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Morphogenesis of the secondary palate in camel (*Camelus dromedarius*)

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Abstract

Thirty camel embryos and fetuses of both sexes, ranging from 0.8 cm to 12.0 cm CVRL, were used in this study. These specimens were collected from Zagazig and Cairo abattoirs. They were fixed and prepared by normal histological technique for light microscopical study. The present study revealed that, the camel palatogenesis was a complicated developmental process, that required two main events; elevation and then fusion of the palatal shelves. The palatal development began at 0.8 cm CVRL camel embryo, and was completed at 5.5-0.6 cm CVRL camel fetus. The two secondary palatine shelves were hanged vertically on both sides of the primitive tongue at 1.5 cm CVRL camel embryo. It began to be elevated from a vertical to horizontal position, above the dorsum of the primitive tongue at 0.2 cm CVRL camel embryo, with the appearance of the primitive palatine rugae at the same age. The primitive rugae appeared as an epithelial thickening at the junction between the lateral surface of the palatine shelves (future oral surface) and the maxillary process, with an apparent condensation in the subjacent mesenchyme. The close contact of medial epithelial edge (MEE) could be detected at 3.5-0.4 cm CVRL camel embryo. For fusion of the palatal shelves to be occurred, there was a breakdown of the MEE, followed by the fusion of the mesenchyme of the two secondary palatine shelves. This fusion took place rostro-caudally in its direction.

Keywords: Morphogenesis, secondary palate, camel.
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التطور الجنينى للحنك الثانوى فى الجمال

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لقد أجريت هذه الدراسة على عدد ثلاثون رأسا لأجنة الجمال، تتراوح أطوالها من ٠.٨ إلى ١٢ سم بمقياس الطول CVR، وأعمارها من ٦٧–٩٨ يوم. وقد تم جمع هذه العينات من مجازر الزقازيق و القاهره، ثم ثبتت العينات وتم تجهيزها للفحص بالميكروسكوب الضوئى. وقد بينت الدراسة أن تخلق الحنك فى الجمال هو عبارة عن عملية مركبة تحتاج إلى حدثين أساسين هما إرتفاع ثم إتحاد الرفوف الحنكية الثانوية. وقد بدأ تطور الحنك الثانوى فى الظهور عند ٠,٨ سم طول، ثم بدأ يأخذ فى الإنتهاء عند ٥,٥ – ٦ سم طول. وقد بدأت هذه العملية بتعليق الرفوف الحنكية الثانوية عموديا على كلا الجانبين للسان البدائى عند ١,٥ سم طول، ثم بدأ فى الإرتفاع من الوضع العمودى إلى الوضع المستعرض فوق ظهر اللسان البدائى عند ٢ سم طول، مع بداية ظهور ثنيات الحنك المستعرضة البدائية فى نفس العمر. وكانت بداية هذه الثنيات عبارة عن زيادة فى سمك النسيج الخلوى المكون لها، عند منطقة إتحاد السطح الوحشى للرفوف الحنكية (السطح الفمى المستقبلى) مع الناتىء الفكى العلوى، مع زيادة تكثيف اللحمة المتوسطة الملاصقة لهذا النسيج الخلوى. وقد بدأ إلتحام الحافة الأنسية الظهارية (MEE) لكل من الرقوف الحنكية الثانوية عند ٣,٥–٤ سم طول. ولكى يحدث هذا الإلتحام بين الرفوف الحنكية الثانوية لابد أن يحدث تكسير لخلايا الحافة الأنسية لها، يتبعها إتحاد اللحمة المتوسطة. وقد بدأ هذا الإلتحام يأخذ فى الحدوث من الجهة الأمامية (المنقارية) لهذه الرفوف متجها إلى الخلف (الإتجاه الذنبى).

Introduction

Palatogenesis is of critical importance to divide the oronasal cavity into two parts; oral and nasal parts. A dysfunction in one of the regulators of this developmental process can lead to cleft palate syndrome. This clefting causes problems with feeding, hearing and dentition. The events and mechanisms responsible for the development of

the definitive palate (hard palate) have been thoroughly studied in human (1-5), in mice (6-9), in rat (10-12). Few studies are carried on different domestic animals (13-16). Meager studies are carried out on the development of the secondary palate in camel.

The aim of the present study was conducted to give detailed description about the morphogenesis of the secondary palate in camel, which may be helpful in the field of biological science.

Material and methods

The present work was carried out on thirty camel embryos and fetuses ranging from 0.8 cm to 12.0 cm CVRL of both sexes. Samples were collected from Zagazig and Cairo abattoirs. The number of specimens with its CVRL, and the suggested ages were estimated according to the CVRL previously cited formula (17). The previous data are postulate in table 1.

The whole embryos and fetuses were fixed immediately using 10% neutral buffered formalin, 1% glycerin and 1% thymol and Bouinś solution. The samples were processed by normal histological technique, sectioned at 5-7 µ thickness, stained with the normal histological stain H&E adopted (19). The nomenclature was adopted as previously described (20), as if it was possible.

Table 1: Number of the collected specimens, their CVRL and estimated age.

Number of specimens	CVRL (cm)	Estimated age (days)
1	0.8	67
3	1.0	68
2	1.2	69
1	1.5	70
1	2.0	71
2	2.5	73
3	3.0	74
1	3.5	75
2	4.0	77
2	5.0	79
1	5.5	80
1	6.0	82
2	7.0	85
1	8.0	87
2	9.0	90
1	10.0	93
2	11.0	95
2	12.0	98

Results

At 0.8 – 1.0 cm CVRL camel embryos, the roof of the stomodeum was bounded laterally by the maxillary process. Broad mesenchymal process (primary palatine process) grew ventrally into the oronasal cavity from the maxillary process, on both sides of the primitive tongue (Fig. 1). The epithelium covering this palatine process was of uniform thickness. The core of this process was formed from undifferentiated mesenchymal tissue. At the oral part of the stomodium, the nasal pit communicated ventrally to the oronasal cavity (Fig. 2). The aboral part of the oronasal cavity was filled completely by the tongue primordia. The roof of this cavity was lined by stratified epithelium, ranged from 3-5 cell layers. It was composed of darkly stained basal columnar cells with elongated nuclei, and 3-5 polyhedral cell layers with lightly stained nuclei (Fig. 3).

Abbreviations of figures

Maxillary process (m), Palatine shelves (p), Primitive tongue (t), Oronasal cavity (on), Meckleś cartilage (M), nasal septum (N), Primitive nasal cavity (n), Primitive oral cavity (o), Medial edge epithelium (MEE), Epithelial seam (ES), Maxillary process (mx).

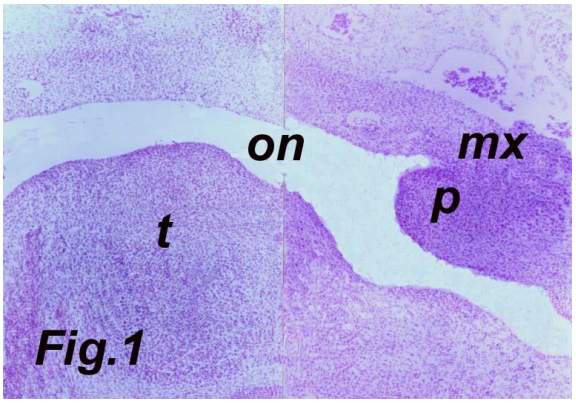

Fig. 1: Coronal section of 0.8 cm CVRL camel embryo at its rostral part showing, the primitive tongue, maxillary process and the broad mesenchymal primary palatine shelf. H&E stain. Obj. X4.

At 1.5 cm CVRL camel embryos, two distinct secondary palatine shelves were hanged vertically from the maxillary process on either sides of the primitive tongue (Fig. 4). The palatine shelves were composed of mesenchymal cells, surrounded by two or three thick layers of undifferentiated epithelial cells.

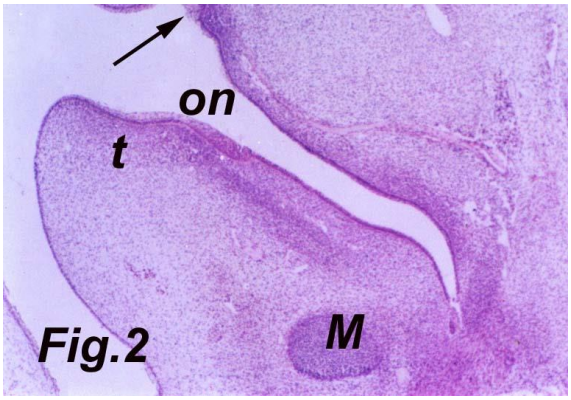

Fig. 2: Longitudinal section of 1.0 cm CVRL camel head embryo showing, the nasal pit (arrow), primitive tongue, Meckelś cartilage and the oronasal cavity. H&E stain. Obj. X4.

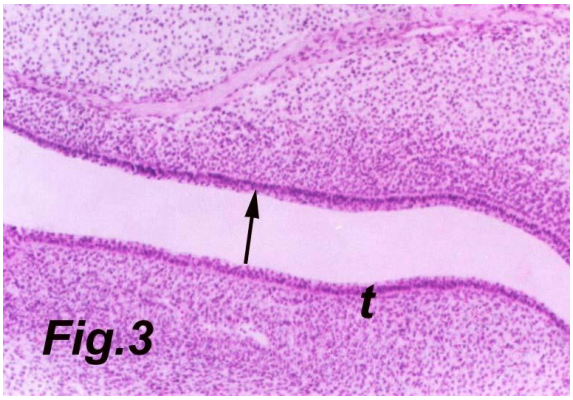

Fig. 3: High magnification to fig. 2 showing the lining epithelium of the roof of the oronasal cavity (arrow) and the primitive tongue. H&E stain. Obj. X10.

At 2.0 – 3.0 cm CVRL camel embryos, The secondary palatine shelves began to elevate from a vertical to a horizontal position above the dorsum of the primitive tongue. This process of fusion of the secondary palatine shelves were happened rostrally, and continued toward the middle and caudal (aboral) parts of the oronasal cavity, dividing it into future primitive oral and nasal cavities (Figs. 5 a, b and c). Also, in this stage the epithelium was thickened at the junction between the lateral surface of the palatine shelves (future oral surface) and the maxillary process, with increased condensation in the subjacent mesenchyme. The latter thickening was considered as the primordia of the palatine rugae. This thickening never reached the tip of the palatine shelves (Figs. 6 a and b).

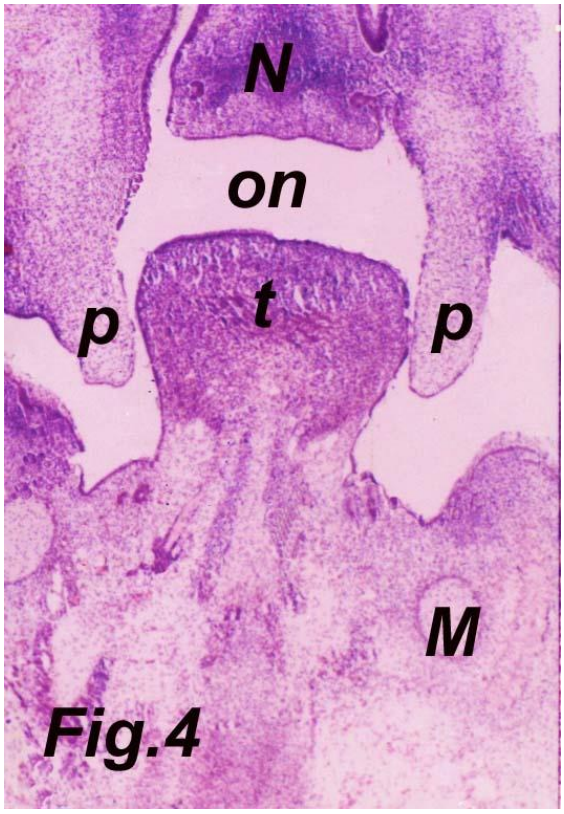

Fig. 4: Coronal section of 1.5 cm CVRL camel head embryo showing, the secondary palatine shelves hanged vertically from the maxillary process, on both sides of the primitive tongue. H&E stain. Obj. X4.

At 3.5 -4.0 cm CVRL camel fetuses, The two palatine shelves were grown horizontally, brings their medial edges into close contact. Prior to the fusion of the palatine shelves, there were epithelial projections from the medial edge epithelium (MEE) of each shelf, and extended across the fusion site of the opposite shelf (Figs. 7 a and b). The initial point of palatal shelves contact was occurred in the rostral region of the oronasal cavity, followed by progressive contact in the mid-region (Figs. 8 a and b), then the caudal part latter on (Fig. 9). At this period, the mid-region of the future palate, even after contact, the mesenchyme in opposing palatine shelves was still separated by an epithelial seam (Fig. 8 b). At this stage, the two palatine shelves were in close contact with each other in one hand, and both were connected with the distal end of the nasal septum in the other hand.

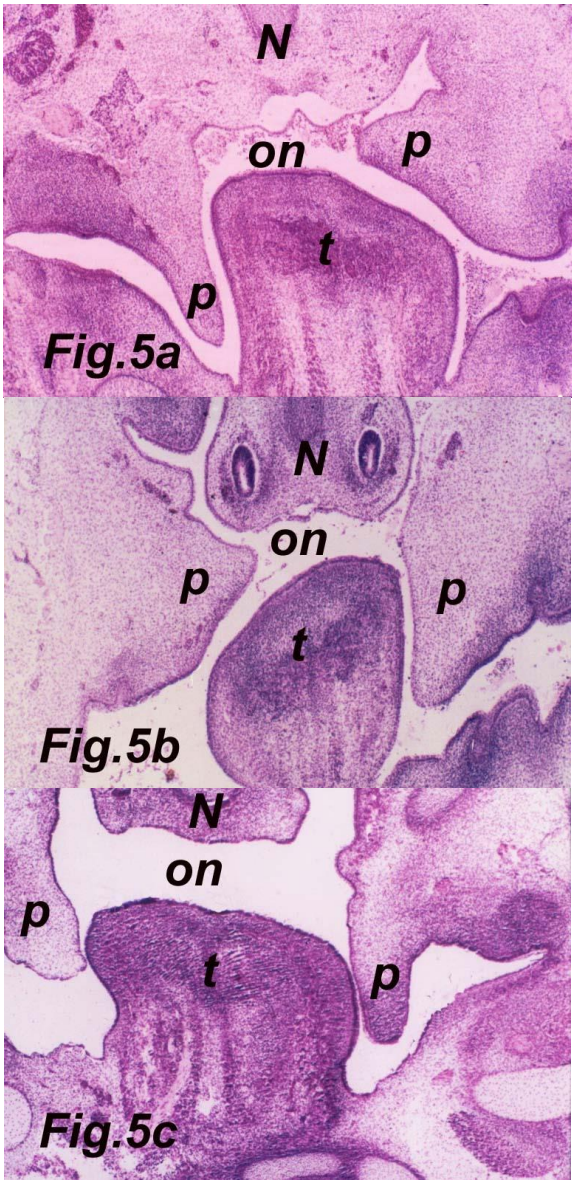

Fig.5. Coronal sections of 2.0 cm CVRL camel head embryos, showing the rostral part of the oronasal cavity (5a), its middle part (5b), and the caudal part of it (5c) to compare between the orientation of the secondary palatine shelves. H&E stain. Obj. X4.

At 5.5 – 7.0 cm CVRL camel fetuses, the fusion between the two secondary palatine shelves was completed caudally (Fig. 10). Also, the osteogenesis of the palatine processes of the premaxilla, maxilla and palatine bones occured through a process of intramembranous ossification. The mesenchymal tissue revealed islets of ossification centers (Fig. 11).

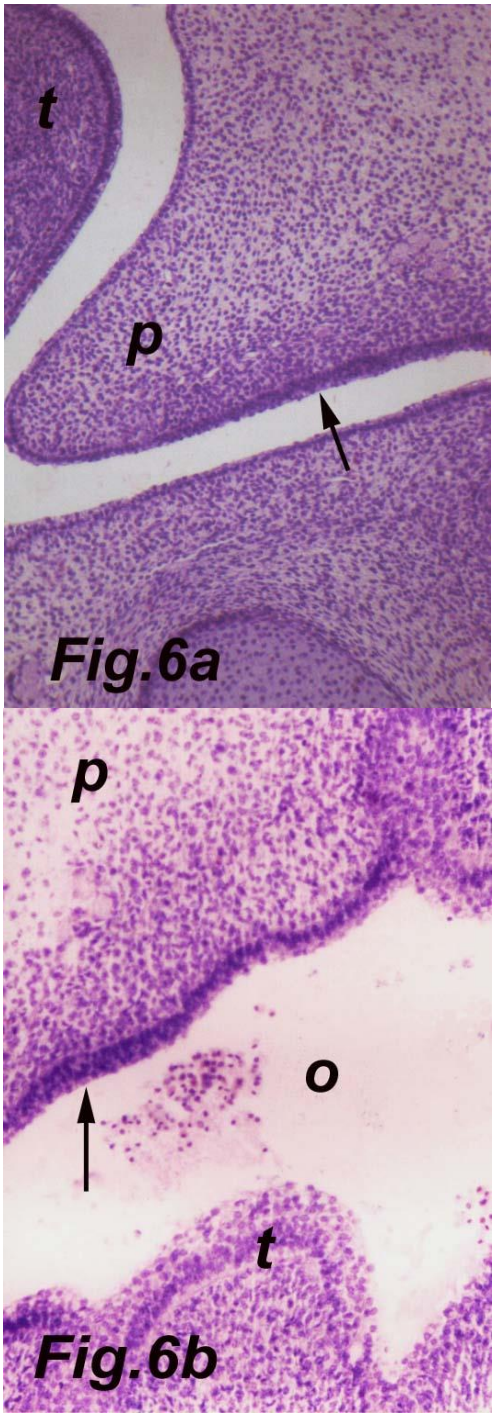

Fig. 6a: Coronal section of 3.0 cm CVRL, camel head fetus showing, a clear epithelial thickening (arrow) at the lateral surface of the secondary palatine shelves. H&E stain. Obj. X4, Fig. 6b: High magnification to fig. 6a showing a clear irregular thickening of the primitive palatine rugae (arrow). H&E stain. Obj. X10.

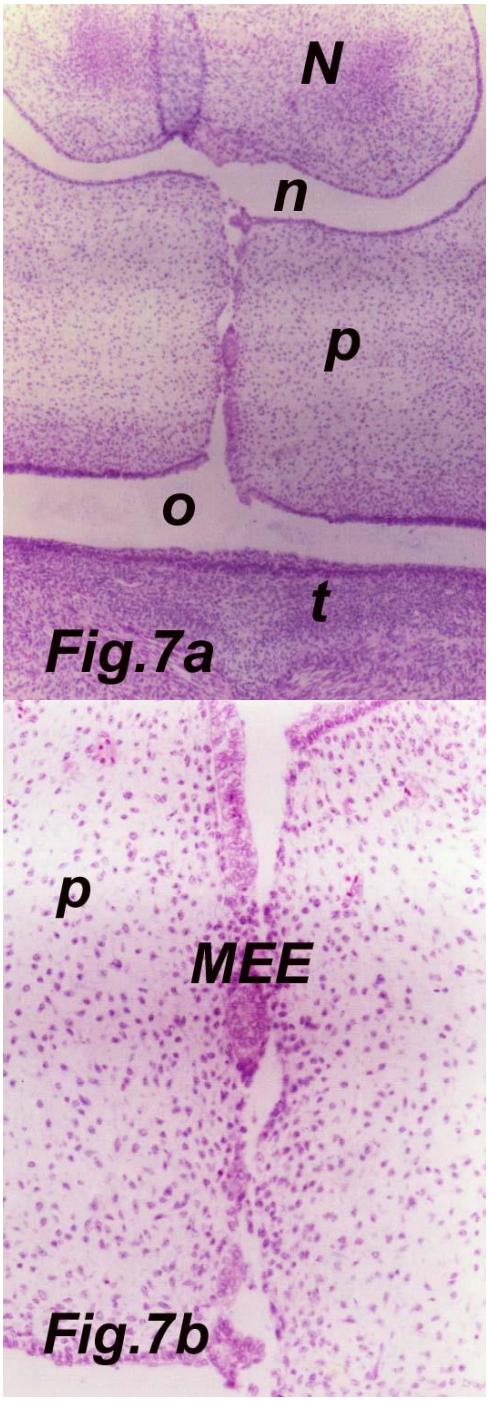

Fig. 7a: Coronal section of 3.5 cm CVRL camel head fetus at its rostral end showing, the horizontal orientation of the secondary palatine shelves, with a clear medial edge epithelium. H&E stain. Obj. X4, Fig. 7b: High magnification to fig. 7a showing, the medial edge epithelium of each secondary palatine shelves. H&E stain. Obj. X10.

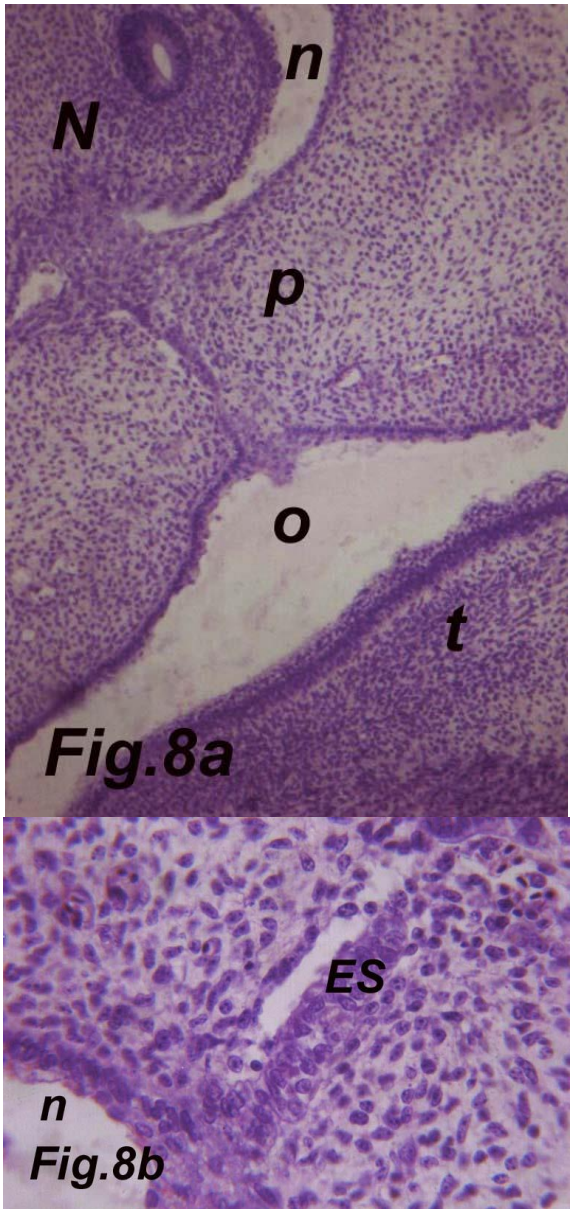

Fig. 8a: Coronal section of 4.0 cm CVRL camel head fetus at its mid-region showing, the fusion between the secondary palatine shelves and the distal end of the primitive nasal septum. H&E stain. Obj. X4, Fig. 8b: High magnification to fig. 8a showing, the epithelial seam (ES). H&E stain. Obj. X10.

At 8.0 -10.0 cm CVRL camel fetuses, the medial edge epithelium (MEE) forming an epithelial seam was finally lost, resulting in mesenchymal continuity of the secondary palate, with clear and numerous osseous plates at the site of fusion (Fig. 12).

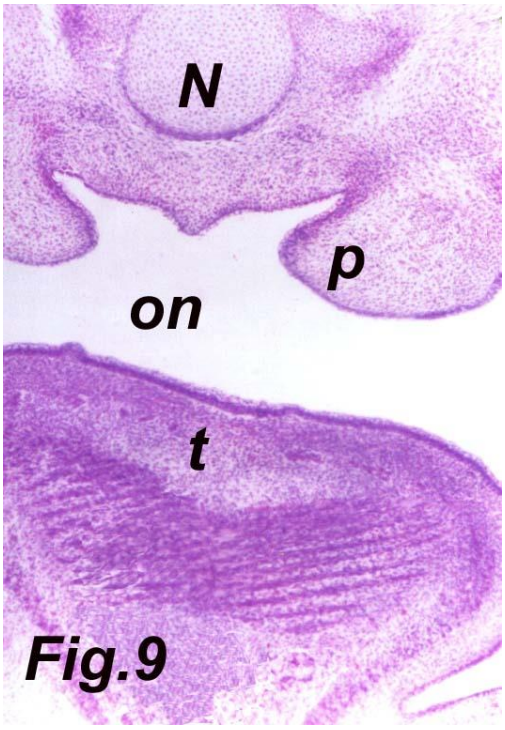

Fig. 9: Coronal section of 4.0 cm CVRL camel head fetus at its aboral-region showing, the horizontal orientation of the two secondary palatine shelves. H&E stain. Obj. X4.

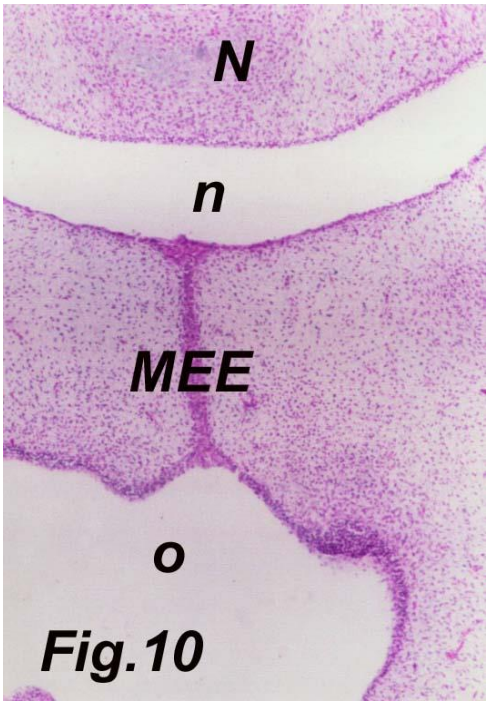

Fig. 10: Coronal section of 5.5 cm CVRL camel head fetus at its aboral-region showing, a clear MEE as a result of fusion of the two secondary palatine shelves. H&E stain. Obj. X4.

Discussion

The current study attempted to increase the information about the normal palatogenesis in camel. The present study revealed that, the palate development was detected at 0.8 cm CVRL, and was completed at 5.5-0.6 cm CVRL camel embryo. On the same line, in human, it begins at the end of 5th week of intrauterine life and completed at 12th week (2). In rat, it begins at 12.5 day mouse fetus (7) and in ferret, at 27 days of gestation (21). The formation of the secondary palate could be traced in human at 9th week, in rat at 16th day, in cat at 32nd day, in dog and pig at 33th day, in horse at the 4th week and in cow at 8th week. These findings are due to species variations, and were tabulated in previous record (14).

The present context revealed that, there were two distinct secondary palatine shelves, hanged vertically on either side of the primitive tongue at 1.5 cm CVRL camel embryo. It is unknown why the shelves attain this vertical orientation. It has been proposed that, the direction of shelves growth are related to the amount of space available in the oronasal cavity during the period of palatogenesis (22,23). The evolution of the large muscular mammalian tongue constrains the shelves to grow vertically, until sufficient space can be created in the oronasal cavity (23).

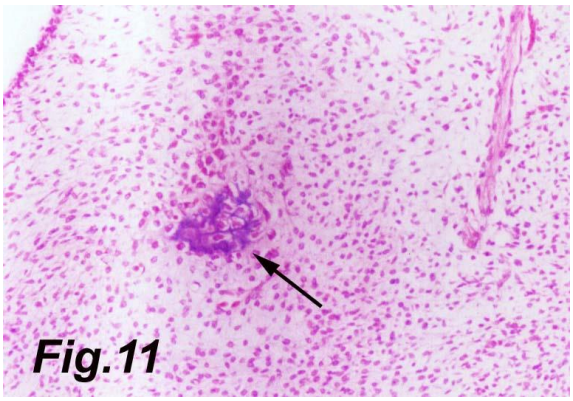

Fig. 11: Coronal section of 6.0 cm CVRL camel head fetus showing, the islets of ossification center (arrow) within the fused secondary palate. H&E stain. Obj. X10.

The present investigation demonstrated that, the palatine shelves began to be elevated from a vertical to horizontal position, above the dorsum of the tongue at 2.0 cm CVRL camel embryo. This elevation is completed within a day as previously mentioned (14). These findings correlated with that reported in human (2), as this event occurs rapidly, possibly in a matter of hours.

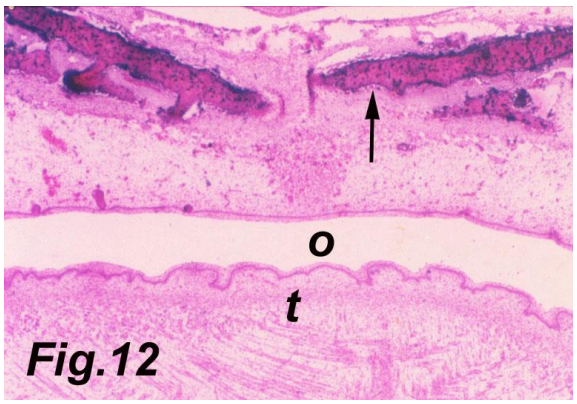

Fig. 12: Coronal section of 12.0 cm CVRL camel head fetus showing, a clear osseous plates (arrow) of the palatine process of the maxillary and palatine bones. H&E stain. Obj. X4.

Several mechanisms have been proposed to account for rapid movement of palatal shelves, from the vertical to the horizontal position, and the source of forces responsible for this reorientation. Two categories of explanation have been provided; either the forces are extrinsic to the shelves (movement of the tongue), or they are generated intrinsically by the shelves mesenchyme, due to the hydration of extracellular matrix component of the mesenchyme (24). The downward movement of the tongue due to the mandibular growth spurt clearing a path for palatal shelves elevation (25,26).

Concerning the close contact of the medial edge epithelium (MEE) at 3.5-4.0 cm CVRL camel fetuses. There have been several theories on the mechanisms of MEE degeneration. The first theory (27) suggested that, it occurred as a result of programmed cell death. The second theory (28) disputed this degeneration on the grounds, that there was no evidence of any cell debris or phagocytic activity at any time during this process. The possibility that the MEE cells migrate into the body of the mesenchyme and transform into mesenchymal cells (Epithelial-mesenchyme interaction) (29).

Regarding to the close contact between the two horizontal palatine shelves and the distal end of the nasal septum at 3.5-4.0 cm CVRL camel fetuses. These findings are supported at 3.5 cm CVRL camel fetus, corresponding to 8.0-10.0 cm CVRL buffalo fetus (15).

In the present study, the primitive palatine rugae firstly observed at 2.0 cm CVRL camel fetus as an epithelial thickening, at the junction between the lateral surface of the vertical palatine shelves and the maxillary process. This is come in parallel to that found in rat embryo at 12th day (10). The consequence of fusion of the two secondary palatine shelves in the present work took its place rostro-caudally in its direction. This result is consistent with that happened in human (5). In contrast, it occurs in the middle region of the shelves, then followed posteriorly, whereas the final part to close in the region of the incisive canals, in hamster (30,31) and in mouse (32).

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