The embryonic development of the sternum in sheep and goats
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Abstract
The study included detection of the number, site and time of appearance of the primary ossification centers in the sternum of Awasi sheep and black goat feti, by using the modified double staining method that formed from alizarin red to detect the calcification which indicate the biginning of ossification process in the developing sternebrae (sternal pieces) and alcian blue to demonstrate the cartilage. The examination of the cleared stained sterna of Awasi sheep feti explained that each sternebra ossify from double ossification centers which fused later. The appearance of ossification centers in the sternum of sheep begins in the 7th sternebra at 56 days old, then in the 3rd, 4th, 5th sternebra at 57–59 days old, then in the 2nd, 1st sternebra at 60–61 days old, while the double ossification centers of the 6th sternebra delayed in it’s appearance till 69–71 days old. The examination of the cleared staining sterna of black goat feti show that each sternebra ossify from single ossification center appear in the center of the sternebra. The appearance of these centers begin in the 7th sternebra at 54 days old, followed by appearance of ossification centers in the 4th, 5th, 6th, 3rd, 2nd, 1st sternebra at 56, 57, 58, 60, 63, 64, days old respectively.

Introduction
The sternum or breast bone is a median segmental bone which completes the skeleton of the thorax ventrally and articulates with the cartilages of the sternal ribs laterally. It consist of variable number of sternebrae; 8 in carnivore, 6 in horse and pig, 7 in ruminants. The first expanded sternebra called the manubrium sterni, while the thin last sternebra called the xiphoid cartilage. Each sternebra of the sternum ossify by a single center of endochondral ossification. The development of sternum in human have been studied by using 15 male and 12 female feti of northwest indian subject, to detect the time of appearance of ossification centers in various segments of human sternum. A cross-sectional sonographic study also used to assess the relationship between gestational age and appearance of various sternal components and establish growth during human gestation. Other studies of the bone development depend either on radiography or staining with bone stains. Some radiographic studies to fetal sternum, describe it’s development and ossification together with the other components of skeleton of sheep and pig feti during the early embryonic period, while the other studies describe the sternum of the newborn and adult in different species of goat and sheep by using radiography. The aim of this work is to investigate and compare the developmental process in the sternum of the Awasi sheep and black goat feti and to assess the relationship between the appearanc of various sternal components and the estimated fetal age.

Materials and Methods
Eighty feti are used in this study; 40 of them are Awasi sheep feti and 40 are black goat feti. All of them were collected from the uteri of prigrant females after routin slaughter. The crown rump length of the feti were measured by using a measuring tape. This length used to find the estimated age of the studied feti by using Richardson formula {2.1*(crown-rump length (cm.) +17)}(11). The estimated age of this feti range between 45-100 days for both animal species. The sterna were separated from these feti after skinning and then prepare by using the following steps:
1- Fixation in 90% ethyl alcohole which give strong staining later.
2- Staining with modified double staining method by using mixed alcoholic solution
of 0.14 % of alizarin red s and 0.3% of alcian blue (12).

3-Maceration by using 2% potassium hydroxide.

4-Clearing by gradual concentration of glycerin in distilled water (13).

Examination of the cleared stained sterna with the dissecting microscope to detect the time that the primary ossification center of each sternebra appeared which distinguished by it’s red stain ,while the cartilage take the blue stain(14). The lengths of the sterna in all studied feti were measured by using vernia and measuring tape. This length took from the beginning of manubrium sterni till the end of xiphoid cartilage directly from the stained sterna. Analyses of variance were maid to these measurements to show the significant variance found in the average of the relative increase in the sternum length of both species among the studied weeks(15).

Results

The study showed that the sternum of Awasi sheep feti consist of 7 sternebrae , although the cartilaginous sterna of early ages give an idea that it consist of 6 sternebrae only this belong to delay separation of the 6th and 7th sternebrae further than the 6th sternebra is small and it’s ossification delay to an older age.Each sternebra of sheep sternum ossify from double ossification centers that united later. The first appearance of these centers occurs in the 7th sternebra at 56-57 days old, while the last appearance of these centers occurs in the 6th sternebra at 69-71 days old. The study ensured that the sternum of black goat feti consist of 7 sternebrae (Fig.1), each of them ossify from central single ossification center. The first appearance of ossification centers occurs in the 7th sternebra at 54 days old, while the last one occurs in the 1st sternebra at 64 days old. The results of statistic analysis revealed presence of significant variance between the average of the relative increase in the sternum length of Awasi sheep feti in the 7th week and the same average in the next studied weeks (8th –14th weeks ) , and also between this average in the 8th and 10th weeks and the same average in the 9th,11th,12th,14th weeks of fetal age (Fig7) . The results showed also presence of significant variance between the average of the relative increase in the sternum length of black goat feti in the 7th week and the same average in the next studied weeks (8th –14th weeks) , and also between this average in the 8th week of fetal age . and the same average in the next studied weeks (9th – 14th weeks) of fetal age (Fig 8).

Fig (1): Sternum of black goat fetus at 50 days old prepared with double staining method (alizarin red & alcian blue). All sternebrae (1-7) are cartilaginous take the blue color. XC: Xiphoid cartilage. (X 3.8)
Fig (2): Sternum of Awasi sheep fetus at 57 days old prepared with double staining method (alizarin red & alcian blue). Notice the appearance of double ossification centers (red color) in the 7th sternebra (7) only. MS: Manubrium sterni. XC: Xiphoid cartilage. (X 3.8)

Fig (3): Sternum of Awasi sheep fetus at 60 days old prepared with double staining method (alizarin red & alcian blue). Notice the appearance of double ossification centers in 7th, 2nd-5th sternebrae (7, 2-5) which take the red color, while the 1st sternebra (1) has single ossification center at this age (X5).
Fig (4): Sternum of black goat fetus at 59 days old prepared with double staining method (alizarin red & alcian blue). Notice the appearance of single ossification centers in $4^{th}-7^{th}$ sternebrae (4–7) which take the red color (X 5).

Fig (5): Sternum of Awasi sheep fetus at 69 days old prepared with double staining method (alizarin red & alcian blue). All sternebrae have united double ossification centers (1 – 5, 7) except the $6^{th}$ sternebra has single center only (6) take the red color (X 5).
Fig (6): Sternum of black goat fetus at 69 days old prepared with double staining method (alizarin red & alcian blue). All the sternebrae (1 – 7) have single ossification centers take the red color (X 4.5).

Table 1: The sequence of appearance of the primary ossification centers in the sternum of sheep and goat feti.

<table>
<thead>
<tr>
<th>Fetal age (day)</th>
<th>The number of the sternebrae that ossification centers appeared in it</th>
</tr>
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<tbody>
<tr>
<td>54</td>
<td>7\textsuperscript{th} - 1</td>
</tr>
<tr>
<td>56</td>
<td>7\textsuperscript{th} - 2, 3\textsuperscript{rd} - 1 (fig 2)</td>
</tr>
<tr>
<td>58</td>
<td>3\textsuperscript{rd} - 2, 4\textsuperscript{th} - 1, 5\textsuperscript{th} - 1</td>
</tr>
<tr>
<td>59</td>
<td>4\textsuperscript{th} - 2, 5\textsuperscript{th} - 2 (Fig 4)</td>
</tr>
<tr>
<td>60</td>
<td>1\textsuperscript{st} - 1, 2\textsuperscript{nd} - 1, 2 (fig 3)</td>
</tr>
<tr>
<td>61</td>
<td>1\textsuperscript{st} - 2</td>
</tr>
<tr>
<td>63</td>
<td>2\textsuperscript{nd}</td>
</tr>
<tr>
<td>64</td>
<td>1\textsuperscript{st}</td>
</tr>
<tr>
<td>69</td>
<td>6\textsuperscript{th} - 1 (Fig 5)</td>
</tr>
<tr>
<td>71</td>
<td>6\textsuperscript{th} - 2 (Fig 6)</td>
</tr>
</tbody>
</table>
Discussion

The results ensure that the number of the sternebrae is 7 in both Awasi sheep and black goat sternum although the 6th sternebra of sheep sternum is almost small in size and delay in it’s ossification. These results vary what said by (9) that the number of the sternebrae in goat sternum either 5, 6, or 7 sternebrae. These depend on the breed of the goat, and agree what said by Wenham that the routin number of sternebrae in sheep feti is 7, with very small 6 sternebra (6).)

The results show that each sternebra ossify from single ossification center in goat sternum and double ossification centers in sheep sternum, these results agree with the previous studies on both goat and sheep sternum (7,9,10). The first appearance of ossification centers in the sternum was in the 7th sternebra in both goat and sheep feti at 54,56 days old respectively. The previous studies of Wenham agree with this results that the first sternebra begin to ossify is the 7th one in sheep feti at 60 days old then the 6th sternebra then the 5th one (6,7), while our results ensure that the 6th sternebra is the
last piece of sternum that begin to ossify at 69 – 71 days old in sheep feti. The results of statistic study showed that the maximum development in sternum length occured in the 7th week of fetal age in both animals, then in the 8th week, then in the 10th week. This result agrees with the previous studies on bone development, especially the studies of bone development in vertebral column in sheep and goat and limb bones of black goat feti (12, 16, 17).

References

التطور الجنيني لعظم القص في الأغنام والفاعز

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الخلاصة

تضمنت الدراسة تحديد عدد ، موقع وموقع ظهور مراكز التهوية في عظم القص لأذجة الأغنام والفاعز، والفاعز الأسود باستخدام الصبغة المزدوجة المحورة والمكونة من صبغة الاليزاز الحمراء لتحديد التكلس والذي يشير بدء عملية التهوية في القطعة القصية المتطورة وصبغة الأليسين الزرقاء لتوسيع العضروف . بين فحص عظم القص المصبوغة والمروعة أن كل قطعة قصية في أذجة الأغنام والعوامي تتظاهر كل مراكز فياقفة في عمر 80 يوماً ثم في القطعة القصية الرابعة في عمر 90 يوماً. ظهور مراكز التهوية في عظم القص يبدو في القطعة الفصية السابعة في عمر 65 يوماً ثم في القطعة القصية الثالثة، الرابعة، الخامسة بعمر 57-69 يوماً ثم في القطعة الفصية الثانية. الأولي بعمر 10-12 يوماً في حين تأخر ظهور مراكز التهوية في القطعة القصية السابعة إلى عمر 77-92 يوماً. أظهر فحص عظم القص المصبوغة والمروحة لأذجة الماعز الأسود أن كل قطعة قصية تظهر مرارا وفجأة في مراكز القصية وفجأة ظهور هذه المراكز بدأ في القطعة الفصية السابعة بعمر 54 يوماً وبليه ظهور مراكز تهوية القطع القصية الرابعة، الخامسة، السادسة، السابعة، الأولى في الأعمار 65، 57، 50، 30، 20، 14 يوماً على التوالي.