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Embryonic development of nasal septum in indigenous sheep

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Abstract

The study included detection the onset time of the chondrification and ossification of nasal septum in indigenous sheep fetuses. Fetuses have been prepared by using stained with modified double staining method in young ages and maceration with potassium hydroxide in older ages. First sign of nasal septum demonstrated as small spot of condensed mesenchyme of medial nasal prominence of Meckel's cartilage (Hyaline cartilage). Chondrification process of nasal septum initiated at 54 day old. The primary ossification centre of the caudal part of nasal septum appeared at 61 day old in the distal caudal end of mesenchyme of Meckel's cartilage. The examination of nasal septum growth from sheep embryos in the (42-63 days) period shows that this is a rapid period of growth is in the end of the first trimester and the first of second trimester of gestation. The increase in the length of the nasal septum is about three folds (from 0.5 cm at 7 week to 1.6 cm at the 10 week of indigenous fetal age. The results of analysis of variance done on the relative increase in the nasal septum length and nasal septum height showed that the greatest relative increase in nasal septum length was at the 7 week, while the greatest relative increase in the nasal septum height was in the 11 week.

Introduction

Studying the development of nasal septum and determine its relationship with the growth of other facial constituents in indigenous sheep foetuses is the main object of this research. In Iraq, there are few studies deals with embryological development in animals. Although indigenous sheep(Awassi, karadi and Arabi breeds) is the dominant breeds in Iraq, so that nasal septum chondrification and ossification haven't been studied yet.The development of growing heads is necessarily an extremely complex process since organs and tissues having completely different functions are growing in close physical association with each other (1). Nasal septum which is the dividing partition between the left and right nostrils is composed of soft, flexible cartilage rostrally and solid inflexible bone caudally. Nasal septum lined with a tightly adherent mucoperichondrium that supplies it with nutrients. Nasal septum plays an active role in embryonic midfacial development (2). The nasal septum develops from a primary growth centre in the midline approximation of the medial nasal prominences of

Mickel's cartilage (3, 4, ,5).There are three data sources for embryological development studies; skeletons specimens, radiographic pictures and ultrasoundgraphy. For instant study, skeleton specimens have been used as it's the only available source in Iraq due to current situation. Furthermore, detecting the onset of nasal septum which starts as tiny spot is quite difficult using radiographic pictures or ultrasoundgraphy. Beside that, still most of the previous literatures in the first appearance of prenatal ossification centres whether in human or animal foetuses skeletons based on specimens cleared in dilute potassium hydroxide and stained with alizarin reds (6, 7, 8, 9, 10, 11 ,12).The aim of this study is to detect the onset time and studying the development of nasal septum will bridge the gap of this kind of studies in Fertile Crescent which Also,it will help to detect if there is any defect or abnormality (nutritional or pathological cause) which in turn can lead to abnormal development or teratogenesis

Materials and Methods

Eighty foetuses were collected from uteri of Indigenous ewes slaughtered in Mosul, North of Iraq. The crown-rump length of each foetus was measured by using vernier and a measuring tape. This length used in Richardson's formula to find the estimated age of each foetus, (13) Estimated age = {(crown-rump length (cm) + 17} × 2.1

The skulls of young foetuses separated and prepared by skinning, fixing in 90 % ethyl alcohol, then staining with modified double staining method using mixed solution of Alizarin reds and Alcian blue (11,12 ,14).The specimens macerated in 2% potassium hydroxide solution, then cleared by using glycerine, and examined by stereoscopic microscope, macrolens and graph paper . The skulls of older ages prepared using maceration with different concentrations of potassium hydroxide according to the age, (15).The growth and development of nasal septum followed up by examining the sagittal sections of nasal

cavity during 14 weeks (7–20 weeks) of foetal age. The examination has been done by taking the following measurements:

- Length of the nasal septum (LN): represented by the horizontal line extended from the anterior end of perpendicular plate of ethmoid bone to the alveolar point (16 and 17).
- Height of the nasal septum (HN): represented by the vertical line extended from the nasion (fronto-nasal point) to the most anterior part of anterior end of perpendicular plate of ethmoid bone (17 and 18) (Fig1).These measurements submit to the statistical analysis to find the variance then followed by the least significant test (19) to demonstrate the significance of variance present in these measurements during the studied period (7–20 weeks) of foetuses age.

Results

The study showed that the first sign of growth of nasal septum was at 43 day old in indigenous sheep foetuses, which distinguished as a small area of condensed mesenchyme of medial nasal prominence of Meckel's cartilage of perpendicular plate of ethmoid bone. The chondrification process initiate in the midline mesenchyme at about 54 days old and the nasal septum cartilage is evident in the sagittal section of nasal cavity (Fig. 2).Week later (61 day old), the initiation of the ossification in the caudal part of the nasal septum was seen as a red locus appeared at the distal caudal end of the mesenchyme of Meckel's cartilage (Fig.3). Ten days later, (72day old) the anterior end of the nasal septum creates a longer external nose. Another ten days later (82 day old) the continuous

growth of nasal septum results in further protrusion of the external nose. The growth of nasal septum correlated with midline facial growth. The increase in the length of the nasal septum is about three folds (from 0.5 cm at 7 week to 1.6 cm at the 10 week of indigenous foetal age. The results of statistic analysis done on the relative increase in the length and height of the nasal septum showed that the greatest average of relative increase in length of nasal septum was in the 7 week of foetal age which significantly differ from the same average in the other studied weeks, while the greatest average of relative increase in height of nasal septum was in the 11 week of foetal age, which significantly differ from the same average in the other studied weeks (Fig 4 and 5).

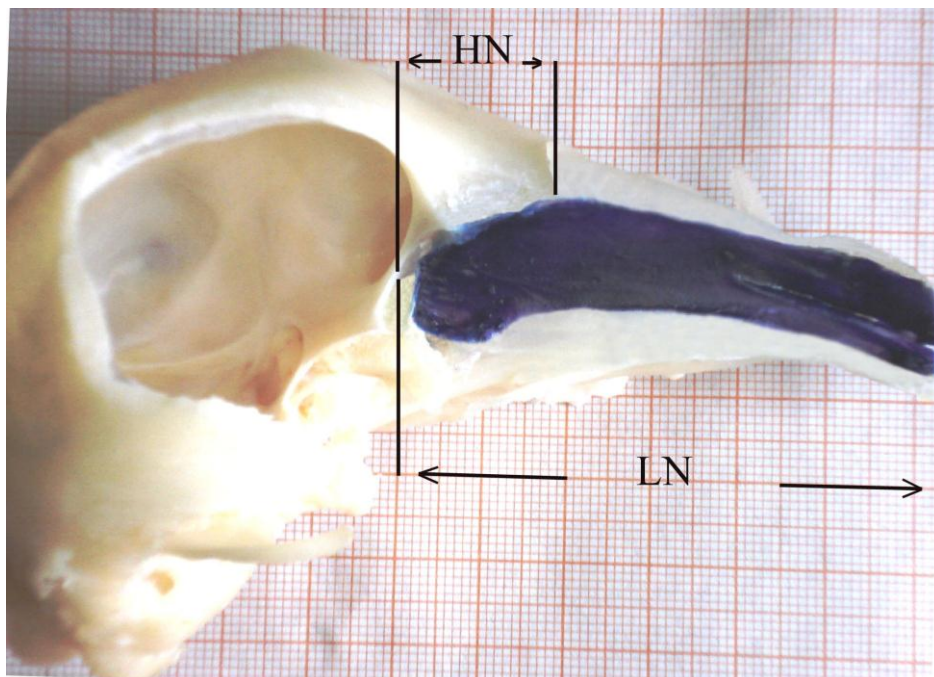


Fig. 1: Skull of indigenous sheep fetus with an estimated age of (145) day old macerated with potassium hydroxide. One side of nasal cavity lifted to demonstrate the nasal septum (dark blue). LN (length of nasal septum), HN (height of the nasal septum).

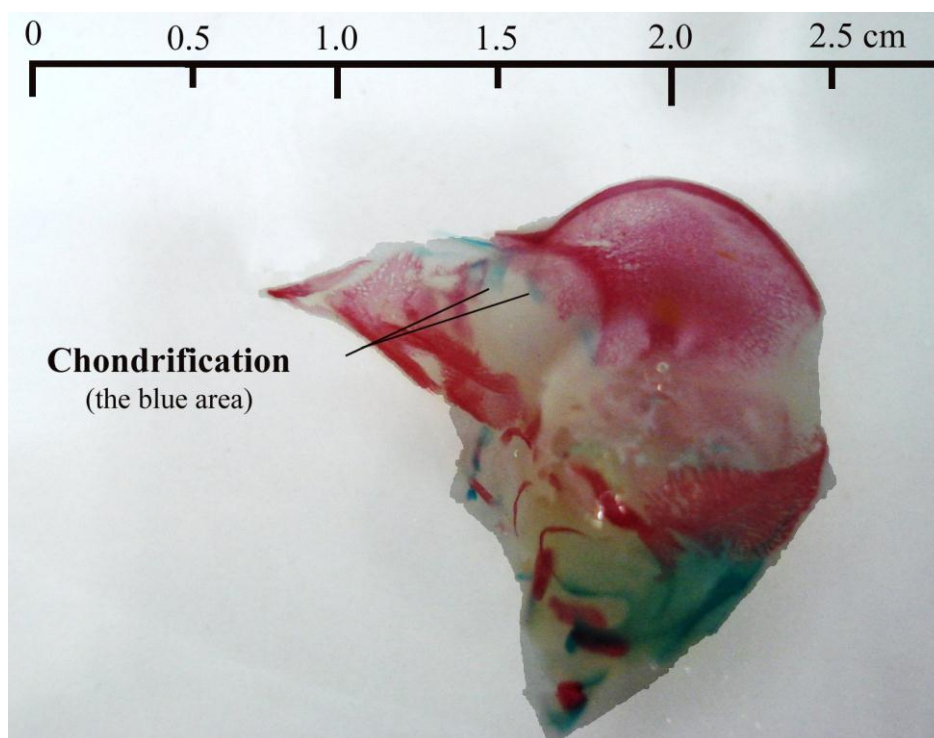


Fig. 2: Skull of indigenous sheep fetus with an estimated age of (54) day old prepared by double staining method. Chondrification in rostral part of nasal septum (blue color).

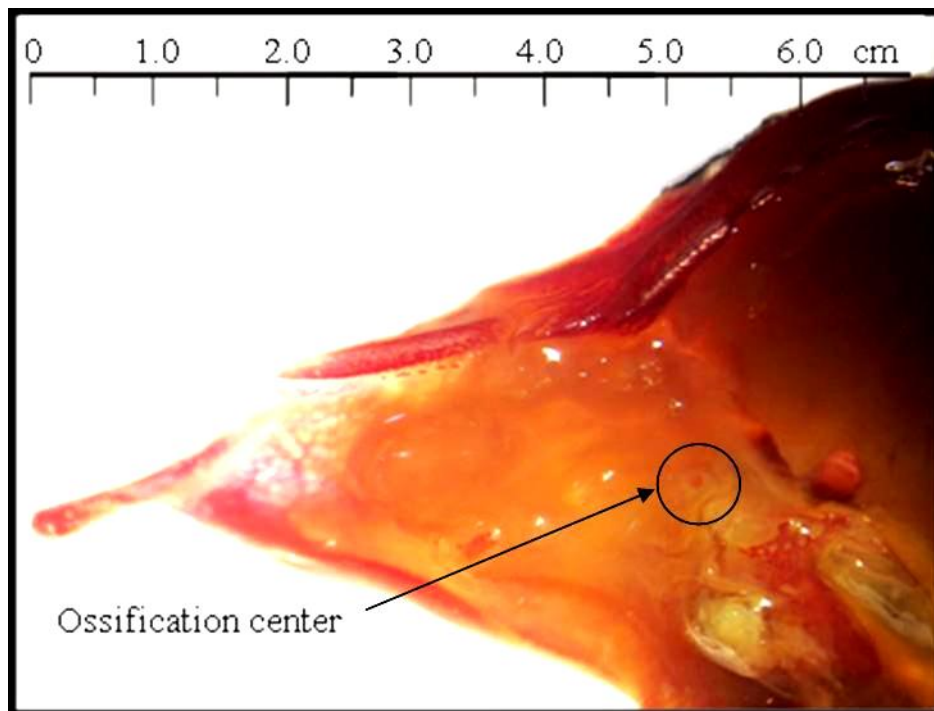


Fig. 3: Skull of indigenous sheep fetus with an estimated age of (61) day old prepared by double staining method. Initiation of caudal part of nasal septum ossification by the intramembranous method (Ossification center).

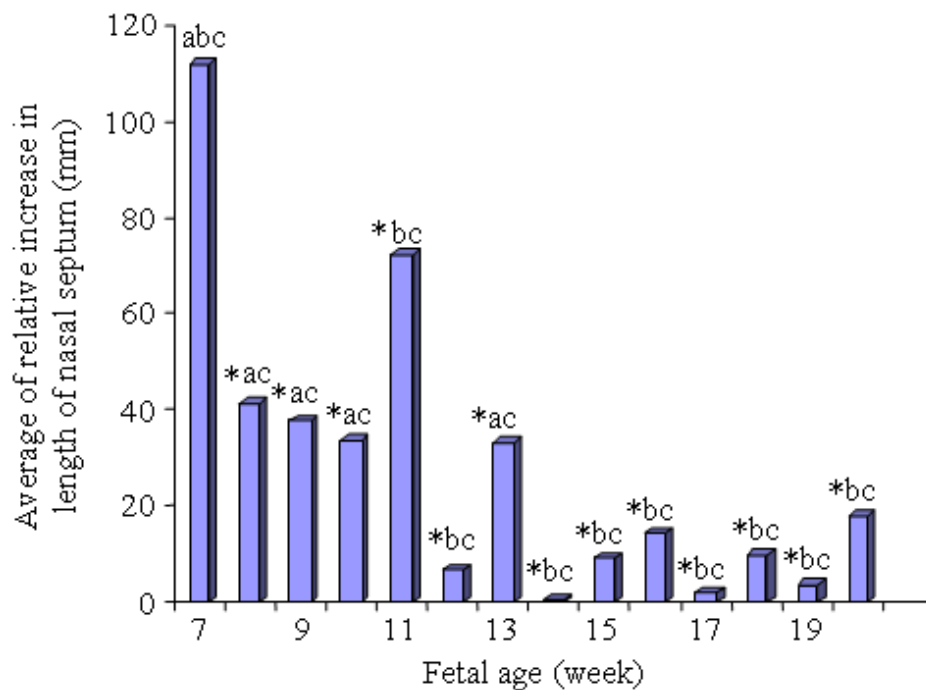


Fig. 4: The relative increase in the length of nasal septum of indigenous sheep fetuses. (*) Differ significantly in this week than in the 7th week at $p < 0.05$. (a) differ significantly in this week than in the 11th week at $p < 0.05$. (b) differ significantly in this week than in the 8th week at $p < 0.05$. (c) differ significantly in this week than in the 18th week at $p < 0$.

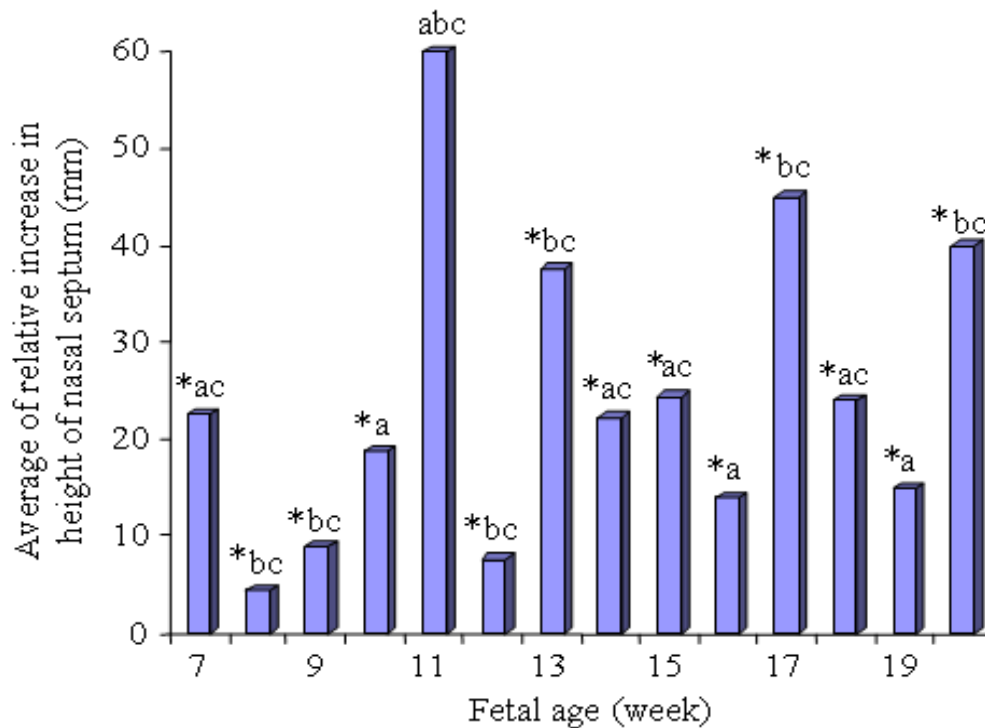


Fig. 5: The relative increase in the height of nasal septum of indigenous sheep fetuses. (*) differ significantly in this week than in the 11th week at $p < 0.05$. (a) differ significantly in this week than in the 17th week at $p < 0.05$. (b) differ significantly in this week than in the 15th week at $p < 0.05$.

Discussion

The results of this study showed important correlation between development of nasal septum and subsequent midfacial growth at 43–140 days old of Indigenous sheep fetuses. This result agree with the study results on human fetuses (2). The early initiative point of nasal septum growth was at 43 day of indigenous sheep fetuses, this results agree with the facial development studies done on human and sheep fetuses (4, 11, 16, 20, 21), and with results of (3, 4) on human and lab animal fetuses (Rat, Rabbit, Guinea pig and Beagle) who suggests that the cartilaginous nasal

septum is a primary growth centre that pushes or thrusts the midfacial bones downward and forward. The results of statistic analysis showed presence of significant variance in the average of the relative increase in length and height of the nasal septum among some of the studied weeks of foetal age, but the maximum development in this septum occurred in 7 and 11 week, this means that the sensitive period of most rapid longitudinal growth in the nasal septum. This result agrees with results of many other studies on the skeleton development of sheep and goat fetuses (14, 17, 20, 21).

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التطور الجنيني للحاجز الانفي في الاغنام المحلية

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الخلاصة

تضمنت هذه الدراسة تحديد المدة الزمنية لحدوث التغضرف والتعظم في الحاجز الانفي لاجنة الاغنام المحلية والتي تم تحضيرها باستخدام الصبغة المزدوجة المحورة في الاعمار الصغيرة والتعطين باستخدام هيدروكسيد البوتاسيوم او يرقات الذباب في الاعمار الكبيرة. إن العلامة الأولى لظهور الحاجز الانفي كانت كبقعة صغيرة في الميزنكايم الكثيف التابع للنتوء الانفي الانسي لغضروف مايكل (غضروف زجاجي), حيث بدأت عملية التغضرف للحاجز الانفي بعمر (54 يوما) من عمر اجنة الاغنام المحلية , في حين بدأ مركز التعظم الابتدائي للجزء الخلفي من الحاجز الانفي بالظهور بعمر (61 يوما) من عمر اجنة الاغنام المحلية وذلك في النهاية الخلفية القاصية للميزنكايم التابع لغضروف مايكل. إن دراسة وتتبع نمو الحاجز الانفي في اجنة الاغنام المحلية التي تراوحت اعمارها بين (42-63 يوما) أظهرت أن الفترة الزمنية الأسرع في النمو هي في نهاية الثلث الأول من الحمل وبداية الثلث الثاني من الحمل . وان الزيادة في طول الحاجز الانفي هي تقريبا ثلاث طيات اسبوعيا حيث تراوحت ما بين (0.5 سم في الاسبوع السابع ووصلت الى 1.6 سم في الاسبوع العاشر) من عمر اجنة الاغنام المحلية , في حين أظهرت نتائج تحليل التباين للزيادة النسبية لطول وارتفاع الحاجز الانفي ان اعلى زيادة نسبية في طول الحاجز الانفي كانت في الاسبوع السابع من عمر اجنة الاغنام المحلية , في حين كانت اعلى زيادة نسبية في ارتفاع الحاجز الانفي في الاسبوع الحادي عشر من عمر اجنة الاغنام المحلية.