

PRENATAL DEVELOPMENT OF VERTEBRAL COLUMN OF SHEEP

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Abstract: The present study included the identification of chronological appearance of primary and secondary ossification centres and their development in vertebrae of sheep. Fifty-seven sheep fetuses irrespective of sex were used for this study. Alizarin red, Alizarin red with alcian blue methods, radiography and histological techniques were used to detect the ossification centres at an early age. The cervical vertebrae in general developed from three ossification centres, but axis developed from four ossification centres. The thoracic vertebrae developed from four ossification centres viz., two for neural arches, one for the centrum and separate ossification centre for dorsal spinous process. The ossification centres for second to fifth dorsal spinous process appeared at 68 days of gestation and the first dorsal spine ossified at 74 days. Ossification centers appeared in all thoracic spines at 181 days. The lumbar vertebrae, sacral vertebrae and first four coccygeal vertebrae were developed from 3 primary ossification centres viz., one each for the neural arches and one separate ossification centre for the body of vertebrae. Further, ossification of transverse processes of lumbar vertebrae were first appeared towards the neural arches and extended laterally. The most caudal of the coccygeal group were developed by single centre of ossification.

Keywords: Ossification, prenatal development, Sheep foetus, Vertebral column

Introduction

The bones of the vertebral column were developed by the endochondral method of ossification from sclerotomic mesenchyme of somites in domestic animals (Mc Geady *et al.*, 2006). The bones of vertebral column of domestic animals develop from more than one ossification centre (Noden and Lahunta, 1985). The ossification time of the vertebral bodies and their neural arches varies from one region to another (Mohammed and Sayed, 1985). As sheep lumbar spines represent viable options for use as models of the human lumbar spine (Wang *et al.*, 2015) the present study was undertaken.

Materials and methods

Fifty seven foetuses were collected from uteri of the ewes slaughtered in and around Tirupati. The crown rump lengths (CRL) of these fetuses were measured with measuring tape to calculate age (Arthur *et al.*, 1989). The age of the fetuses ranged between 40-140 days. The vertebral columns of small (CRL between 3.5 and 14cm) fetuses were

stained with alizarin red (Humason, 1962) and alcian blue with alizarin red stains as explained by Farzane Sadeghi (2015). The stained specimens were examined to detect the primary ossification centers. The vertebral column of the fetuses size more than 14 cm CRL were used to take radiographs in dorsoventral, lateral and oblique views at exposure factors 65kVp (kilo Volt power) and 10mAs (milli Ampere per second) and 60kVp and 12 mAs by using digital x-ray unit (Genius 100). For those fetuses, where the above two methods were not feasible i.e. CRL below 3.5 cm were fixed in 10% Neutral buffered formalin and processed for serial paraffin sections of 5-6 μ . They were stained by routine Haematoxylin and Eosin method (Singh and Sulochana, 1996) to study the ossification process in bones of vertebral column in sheep. Some specimens were macerated in diluted solution of potassium hydroxide 2-10% for about 6-24 hours for digestion of muscles.

Results and discussion

The vertebral column of sheep in general showed seven cervical, thirteen thoracic, six lumbar and four sacral vertebrae and five coccygeal vertebrae. In the present study one variant with eight cervical vertebrae (Figure.1) and two variants, one with seven lumbar vertebrae and one with five lumbar vertebrae were observed but according to Wenham (1977) in general seven lumbar vertebrae with occasional sacralisation of the seventh lumbar vertebra in sheep. The centrum of each vertebra during its development showed a pair of foramina on the dorsal surface.

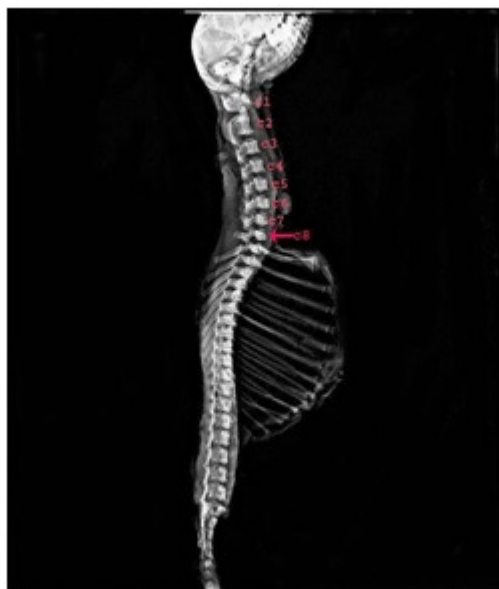


Figure 1: radiograph of 136days sheep foetus showing 8 cervical vertebrae.

Cervical vertebrae:

The mesenchymal aggregate associated with the 5th somite, forms the primordial body of first cervical vertebra in domestic animals (Noden and de Lahunta, 1985). Atlas of sheep showed total three ossification centers viz., two for neural arches and one ossification center for ventral arch. The two neural arches of atlas appeared at 48 days of gestation in cartilaginous lateral masses. The ossification centers of neural arches of the atlas were largest among the seven cervical vertebrae and showed notches on anterior part and lateral aspect of the wing (Figure.2). They were converted into intervertebral and alar foramens at an estimated age of 77 days of gestation.

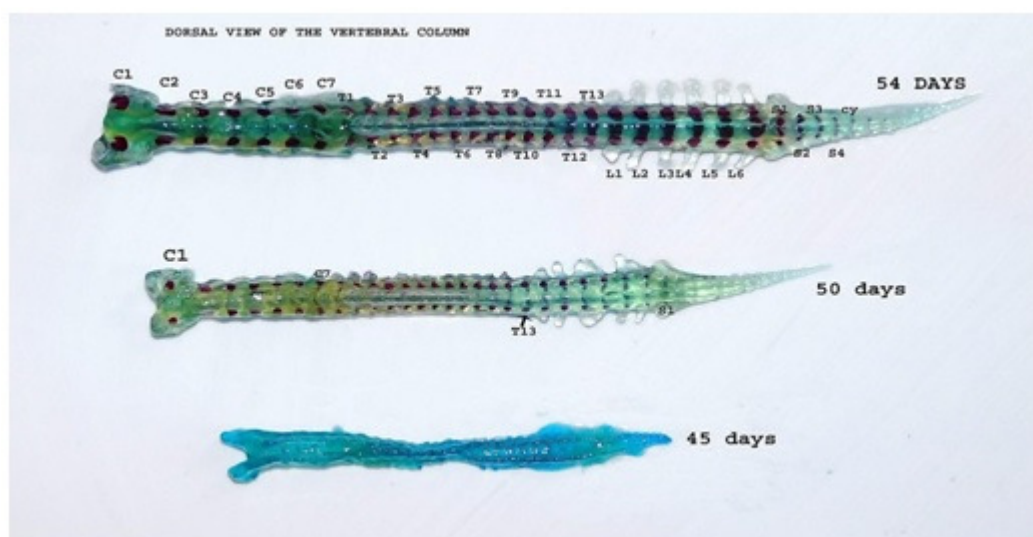


Figure 2: Dorsal view of vertebral column of 45 days, 50 days and 54 days sheep fetus. (Red color: bone; blue color: cartilage)

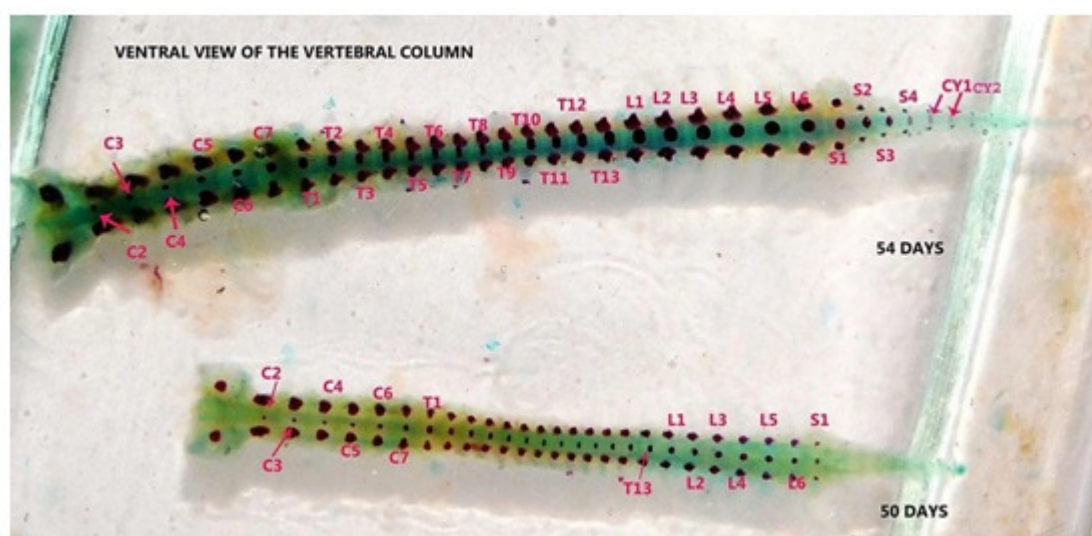


Figure 3: ventral view of vertebral column of 50 day and 54 days sheep fetus.

The size of the ossification centers for the bodies of the 2nd to 7th cervical vertebrae increased in size till 54 days (Figure.3), at 65 days age ossification centers for the centrum of the 2nd to 7th vertebrae were almost equal and after 70 days body of the second cervical vertebrae became larger and thicker. One secondary ossification center for the ventral arch of atlas was noted for the first time at 87 days. Getty (1975) noted four loci of ossification in atlas, of which 2 were noted for ventral arch one on either side for lateral mass, wing and half of the dorsal arch in domestic animals. Ossification center for the ventral arch of the atlas was the last to appear among the all loci of ossification centers of the cervical vertebrae. No anterior or posterior epiphyses were seen in atlas. The vertebral ossification begins at the 6th week of gestation in dog (Noden and de Lahunta, 1985).

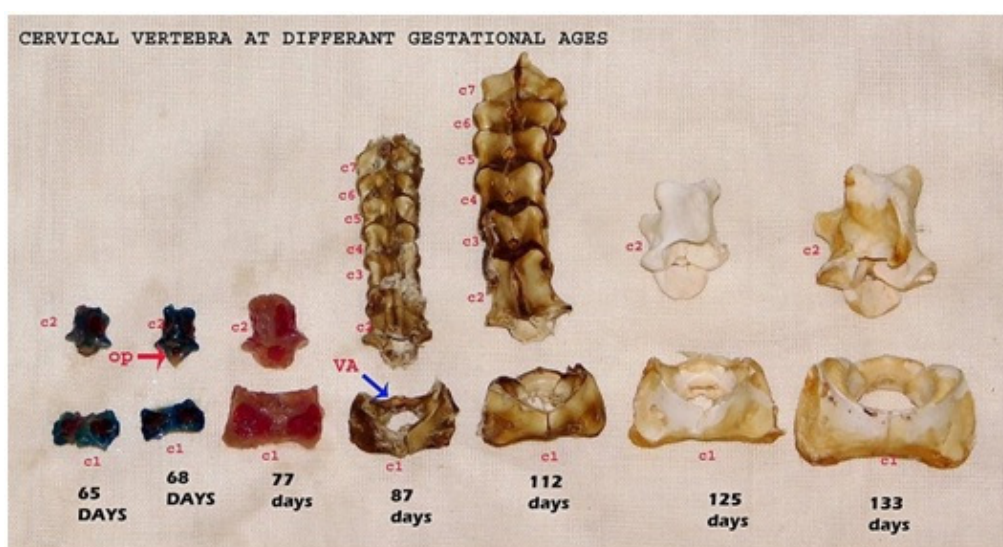


Figure 4: cervical vertebrae showing locus of ossification for odontoid process (OP) of axis and ventral arch (VA) of atlas. Atlas (C1), Axis (C2).

Axis in sheep showed total four primary ossification centres viz., one each for neural arches, one for body and one separate ossification centre for odontoid process. In equines 6 or 7 centres of ossification for axis were noted (Getty, 1975). The loci of ossification for neural arches and body of axis were appeared first at 50 days of gestation in sheep (Figure.3). The locus for odontoid process was noted first time in anterior cartilaginous mass of axis at 68 days of gestation in sheep (Figure.4). It was placed caudal and ventral to the wings of the atlas and this process was easily identified from lateral view of radiograph. The odontoid process (dens) ossified at an estimated age of 80 days and 43 days in bovine foetus (Lindsay, 1972) and feline foetus respectively (Boyd, 1976).

The 3rd to 7th cervical vertebrae developed from three primary centres of ossification, one each for the lateral walls of the neural arches (Figure. 2) and one for the

body of the vertebrae at 50 days of gestation (Figure.3). Anterior and posterior articular processes were developed from the lateral aspect of lamina and the transverse process of both sides of cervical vertebrae from 3rd to 7th extended dorsally and ventrally from the area of attachment of pedicle with body and subsequently ossification was extended from the neural arches to cartilaginous spines. Two halves of the lamina got fused at 112 days in sheep fetus. In dog and cat, which were born in a relatively immature condition these ossification centers do not fuse dorsally until after birth (Noden and Lahunta, 1985) whereas, fusion of the neural arches in human fetuses between 9th and 10th weeks of gestation (Grzymistawska and Wozniak, 2010).

Thoracic vertebrae:

In the present study four primary locus of ossification were noted for thoracic vertebrae viz., two for neural arches, one for centrum and a separate ossification center for dorsal spinous process of thoracic vertebrae. The cartilaginous masses for the bodies of thoracic vertebrae were noted initially at 47 days of gestation in sheep fetus. The size of the ossification centre for centrum going to decrease from T1 to T11 and later on the size is going to increase till T13. Two secondary ossification centers for the anterior and posterior extremities separately, which were developed in later part of life. Hautier *et.al.* (2010) stated that neural arches ossify faster than centra throughout the spine in mammals. The bodies of thoracic and lumbar vertebrae ossify earlier to that of other regions in mammals. The ossification of dorsal spinous process of thoracic vertebrae was noted in cranio-caudal sequence with the exception of first dorsal vertebra. The ossification centres were firstly noted in dorsal spinous process of T₂, T₃, T₄ and T₅ vertebrae at 68 days of gestation, whereas in first dorsal spine ossification began at 74 days. The ossification centre in 6th and 7th dorsal spine was reported at 78 days, 8th and 9th dorsal spine at 80 days (Figure 5), 10th and 11th spines ossified at 93 days of gestation further ossification appeared in all thoracic spines at 140 days.



Figure 5: vertebral column of 65 days, 68 days and 74 days,78 days and 80 days sheep fetus showing locus of ossification for spinous processes (T:Thoracic)

In sheep fusion of neural arches of thoracic vertebrae was first noted at 105 days of gestation. The dorsal spines of thoracic vertebrae from 4-8 fused with the neural arches at 112 days in sheep. The spinous process of 13th thoracic vertebra developed from the fused neural arches like those of the spinous process of cervical and lumbar vertebrae. The transverse processes were developed as lateral outgrowth of neural arches of thoracic vertebrae. First thoracic vertebrae's transverse process bear flat articular area on its dorsal aspect. The fusion of neural arches was quite early i.e. at 9th week in human foetuses (Grzymistawska and Wozniak, 2010). Two secondary ossification centres for the extremities of centrum were noted at 112 days in sheep foetus.

Lumbar Vertebrae:

The lumbar vertebrae were developed from three primary ossification centres viz., one each for the neural arches and one separate ossification centre for the body of vertebrae at 49 days of gestation and they were more distinct at 50 days of gestation (Figure.2, 3). Bagnall *et.al.* (1977) stated that ossification in the centrum appeared first in upper lumbar region whereas lower lumbar region develops slowly. In sheep transverse processes of lumbar vertebrae were cartilaginous till 64 days. Further, ossification of transverse processes of vertebrae has first appeared towards the neural arches and extended laterally. The forward inclination of transverse process is more prominent in the last lumbar vertebrae. No separate ossification centre for dorsal spine in lumbar region of sheep. The fusion of neural arches and ossification of dorsal spines from the neural arches of lumbar vertebrae was appeared at 133 days in sheep foetus. Similarly Rajtova (1968) reported that in guinea pig dorsal spinal processes of lumbar vertebrae developed by ossification from the neural arches and they are finally visible on the 41st day of fetal development in guinea pig.

Sacrum:

In sheep 3 primary ossification centers were noted for each sacral vertebra i.e. two ossification centres for neural arches and one for centrum, The locus of ossification for centrum and neural arches of S₁ identified at an estimated age of 50 days and centrum and neural arches of S₂, S₃ and S₄ appeared at 54 days (Figure 2, 3). Cranial and caudal epiphysis in sheep developed from two secondary ossification centers. In the present study fusion of neural arches in sheep was noted at 136 days. At this age sacral spines were distinct and they were not fused. Getty (1975) observed fusion of sacral vertebrae in cranio-caudal sequence and fusion is not completed until adult age in equines.

Coccygeal Vertebrae:

In sheep the first four coccygeal vertebrae developed each from 3 centers of ossification i.e. one for the centrum and two for the neural arches. However the caudal of the group was developed by only one center of ossification. In the present study ossification centers for the body of first two and neural arches of first four coccygeal vertebrae was noted at 54 days. At 60 days ossification was noted in nine coccygeal vertebrae. First 4 or 5 coccygeal vertebrae consisted of body and anterior and posterior extremities of centrum at 136 days of gestation. No extremities could be seen in the most distal segments of coccygeal vertebrae. Whereas, in bovine fetus the loci of all neural arches and all the centrum from C₂ to CY₅ appeared by the end of the 8th to the 10th week (Lindsay, 1972).

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