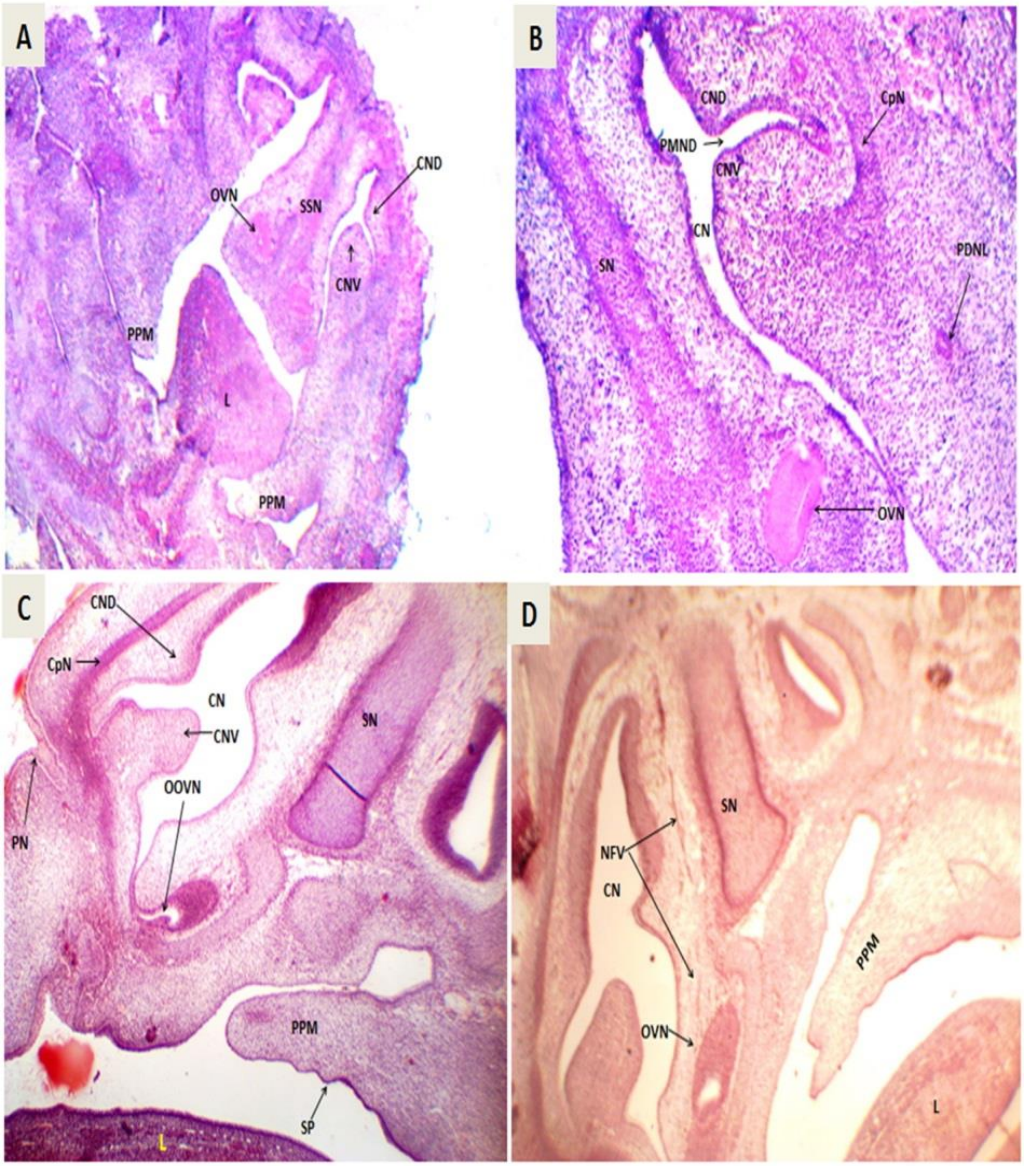

Figure 2: (A) A photomicrograph of C. S. of the head of the rabbit Embryo of 14 days old showing; the primordia of the secondary Septum nasi (SSN), primordia Concha nasalis ventralis (CNV), Concha nasalis dorsalis (CND), Processus palatinus medianus (PPM), primordia of Organum vomeronasale (OVN) and lingua (L) (H.E. stain 40X). **(B)** A photomicrograph of C. S. of the head of the rabbit Embryo of 14 days old showing; the primordia of the Septum nasi (SN), Cavum nasi (CN), primordia Concha nasalis ventralis (CNV), Concha nasalis dorsalis (CND), primordia of Ductus nasolacrimalis (PDNL), primordia of Organum vomeronasale (OVN), Primordial of Meatus nasi dorsalis (PMND) and Capsula nasalis primitivae (CpN). (H.E. stain 100X). **(C)** A photomicrograph of L. S. of the head of the rabbit Embryo of 16 days old showing; the primordia of Cavum nasi (CN), Septum nasi (SN), primordia Concha nasalis ventralis (CNV), Concha nasalis dorsalis (CND), Ostium of Organum vomeronasale (OOVN), primordia of Capsula nasalis primitivae (CpN). Processus palatinus medianus (PPM), primordia secondary palate (SP) and lingua (L). (H.E. stain 40X). **(D)** A photomicrograph of L. S. of the head of the rabbit Embryo of 16 days old showing; Neurofibra vomeronasale (NFV), Organum vomeronasale (OVN), Septum nasi (SN), Processus palatinus medianus (PPM), Cavum nasi (CN) and lingua (L). (Silver I. stain 40X)

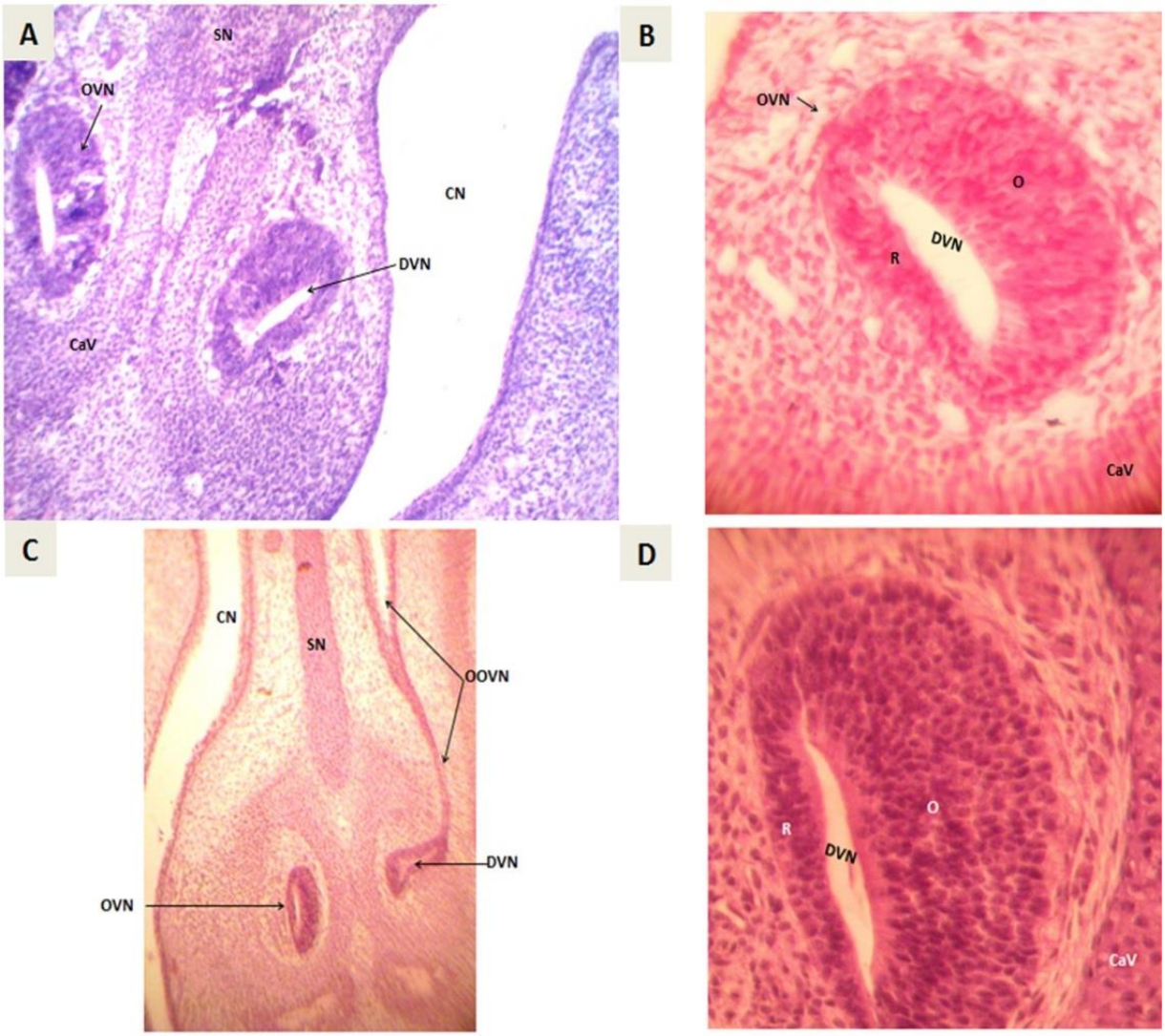

Figure 3: (A) A photomicrograph of C. S. of the head of the rabbit Embryo of 16 days old showing; Ductus vomeronasalis (DVN) slit like opening was lined by thin epithelium ventrolaterally and thick epithelium dorsomedial, Organum vomeronasale (OVN), Cartilago vomeronasalis (CaV), Septum nasi (SN), Cavum nasi (CN). (H.E. stain 100X). **(B)** A photomicrograph of C. S. of head of the rabbit Fetus of 18 days old showing; the lining Epithelium of Ductus vomeronaslis (DVN), Cartilago vomeronaslis (CaV), Organum vomeronasale (OVN). Was differentiated into thick Tunica mucosa olfactoria vomeronaslis(O) and thin Tunica mucosa respiratoria vomeronaslis(R). (H.E. stain 400X). **(C)** A photomicrograph of C. S. of head of the rabbit Fetus of 20 days old showing; The rostral opening Organum vomeronasale (OOVN) opened directly into the floor of Cavum nasi (CN), Septum nasi (SN), Ductus vomeronasalis (DVN). (H.E. stain 100X). **(D)** A photomicrograph of C. S. of head of the rabbit Fetus of 20 days old showing; lumen of Ductus vomeronaslis (DVN), a thick medialTunica mucosa olfactoria vomeronaslis(O) and thin lateral Tunica mucosa respiratoria vomeronaslis(R). Cartilago vomeronaslis (CaV), (H.E. stain 400X)

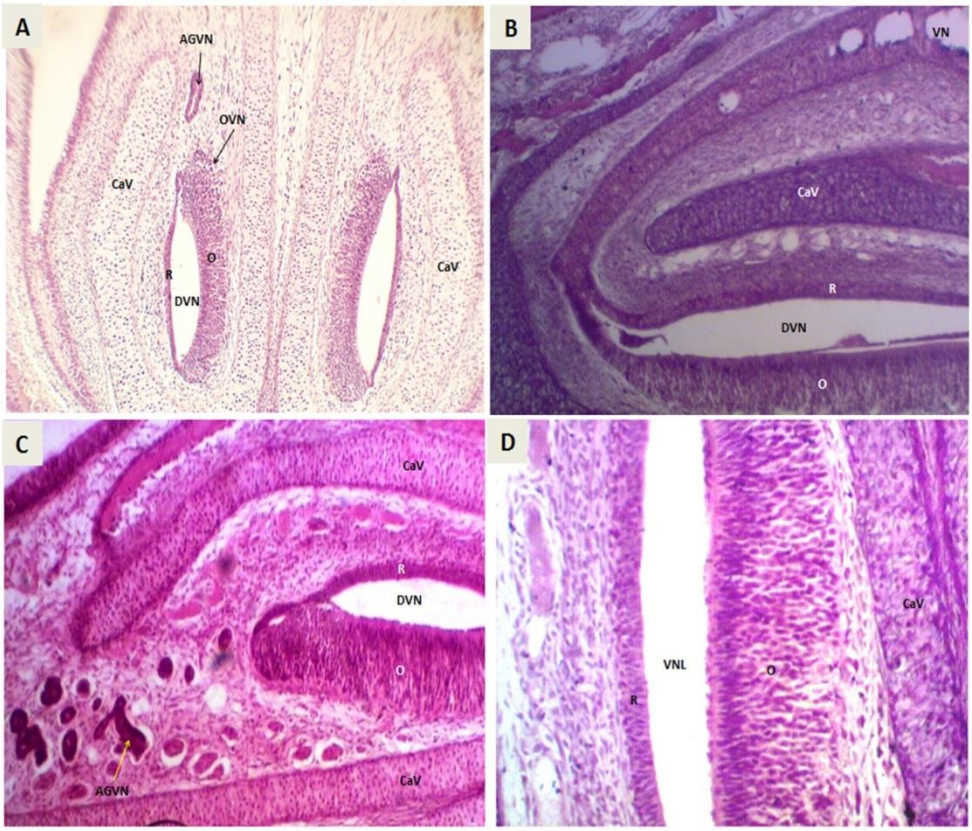

Figure 4: (A) A photomicrograph of C. S. of the head of the rabbit Fetus of 22 days old showing; lumen of Ductus vomeronaslis (DVN), a thick medialTunica mucosa olfactoria vomeronaslis(O) and thin lateral Tunica mucosa respiratoria vomeronaslis(R). Cartilago vomeronaslis (CaV), the Primordia Acinus glandula vomeronasalis (AGVN) were determined at the dorsal commissure of Organum vomeronasale (OVN). (H.E. stain 100X). **(B)** A photomicrograph of C. S. of the head of the rabbit Fetus of 28 days old showing; Ductus vomeronaslis (DVN), a thick medialTunica mucosa olfactoria vomeronaslis(O) and thin lateral Tunica mucosa respiratoria vomeronaslis(R). Cartilago vomeronaslis (CaV), was opened into the rostral of ventral part of Septum nasi, the mucous membrane was of stratified columnar type and the rostral opening of the duct blended directly with that of Vestibulum nasi (VN). (H.E. stain 400X). **(C)** A photomicrograph of C. S. of the head of the rabbit Fetus of 28 days old showing; Ductus vomeronaslis (DVN), Tunica mucosa olfactoria vomeronaslis(O),Tunica mucosa respiratoria vomeronaslis (R). Cartilago vomeronaslis (CaV) and Acinus glandula vomeronasalis (AGVN). (H.E. stain 400X). **(D)** A photomicrograph of C. S. of the head of the rabbit Fetus of 28 days old showing; of Ductus vomeronaslis (DVN), a thick medialTunica mucosa olfactoria vomeronaslis(O) and thin lateral Tunica mucosa respiratoria vomeronaslis(R). Cartilago vomeronaslis (CaV), (H.E. stain 400X).

Discussion

The present investigation showed that, the thickened epithelium of the nasal placode was invaginated to make the nasal pits (olfactory pits) at 11 days old of rabbit embryo. The primordia of VNO was performed at the medial aspect of the nasal pit as a thickening of the epithelium a result which came in agreement with, (6) in hamster, (7) in rabbit and (30) in rat whose mentioned that, the vomeronasal organ was embryologically derived from the olfactory placode. In human, early during the fifth week, the ectoderm in the upper one-third of each enlarging nasal sac became thickened and developed into the olfactory epithelium (19, 32). On the contrary in mammals (15) mentioned that the vomronasal organ originated from the medial wall of the olfactory pit shortly after the middle of the embryonic period.

In the present investigation, there was a clear invagination, the epithelium lined the pit was similar to that of the placode, and merged

steadily with the general ectoderm contiguous the outside opening, which majorities towards the mesenchyme and causes a slight recess on the olfactory purse at 11 days old of rabbit embryo. Later on, cellular bud grew dorsally, caudally, and to the midline on both sides and formed the primordia of the vomeronasal groove these results were in accordance with (32) in hamster whose compared between the vomeronasal sensory and the olfactory epithelia and noticed that, the both epithelia were divergently derived from the olfactory placodes.

At 13 days old embryo VNO appeared as arrangement of bilateral undifferentiating epithelial thickenings on rostroventral region of the nasal septum. The organ was enclosed via the immature vomeronasal cartilage, which appeared as but densely arranged in small amorphous cells. The latter cells were suggestive of their future dispositions. The medial and lateral sides of the nostrils showed the miniature of nasal cartilages. These results were in disagreement with (33) who stated that, the vomeronasal organ appear as two blind epithelial like tubes in the ventral aspect of the nasal septum at nasal vestibule at sixteenth prenatal day of rat. While our findings were in accordance with (7, 8) in rabbit, (9,34) in rat, (21) in goat, (19, 31, 35) in human, (36) in mammals and (37) in animals.

The present work revealed that, the (future) VNO was in contact with the nasal cavity at 13- 28 days old of the rabbit embryos and fetuses. This result could not met with the available literature in the rabbit or the other animals and human expect (16) in primates whose mentioned that, one exception occurred in the largest fetal Tarsius (25 mm crown–rump length), in which the vomeronasal organ communicated with the nasal cavity alone.

Conclusion

To the best of our knowledge, this may be the first description to the designing, categorize, define and demonstrate the normal explaining of the onset time of first appearance and origin as well as developmental changes of vomeronasal organ of the rabbit and its allied structures

Conflict of interest

The authors declare that they have no conflict of interest.

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