

Prenatal Development of the Nasal Conchae (Conchae nasales) in Rabbits (*Oryctolagus cuniculus*)

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With 13 figures.

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Abstract

Nasal conchae increase the surface area of these cavities, thus providing for rapid warming and humidification of air and by regulating nasal airflow, play a role in immune surveillance because they are the first intranasal structures to come into contact with outside air. The present study aimed to depict the onset time of the first appearance and origin as well as developmental changes of Nasal conchae.

This study was carried out on 116 rabbit embryos and fetuses of both sexes. All specimens of the heads assembled for normal histological technique, stained with H&E. Some were processed for scanning E/M.

At day13: The primitive nasal cavity was surrounded by a condensation of mesenchymal tissue giving rise to the miniature of the nasal

capsule (Fig.8). As well, the anlage of the ventral nasal concha was detected in the lateral nasal wall. **At day14:** The secondary nasal septum appeared as a wedge-like structure formed of mesenchymal connective tissue. It was covered ventrally by a thin layer of undifferentiated epithelium which faced the dorsum of the tongue through the primitive choana. **At D16:** the nasal cavity and the dorsal and ventral conchae plugged inside the nasal cavity and separated by the middle meatus and surrounded externally by nasal capsule. The caudodorsal part of the future nasal cavity demonstrated many folds forming the future ethmoidal conchae. **At Day 18:** there were many processes which were differentiated into ectoturbinates and endoturbinates in the ethmoidal region. **At Day 20:** At the ethmoidal region of

nasal cavity showed nerve bundles extended from the covering epithelium toward the olfactory bulb. **At Day 22:** The core of fold contained the cartilaginous extension of ventral nasal conchae was clear. The cells of the olfactory mucous membrane were differentiated and the shape of the nuclei of its cells was tapered in the basal cell layer or rounded or oval in the intermediate layers. **At Day 25:** The ethmoidal conchae had primary and secondary branches housing in between ethmoidal meatuses. The top of the ethmoidal conchae was capped by olfactory epithelium

Keywords: conchae, mesenchymal tissue, ethmoidal concha, primitive

Introduction

Nasal conchae increase the surface area of these cavities, thus providing for rapid warming and humidification of air and by regulating nasal airflow, play a role in immune surveillance because they are the first intranasal structures to come into contact with outside air. The present study aimed to depict the onset time of the first appearance and origin as well as developmental changes of Nasal conchae. In rat, Vidic (1971) stated that, at 14-day old fetus, all the turbinates and

nasal capsule were already differentiated and at 16 day of pregnancy the lateral nasal wall was initiated. In hamster, Kuramoto, et al., (1978) recorded that, nasoturbinates, maxilloturbinate and endoturbinates I, II and III had already been formed by 11 days of gestation.

Andersen and Matthiessen (1967) proved that, the primordium of concha nasalis ventralis was observed in 21 mm CRL human embryo. Snell (1975) mentioned that, Conchae nasals were developed at the second month. William, Smith and Treadgold (1984) revealed that, the nasal conchae first obviated as a number of elevations on the lateral nasal wall and soon was coalesced to form the nasal conchae. Bingham, Wang, Hawke and Kwoky (1991) stated that, the human three turbinates (Inferior, middle and superior) arised as soft tissue swellings (Preturbinates) by 8 weeks' gestation. De Arreola, Serna, Parra and Salinas (1996) concluded that, the seven weeks of the human gestation showed the first buds of the three turbinates and at ninth week the precartilaginous nucleus of the inferior turbinate was observed.

In human, Neskey et al., (2009) studied that, at 15 to 16 weeks' gestation, the inferior, middle, and

superior turbinates are well formed. Additionally, the primordial maxillary sinus is surrounded by a sleeve of cartilage and has grown from the space lateral to the uncinata, the ethmoid infundibulum, toward the apex of maxilla inferiorly. Caudal protrusions from the ethmoid infundibulum continue to enlarge and will become the caudal ethmoid cells. The development of the lateral nasal wall is close to complete by 24 weeks gestation. By this time, the superior and middle turbinates have developed and ossified from the ethmoid bone, while the inferior turbinate has emerged from two origins, the maxilla and the lateral cartilaginous capsule. Based on the initial mucosal thickening, turbinate development appears to be a primary process, and meatal ingrowth occurs secondarily. Som and Naidich (2013) revealed that, by the eighth week of the human gestation, the inferior and middle turbinate anlagen formation was followed by the superior turbinate anlagen.

In camel, Ahmed (1988) stated that, the anlage of the nasal conchae were detected at 17mm CVRL, these structures arose from lateral nasal capsule. The primitive dorsal and ventral spiral lamellae of ventral nasal concha was appeared

at 75 mm CVRL. Its ossification took place from the basal palate. Then complete ossification of the ventral spiral lamella at 650 mm CVRL followed by the ossification of dorsal spiral lamella at 750 mm CVRL.

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 rabbits. The rabbits were obtained from the rabbit farm of Faculty of Agriculture, Zagazig University, Egypt.

Rabbits were housed for one week before experiment for acclimatization. Standard pellet ration (El-Nasr Chemical Company, Cairo, Egypt) and were given and let 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 females were served by natural mating and each female housed individually in stainless-steel cages in environmentally controlled rooms and each maternal rabbit was given about 100g-day of certified rabbit nutrition free access to water. The pregnant rabbits 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 were done. The obtained embryos and fetuses were classified into two groups 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 the 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 Drury and Wallington (1980), Alomaisi et al., (2018), Alomaisi et al., (2019).

After all specimens assembled for normal histological technique, all specimens were dehydrated in ascending grades of alcohol, cleared in three changes of benzene and embedded in paraffin wax. Paraffin

sections of 5-7 μ thickness were obtained and stained by different histological stains such as: Hematoxylin and Eosin (H&E) stain for general histological demonstration and silver impregnation Drury and Wallington (1980), Suvarna, Layton and Banrcoft (2019).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 (Cheville and Stasko, 2014). The were dehydrated Prenatal development of the vomeronasal organ in rabbit 625 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 re fixed 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 Alomaisi et al., (2018), Alomaisi et al., (2019).

The nomenclature used along the course of this study was adopted to Nomina Anatomica Veterinaria (2017), Nomina Embryologica Veterinaria (2017) and Nomina Histologica Veterinaria (2017).

Results

Rabbit embryo of 13-days old:

The primitive nasal cavity was surrounded by a condensation of mesenchymal tissue giving rise to the miniature of the nasal capsule (Fig.1). As well, the anlage of the ventral nasal concha was detected in the lateral nasal wall.

Rabbit embryo of 14-days old:

The secondary nasal septum appeared as a wedge like structure formed of mesenchymal connective tissue. It was covered ventrally by a thin layer of undifferentiated

epithelium which faced the dorsum of the tongue through the primitive choana (Fig.2).

Rabbit embryo of 16-days old:

the nasal cavity and the dorsal and ventral conchae plugged inside the nasal cavity and separated by the middle meatus and surrounded externally by nasal capsule (Fig.3). As well as, there was a deep fissure forming the future middle meatus dividing the epithelium into dorsal and ventral nasal concha (Fig.3). The most caudal part of the primitive nasal cavity constituting the olfactory region was lined by thick layer of stratified columnar epithelium. The submucosa showed well marked nerve bundles extended through the ethmoidal area and interspersed by blood spaces (Fig.4). The caudo-dorsal part of the future nasal cavity demonstrated many folds forming the future ethmoidal conchae. They were reinforced by a dorsal extension of chondrogenic tissue derived from the dorsal part of nasal capsule and housing between them ethmoidal meatuses (Fig.5). They were lined by a thick layer of stratified epithelium (Fig.6).

Rabbit fetus of 18-days old:

At the ethmoidal region of the nasal cavity, there were many processes

which were more differentiated olfactory epithelium. The latter were covered by well developed olfactory epithelium clear basement membrane. The latter were covered by well developed olfactory epithelium (Fig.7). At the rostral part of the primitive nasal cavity, the ventral end of the secondary nasal septum and the free ends of the palatine shelves fused (Fig.8).

Rabbit fetus of 20-days old: At the ethmoidal region of nasal cavity there were nerve bundles extended from covering epithelium toward the olfactory bulb (Fig.9).

Rabbit fetus of 22-days old: The core of a fold contained the cartilaginous extension of ventral nasal conchae was observed. The cells of the olfactory mucous membrane were differentiated and the shape of the nuclei was tapered in the basal cell layer or rounded or oval in the intermediate layers while, the nuclei of the superficial cell layer were deeply stained and elongated. The olfactory epithelium and its underlying mesenchyme contained the primordial of the olfactory (Bowman's) glands (Fig.10).

Rabbit fetus of 25-days old: The ethmoidal conchae had primary

and secondary branches housing in between ethmoidal meatuses (Fig.10). The top of the ethmoidal conchae was capped by olfactory epithelium (Fig.11).

Discussion

The present study showed that, there was a deep fissure (primitive middle meatus) dividing the epithelium into anlage of dorsal and ventral nasal conchae at 14-days old of the rabbit embryo. These results are not in a line with that of Seham-Gabr (2015) in rabbit also, whose mentioned that the first appearance of the dorsal and ventral conchae was at 16- and 19-day of gestation. But these results agreed with the statement of Roongruangchai, et al., (2006) In rabbit. Also, Noden and De Lahunta (1985) in mammals, McGeady, et al., (2017), in animals and, Ahmed (1988) in camel.

In the animal of this study, the caudo-dorsal part of the future nasal cavity demonstrated many folds forming the future ethmoidal conchae at 16 days old of the rabbit embryo these results were in accordance with Vidic (1971) in rat stated that, at 14 day old fetus, all the turbinates and nasal capsule were already differentiated and at 16 day fetuses. Also, Kuramoto, et

al., (1978) in hamster recorded that, nasoturbinate, maxilloturbinate and endoturbinate I, II and III had already been formed by 11 days of gestation. As well, Neskey et al., (2009) in human, mentioned that, at 15 to 16 week of gestation, the inferior, middle, and superior turbinates are well formed. , Nevertheless this findings completely contradicts with Seham-Gabr (2015) whose stated that in rabbit, the first development of the ethmoidal conchae was at 30 day or at birth not before that but it differentiated at one week old after birth.

The present finding revealed that, the conchae in rabbit fetus were divided into dorsal, ventral and ethmoidal conchae, the ethmoidal conchae had primary and secondary branches housing in between ethmoidal meatuses. The top of the ethmoidal conchae was capped by olfactory epithelium at the age of 25 days of rabbit fetus these results were completely conflicted with the statement of Özkadif, and Eken, (2012) in rabbits whose stated that, dorsal, ventral and middle nasal conchae and endoturbinalia were determined on lateral nasal septum. The dorsal nasal concha was on top of cavum nasi, middle nasal concha was the oro-ventral third of the dorsal nasal concha, the ventral

nasal conchawas aboro-ventral third of the dorsal nasal concha Endoturbinalia was located ventral to the middle nasal concha.

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 first appearance and origin as well as developmental changes of Nasal conchae (Conchae nasales) of the rabbit and its allied structures.

Conflict of interest

The authors declare that they have no conflict of interest.

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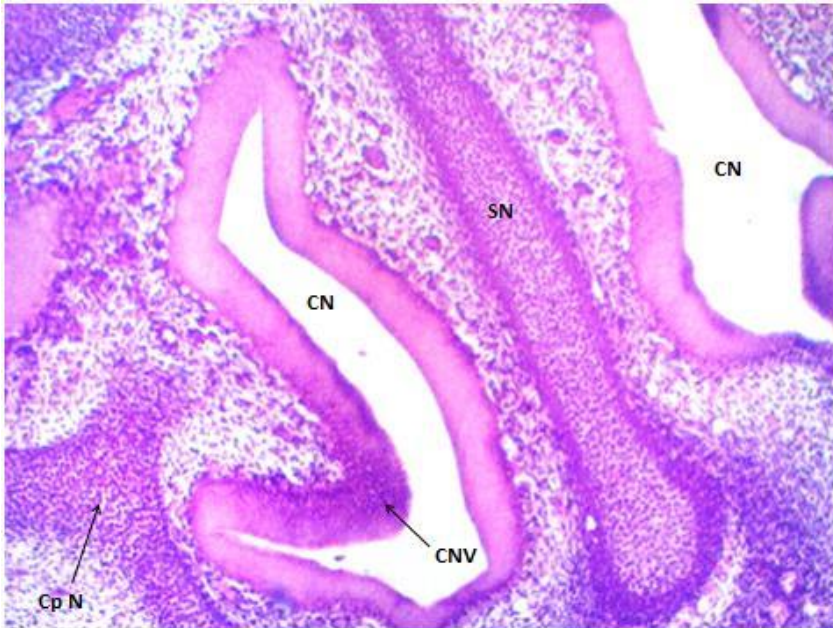


Fig (1): A photomicrograph of C. S. of the head of the rabbit Embryo of 13-days old showing: the primitive Cavum nasi (CN), Septum nasi (SN), primordia of Concha nasalis ventralis (CNV) and primordia of Capsula nasalis primitivae (CpN). (H.E. stain 100X)

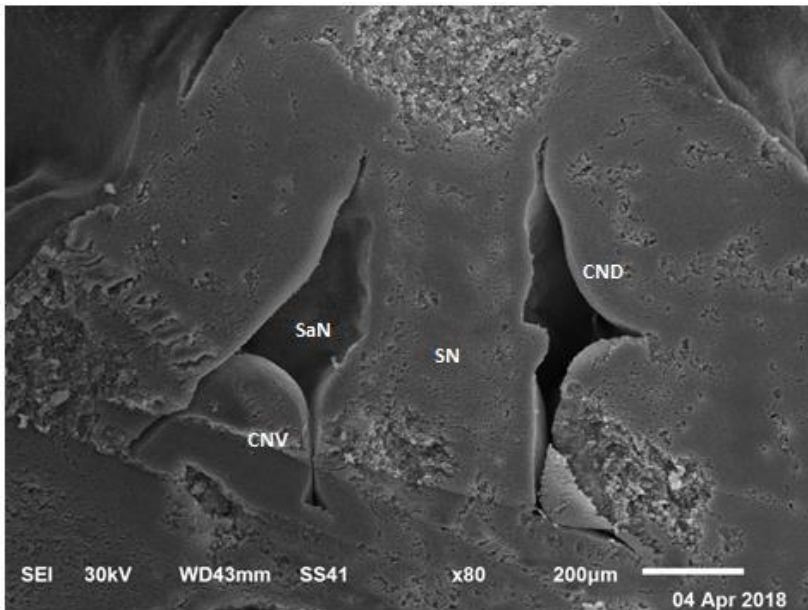


Fig (2): A scanning electron micrograph (SEM) of head of rabbit Embryo of 14-days old showing: the primordia of the Saccus nasalis (SaN), Septum nasi (SN), primordia Concha nasalis ventralis (CNV) and Concha nasalis dorsalis (CND).

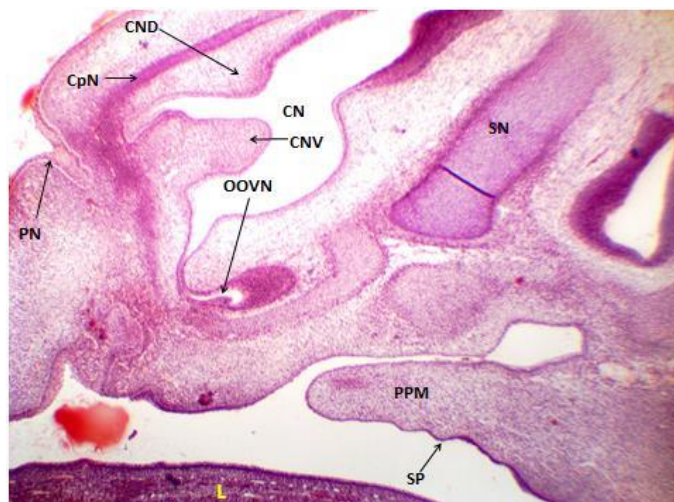


Fig (3): A photomicrograph of L. S. of the head of the rabbit Embryo of 16-days old showing: the primordia of Cavum nasi (CN), Septum nasi (SN), primordia Concha nasalis ventralis (CNV), Concha nasalis dorsalis (CND), Ostium of Organum vomeronasale (OOVN), primordia of Capsula nasalis primitivae (CpN). Processus palatinus medianus (PPM), primordia secondary palate (SP) and lingua (L). (H.E. stain 40X).

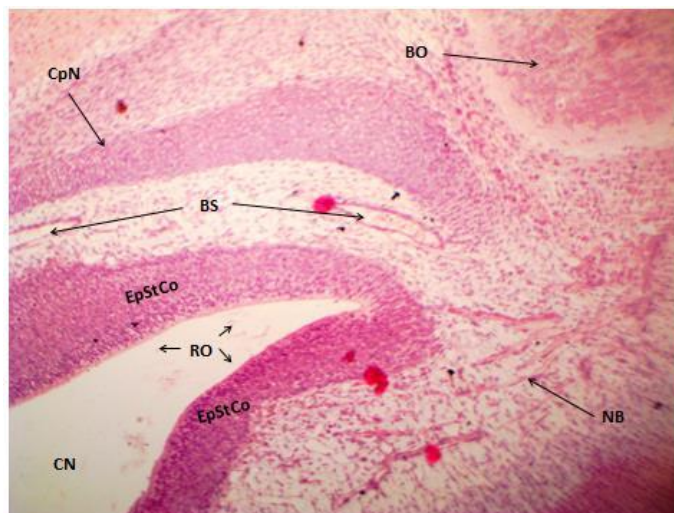


Fig (4): A photomicrograph of C. S. of the head of the rabbit Embryo of 16-days old showing: the caudal part of the primitive Cavum nasi (CN), Regio olfactoria (RO) was lined by thick layer of Epithelium stratificatum columnare (Ep StCo), Nerurofibra (NF), Bulbus olfactorius (BO) and interspersed by blood spaces (BS), Capsula nasalis primitivae (CpN). (H.E. stain 400X).

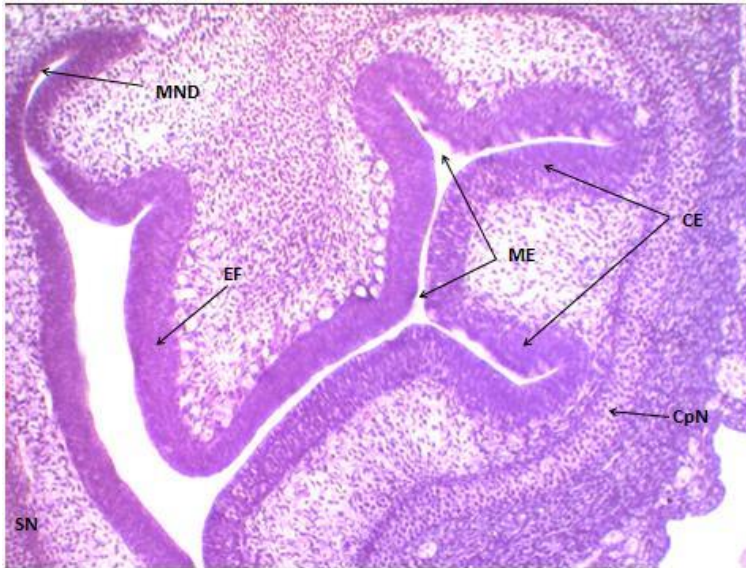


Fig (5): A photomicrograph of C. S. of the head of the rabbit Embryo of 16-days old showing: Ethmoidal folds (EF), Conchae ethmoidales (CE). the dorsal part of Capsula nasalis primitivae (CpN) and Meatus ethmoidales (ME). (H.E. stain 100X).

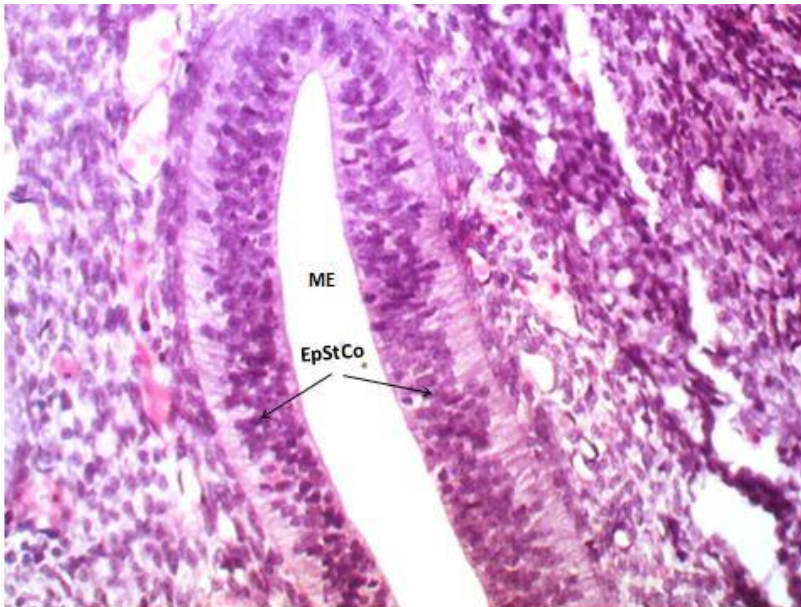


Fig (6): A photomicrograph of C. S. of the head of the rabbit Embryo of 16 days old showing: Meatus ethmoidales (ME), thick layer of Epithelium stratificatum columnare (Ep StCo). (H.E. stain 400X).

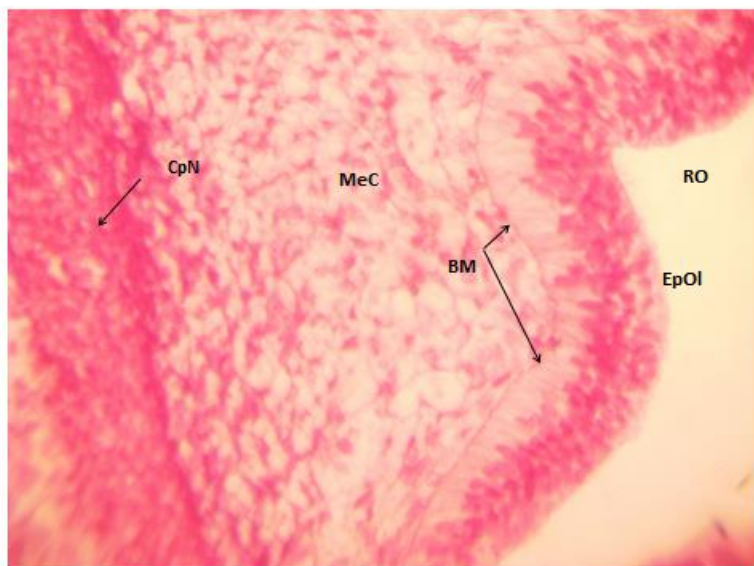


Fig (7): A photomicrograph of head of the rabbit Fetus of 18 days old showing; Epithelium olfactorium (EpOl), Capsula nasalis primitivae (CpN), Regio olfactoria (RO), mesenchyme cells (MeC), base-ment membrane (BM). (H.E. stain 400X).

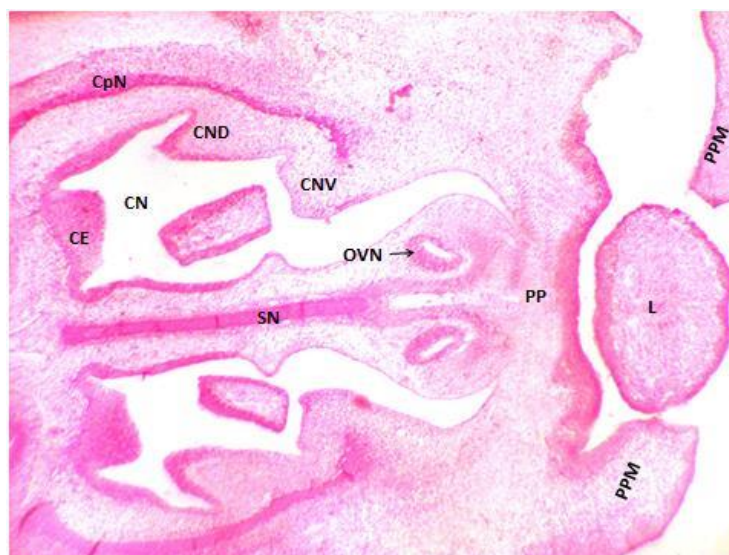


Fig (8): A photomicrograph of coronal S. of the head of the rabbit Fetus of 18-days old showing: primitive Cavum nasi (CN), Septum nasi (SN), Concha nasalis ventralis (CNV), Concha nasalis dorsali (CND), Conchae ethmoidales (CE) Organum vomeronasale (OVN), primordia of Capsula nasalis primitivae (CpN). primary palate (PP), Processus palatinus medianus (PPM) and lingua (L). (H.E. stain 40X).

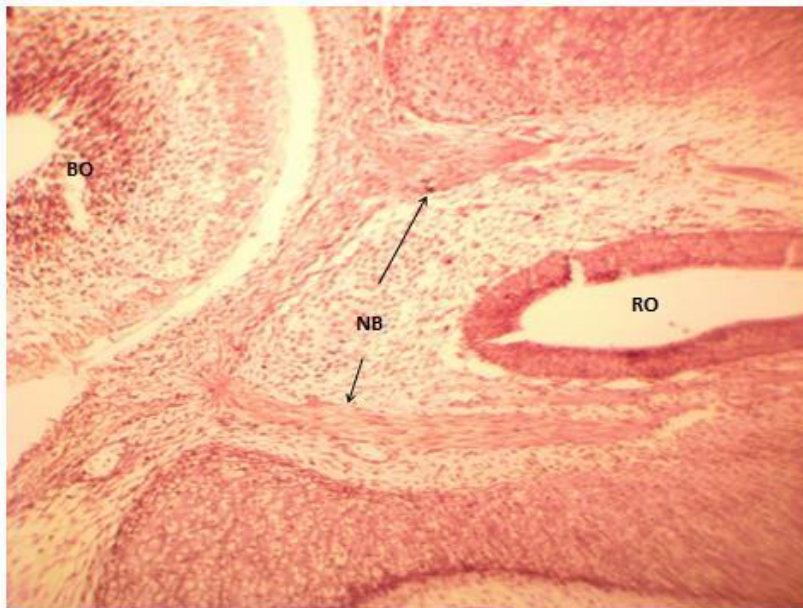


Fig (9): A photomicrograph of C.S. of head of the rabbit Fetus of 20-days old showing: Region olfactoria (RO), the Regio olfactoria (RO) (ethmoidal region) of Cavum nasi had clear Nerurofibra (NB) extended from covering epithelium toward the Bulbus olfactorius (BO). (Silverl. stain 100X).

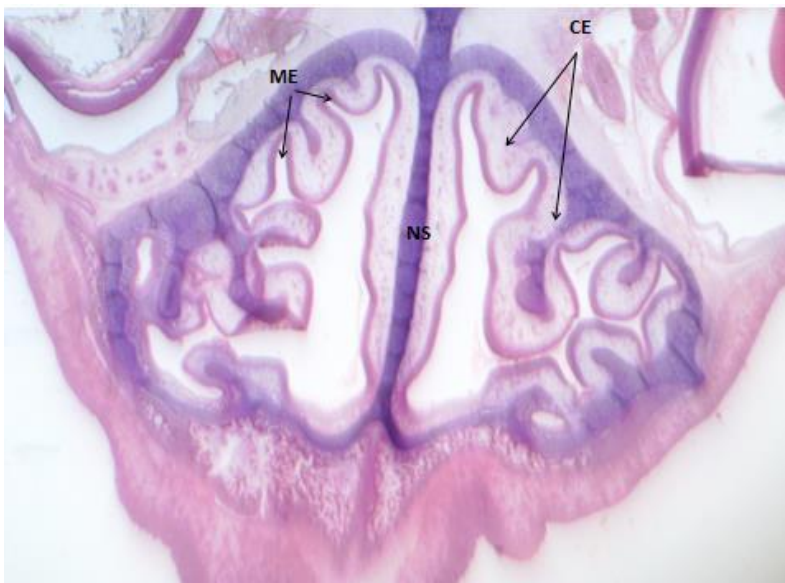


Fig (10): A Stereo-microscopic of C.S. of head of the rabbit Fetus of 25-days old showing: The Conchae ethmoidales (CE) were branched into primary and secondary branches, Septum nasi (SN), Meatus ethmoidales (ME). (H.E. stain 10X).



Fig (11): A photomicrograph of C.S. of the head of the rabbit Fetus of 25-days old showing: the top of the Conchae ethmoidales (CE) capped by Epithelium olfactorum (EpOI), Septum nasi (SN). (H.E. stain 40X).



Fig (12): A photomicrograph of C.S. of the head of the rabbit Fetus of 25 days old showing: Conchae ethmoidales capped by Epithelium olfactorum (EpOI), Neurofibra olfactoria (NO). (H.E. stain 100X).

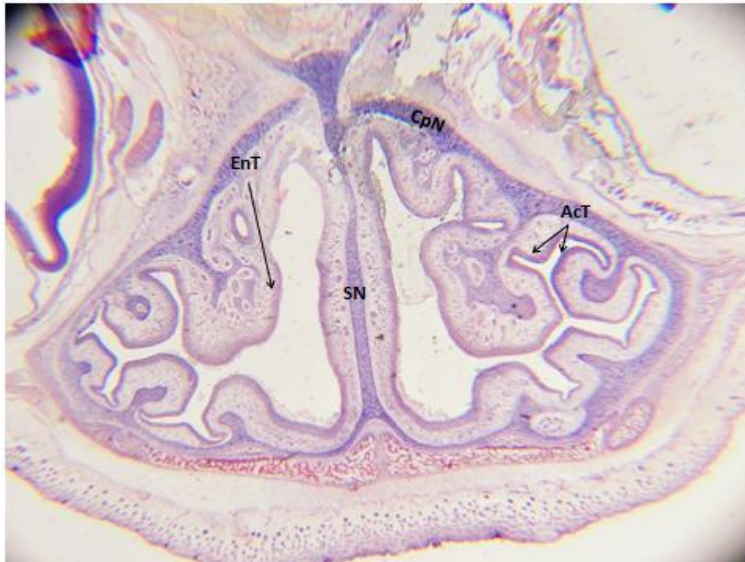


Fig (13): A Stereo-microscopic of C.S. of the head of the rabbit Fetus of 28-days old showing: The endotubinate (EnT) and actourbinate (AcT)of conchae ethmoiales, Capsula nasalis primitivae(CpN), Septum nasi (SN). (H.E. stain 10X).

Animals of this issue

Rabbit (*Oryctolagus cuniculus*)



Kingdom: Animalia & Phylum: Chordata & Class: Mammalia & Order: Lagomorpha & Family: Leporidae (in part) & Genus: *Oryctolagus*

The rabbit's long ears, which can be more than 10 cm (4 in) long, are probably an adaptation for detecting predators. They have large, powerful hind legs. The two front paws have 5 toes, the extra called the dewclaw. The hind feet have 4 toes. They are plantigrade animals while at rest; however, they move around on their toes while running, assuming a more digitigrade form. Wild rabbits do not differ much in their body proportions or stance, with full, egg-shaped bodies. Their size can range anywhere from 20 cm (8 in) in length and 0.4 kg in weight to 50 cm (20 in) and more than 2 kg. The fur is most commonly long and soft, with colors such as shades of brown, gray, and buff. The tail is a little plume of brownish fur (white on top for cottontails). Rabbits can see nearly 360 degrees, with a small blind spot at the bridge of the nose.

Source: Wikipedia, the free encyclopaedia