Embryonic Differentiation of The Nasal Cavity of One-humped Camel (*Camelus dromedarius*)

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Abstract

The current study was carried out on thirty one camel embryos and fetuses with crown vertebral rump lengths (CVRL) ranging between (15-100) mm in order to follow-up the prenatal developmental stages of the nasal cavity. The microscopic examination of the obtained specimens revealed that the camel nasal cavity began as deep invaginations (nasal pits) which appeared in the rostral portion of the embryonic head. These invaginated pits became deeper and enlarged forming the primitive nasal cavities, which were separated from the primitive oral cavity by the oronasal membrane. This membrane then broken down and allowed both cavities to communicate with each other by the primitive choanae. Two shelf-like outgrowths grew ventrally from the maxillary prominences to eventually form the secondary palate. These shelves were initially directed obliquely and ventrally inside the stomodeum in a vertical direction on each side of the developing tongue. Rostral to the developing nasal septum, a ventral
evagination of the lining ectoderm was noticed on both sides of the nasal septum forming the epithelial lining of the vomoronasal organ meanwhile the surrounding mesenchyme developed to be a curved plate of its hyaline cartilage. Two projections were noticed on the lateral wall of each nasal cavity to form the dorsal and ventral conchae. The ectodermal lining of the dorsal and lateral aspects of the nasal cavities differentiated into thick olfactory epithelium while that of the ventral and medial aspects became thin respiratory epithelial coat. Based on the above-mentioned findings, it is noticed that the development of the nasal cavity in one-humped camel was histologically in succession.

Keywords: Nasal cavity, Camel, Embryos, Fetuses

Introduction

Camel is considered as a very important animal, it lives in several countries; Middle East and North Africa as well as many other countries all over the world, but it received little attention when compared with other domestic animal species. It has the ability to tolerate the harsh climate of the desert as it is subjected to the high temperature and the burning sun rays (Bigham et al., 2007). Camels become an interesting field of research to the complete understanding of their development, anatomy and physiology (Fowler, 1997). The rapid rise in the last decade of camel domestication due to their meat and milk (Konuspayeva et al., 2009), needs more knowledge for its effective management.

The embryonic developmental stages of the nasal cavity are based on the classical work of His (1901). This initial work has been supplemented and extended by the studies of (Hochstetter, 1944; Hochstetter, 1950; Peter, 1913; Peter, 1950; Boyd, 1933; Streeter, 1948; Politzer, 1953) and others.

From the available literature, there is no similar report has been found concerning the histo- and morphogenesis of the camel nasal cavity. Therefore, the present work was undertaken with the aim of assessment the integrated histological changes associating with the highlights on morphogenesis of the nasal cavity throughout the early prenatal life of the one-humped camel. Our investigation aimed to document the major landmarks and the time course in the prenatal development of the nasal cavity in dromedary camel and its accommodation with the surrounding hard environment of the desert, and to compare our results with those reported in other domestic animal species as well as humans. The present work may provide a model for studying the developmental morphology of nasal cavity as a potential target organ in camel, in an attempt to understand its accommodation with the environment of the desert.

Material and methods

Specimens collection:

The present study was carried out on apparently healthy she-camels, which are subjected to veterinary inspection according to the Egyptian laws, and using standard animal ethics approved by the Egyptian government. Thirty-one embryos and fetuses of the one-humped camel were used. The crown vertebral rump length (CVRL) ranged from 15 mm to 100 mm. Seven embryos for the stage 15-35 mm, eleven fetuses for the stage 40-65 mm, and thirteen fetuses for the stage 70-100 mm were used. All embryos and fetuses were collected from El-Basateen (Cairo) and Belbes (El-Sharqya) abattoirs directly after

slaughtering of the pregnant animals.

Tissue processing:
The whole embryonic and fetal heads were immersed directly in 10% neutral buffered formalin for a minimum of one month at room temperature. Following complete fixation of the specimens, the formalin-fixed specimens were then preserved in 70% ethyl alcohol. The preserved samples were dehydrated using a graded series of ethanol (75%, 80%, 90%, 95% and three changes of absolute alcohol), subjected to three changes of xylene, and then routinely embedded in paraffin wax. The paraffin-embedded specimens were serially sectioned at 5 – 7 µm thickness and the sections mounted on glass microscope slides. The paraffin sections were subjected to Harris haematoxylin and Eosin (H&E) for histomorphological examination according to Bancroft and Gamble (2008).

Photomicrography:
Photomicrographic images were taken using Olympus BX41 research optical photomicroscope fitted with an Olympus DP25 digital camera. The magnification scale bar was reported on the obtained photomicrographs.

Results

15 - 35 mm CVRL stage (Fig.1)

At this embryonic stage, the nasal pit was observed as a deep invagination comprised stratified epithelial cells appeared in the rostral end of the embryonic head. This pit became deeper due to its downward growth into the underlying mesenchyme as well as the growth of the surrounding nasal swellings (Fig 1A,C). Two shelf-like outgrowths from the maxillary prominences grew ventrally to eventually form the secondary palate. These shelves were initially directed obliquely and ventrally inside the stomodeum in a vertical direction on each side of the developing tongue **(Fig 1D).** Rostral to the developing nasal septum, a ventral evagination of the lining ectoderm was noticed on both sides of the ventral end of the nasal septum (Fig 1E). A projection on the lateral wall of each nasal cavity appeared which represent the developing ventral concha (Fig 1F).

40 - 65 mm CVRL stage (Fig 2)
The lateral palatine processes during this stage were elevated to become horizontally oriented and grew towards each other to fuse at the midline, thus forming the secondary palate. These two fused shelves also were fused dorsally with the nasal septum (Fig 2A). The developing tongue moved ventrally as its development proceeded, and the lateral palatine processes ascend to locate horizontally dorsal to the tongue (Fig 2A). The two palatine

shelves became in close contact with the distal edge of the nasal septum. The secondary palate also appeared to fuse rostrally with the primary palate (Fig 2B). The nasal septum appeared as a downward growth from the dorsal mesenchyme then continued to grow ventrally until it met the secondary palate (Fig 2C). The nasal pit enlarged to form the primitive nasal cavities (nasal sacs), which grew dorsocaudally, ventral to the developing brain. Each sac was separated from the primitive oral cavity by the oronasal membrane (Fig 2B,C) which soon break down to allow the nasal and oral cavities to communicate with each other via the primitive choanae which were presented caudal to the primary palate.

The epithelium of the vomoronasal organ was developed (Fig 2D), from which the epithelium of the lateral wall became thickened and formed the olfactory portion, meanwhile the epithelium of the medial wall remained thin and formed the respiratory epithelium (Fig 2E,F). Another projection appeared in the lateral wall of the dorsal part of the developing nasal cavity, which tends to become the dorsal nasal concha**.**

70 – 100 mm CVRL stage (Fig 3,4)

The primitive nasal cavities (nasal sacs) developed into the definitive nasal cavities **(**Fig 3A-C) and formed the distinct, elongated basal plate then rolled forming the scrolled plates of the conchae. The concha is a lamina formed from a mesodermal core and ectodermal epithelial lining (Fig 3B,C). The interior mesenchyme of the nasal septum is differentiated into hyaline cartilage meanwhile the mesechyme that surrounded the epithelium of the vomoronasal organ developed to a curved plate of hyaline cartilage (Fig 2C).

The ectodermal epithelial lining in the dorsal and lateral aspects of the nasal cavities became specialized for olfaction (Fig 4A) while the epithelium in the ventral and medial aspects became respiratory epithelium, which tends to be psuedostratified columnar ciliated epithelium without goblet cells at this point in development (Fig 4B). Two types of cells were noticed among respiratory epithelium; ciliated tall cells and basal ones. Concerning the olfactory epithelium, it is made up of highly mitotic densely packed cells having different shapes and sizes with large spherical euchromatic nuclei and eosinophilic cytoplasm**,** the epithelium was adjacent to a wide subepithelial mesenchymal layer, which was predominantly cellular.

Discussion

Although many investigators have recorded the morphogenesis of different organs in dromedary camels (Bareedy et al., 1982; El- Hariri et al., 1988; Marai et al., 1990; Konsowa, 2009; Farouk et al., 2012;

Osman et al., 2014 and Abdo et al., 2014), prenatal developmental studies of the camel nasal cavity have not been reported previously in our available literatures.

The nasal pit (groove) is formed by the maxillary process growing medially across the open naso-buccal channel and fusing with the medial nasal process (His, 1901). Our investigation showed that, the nasal pit at 15 mm CVRL stage was present in the form of a deep groove as a result of its downward growth into the underlying mesenchyme, as well as growth of the surrounding nasal swellings. On other hand, Della (1907) reported that the nasal grooves never open directly into the stomodeum and there is, therefore, no naso-buccal channel across which the maxillary process can grow in the way indicated by His (1901).

The primitive nasal cavities (nasal sacs) grow dorsocaudally ventral to the developing brain, and each sac is separated from the primitive oral cavity by the oronasal membrane, which soon breaks down allowing the nasal and oral cavities to communicate with each other by the primitive choanae that are located caudal to the primary palate. His (1901) named the communicating oral and nasal cavities, the naso-buccal channel.

The primary palate is derived from the intermaxillary segment, which is formed by fusion of the medial nasal processes, which also forms the incisive bone. Quinn et al. (2006) mentioned in domestic animals that the oronasal membrane becomes the primary palate after degeneration of its caudal part, which forms the primitive choanae. The secondary palate (the main part of the definitive palate) is formed by two shelf-like outgrowths from the maxillary prominences. These outgrowths, the palatine shelves, are initially oriented obliquely and ventrally, deep inside the stomodeum in a vertical direction on each side of the tongue. Then, as the jaw develops, the tongue moves down and the palatine processes ascend or elevate to assume a horizontal orientation, dorsal to the tongue. The now horizontally positioned palatine processes grow toward each other and fuse at the midline, forming the secondary palate. Our observations are the same as those recorded by Konsowa (2009) in camel, Quinn et al., (2006) in domestic animals in general and Thomas (2009) in human. The secondary palate fuses rostrally with the caudal edge of the primary palate, at the same time as the palatine shelves fuse dorsally with the nasal septum. These findings are on a line with that of Quinn et al. (2006) in domestic animals and Thomas (2009) in human. This fusion of the primary and secondary palates shifts the choanae to be located at the caudal end of the hard palate.

The medial edges of the palatine shelf were covered with ectodermal cells in the 3.5 - 4.0 cm CVRL camel fetuses. The two edges met and fused. There are several theories on the mechanisms of degeneration of the epithelial lining located on the adjacent shelf edges that allow fusion to take place on the midline. The first suggests that, cell loss occurred as a result of programmed cell death (Shapiro and Sweney, 1969). The second theory states that the epithelial cells may migrate into the body of the mesenchyme and become transformed into mesenchymal cells; epithelio-mesenchymal interaction (Gartener et al., 1978) and (Fitchett and Hay, 1989). This would result in a single secondary plate covered by epithelium dorsally and ventrally and with a core of mesenchyme.

The nasal septum appeared as a downward growth from the merged medial nasal prominences then continued to grow ventrally until it met the secondary palate. Similar findings were reported by (Thomas, 2009) in human development, while (Quinn et al., 2006) stated that in domestic animals, the nasal septum developed from the dorsal wall of the nasal cavity.

On both sides of the ventral end of the nasal septum, the vomoronasal organ developed as a membranous tube surrounded by a curved plate of hyaline cartilage medially. The olfactory epithelium of the lateral wall of the tube was thick while the respiratory epithelium of the medial wall was thin. These observations were consistent with the observations reported by (Quinn et al., 2006) in domestic animals. The epithelium of the vomoronasal organ developed from ventral evagination of the ectodermal lining of the floor of the nasal cavity on each side of the developing nasal cavity. The mesenchyme surrounding the ectodermal evagination differentiated into the curved cartilaginous plate. These results are comparable to those reported by Kostov (2007) in domestic animals.

The concha consisted of a lamina formed from a mesodermal core and ectodermal lining like that noted by (Quinn et al., 2006) in domestic animals. The dorsal and ventral conchae developed as elevations on the lateral nasal wall of each nasal cavity then initially elongated to form the basal plate then rolled to form the scroll plate.

The ectodermal epithelium in the dorsal and lateral sides of the nasal cavities became specialized to form for olfactory epithelium and it was noted that the cells in the olfactory epithelium had vesicular nuclei. In the ventral and medial aspects of the nasal cavity, the ectodermal epithelium developed into respiratory epithelium, which was psuedostratified ciliated in type without goblet cells. These observations are consistent with the results reported by

(Quinn et al., 2006) in domestic animals and for human by (Thomas, 2009). The air-conducting portions of the respiratory tract conditions the air as it passes through and which provides protection from dust and airborne infection and help in the adaptation of the dromedary camel with the adverse environmental conditions (Abdel-Salam et al., 2014).

In the present study, the olfactory epithelium appeared comparatively thicker than that of respiratory one. These findings are consistent with those reported by (Abdel-Salam et al., 2014) for nasal cavity of adult camels.

Conclusion

According to the available literatures, this study was the first to provide a detailed description of the development of the nasal cavity of the camel. We documented that the nasal cavity of the camel develops in a manner that is consistent with the pattern of development seen in other domestic animal species as well as in humans, which represent an adaptive modification in relationship to harsh environment.

Conflict of interest

None of the authors has any financial interest or any possible conflict of interest related to the manuscript.

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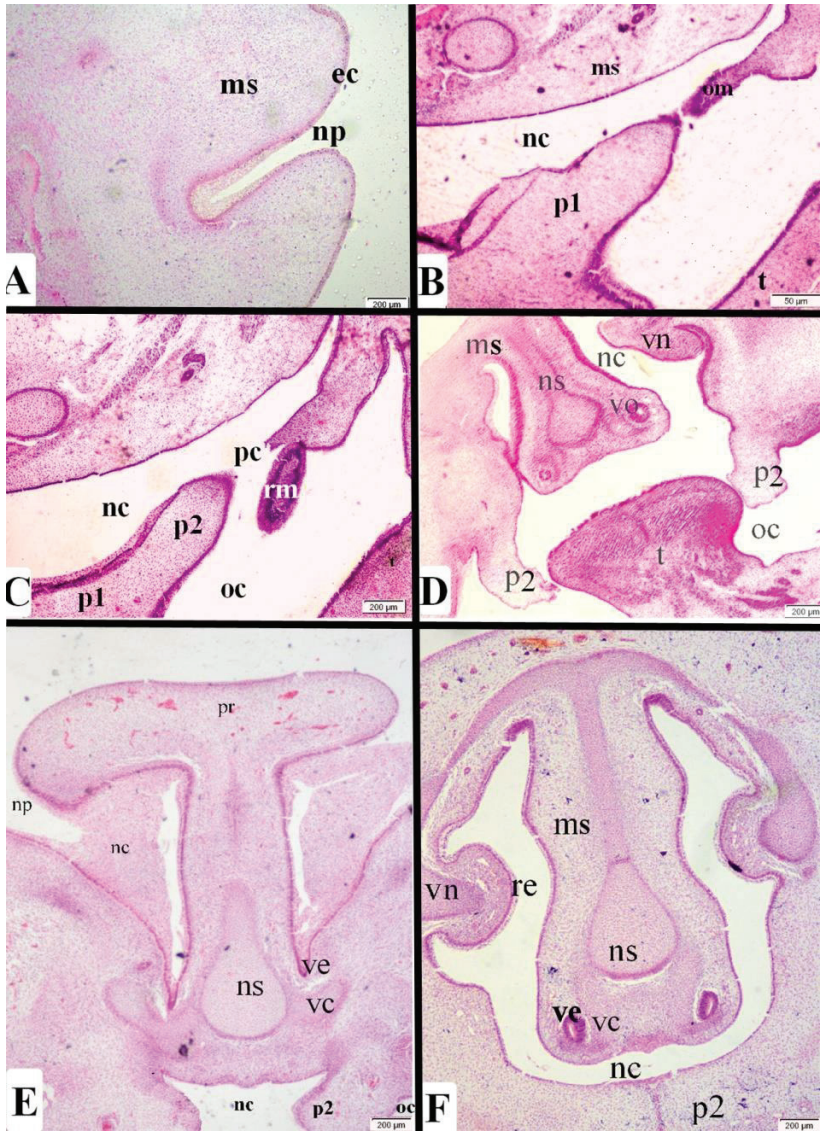

Fig (1): Photomicrographs of transverse sections of camel embryos A, B, D (15 mm CVRL), C (25 mm CVRL), E (30 mm CVRL), F (35 mm CVRL) showing; ectoderm (ec), mesenchyme (ms), nasal pit (np), oronasal membrane (om), primitive nasal cavity (nc), primary plate (p1), tongue (t), secondary plate (p2), primitive choanae (pc), ruptured oronasal membrane (rm), oral cavity (oc), nasal septum (ns), vomoronasal organ (vo), ventral nasal concha (vn), frontonasal prominence (pr), vomoronasal organ epithelium (ve), vomoronasal organ cartilage (vc). H&E stain.

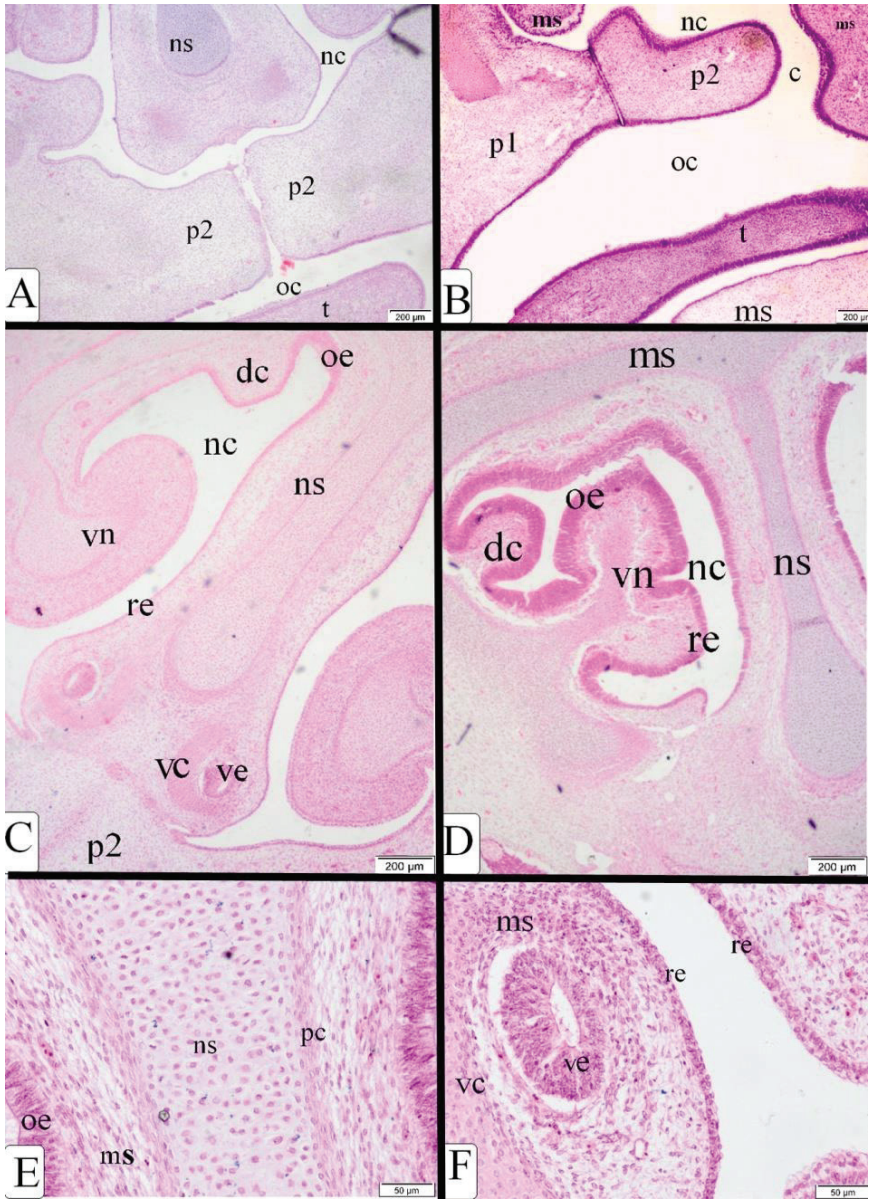

Fig (2): Photomicrographs of sagittal sections of camel fetuses A, B (40 mm CVRL), C (50 mm CVRL), D,E (60 mm CVRL) and F(65 mm CVRL) showing; nasal septum (ns), primitive nasal cavity (nc), secondary plate (p2), oral cavity (oc), tongue (t), primary palate (p1), mesenchyme (ms), dorsal nasal choanea (dc), ventral nasal choanea (ve), olfactory epithelium (oe), respiratory epithelium (re),vomoronasal organ cartilage (vc),vomoronasal organ epithelium (ve), perichondrium (pc). H&E stain.

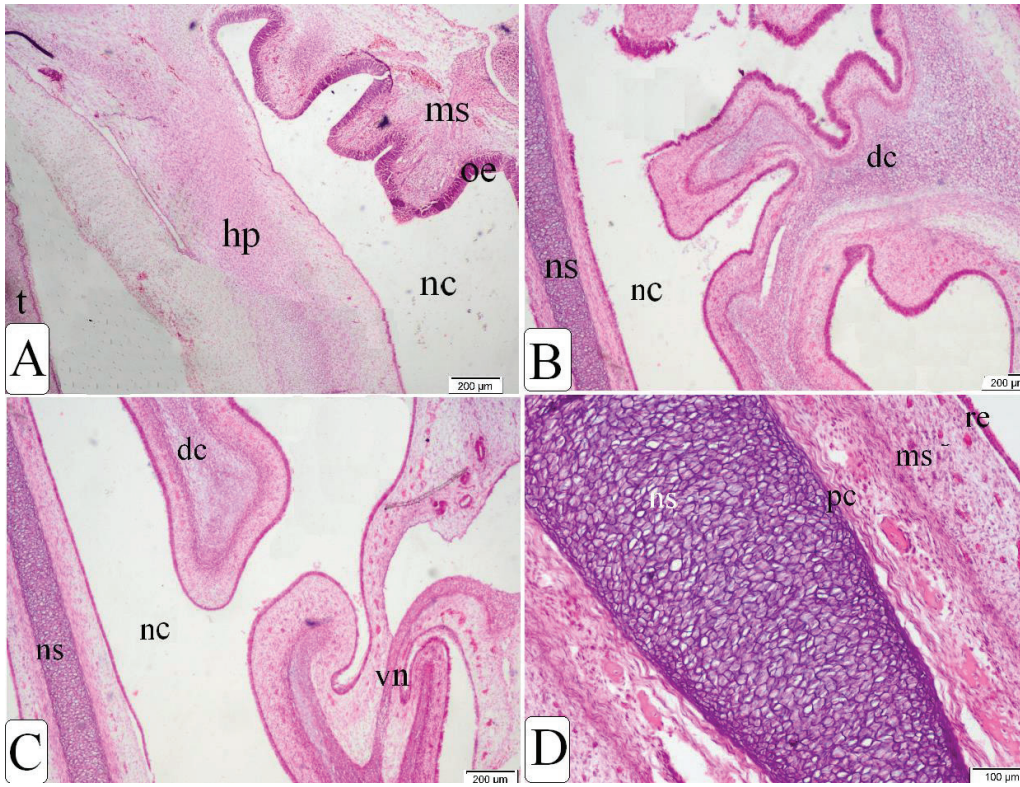

Fig (3): Photomicrographs of transverse sections of camel fetal heads A (70 mm CVRL), B (80 mm CVRL), C,D (90 mm CVRL), showing; tongue (t), oral cavity (oc),hard palate (hp), mesenchyme (ms), nasal cavity (nc), olfactory epithelium (oe), nasal septum (ns), respiratory epithelium (re), dorsal nasal concha (dc), ventral nasal concha (vn), perichondrium (pc). H&E stain.

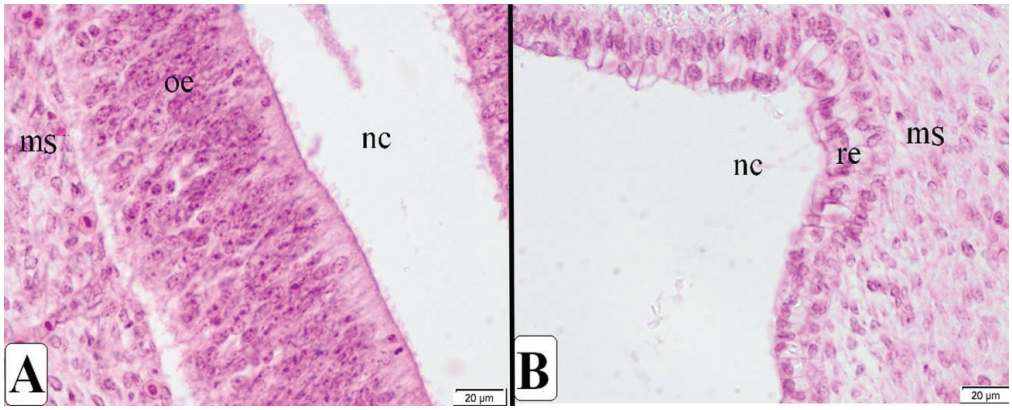

Fig (4): Photomicrograph of transverse section of camel fetal head (100 mm CVRL) A (olfactory region), B (respiratory region) showing; mesenchyme (ms), olfactory epithelium (oe), nasal cavity (nc), respiratory epithelium (re). H&E stain.