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Maitri J Patel

Ph.D. Scholar, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat, India

Hardi N Patel

MVSc. Scholar, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat, India

MM Chudasma

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Rajpur, Himmatnagar, Gujarat, India

NH Joshi

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Rajpur, Himmatnagar, Gujarat, India

MC Desai

Professor and Head, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat, India

KB Patel

Professor, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat, India

Corresponding Author:

Maitri J Patel

Ph.D. Scholar, Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat, India

Prenatal appendicular skeletal development in the buffalo by radiographic study: The pelvic limb

Maitri J Patel, Hardi N Patel, MM Chudasma, NH Joshi, MC Desai and KB Patel

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Abstract

The present radiographic study was carried out on 58 buffalo fetuses from CR 2.00 cm to full term. The chronological order of first radiographic appearance of ossification centers in the pelvic limb of buffalo fetuses was observed as ilium; ischium; diaphysis of femur, tibia, large metatarsus; proximal phalanx and middle phalanx at 97 days. This was followed by center of distal phalanx at 106 days, body of calcaneus at 127 days, pubis at 152 days. The centers for distal epiphysis of femur; proximal epiphysis of tibia; second and third tarsus; and central and fourth tarsus first appeared at 226 days. The ossification centers of distal epiphyses of tibia and large metatarsus, and proximal epiphyses of first and second phalanges, were appeared at 250 days. The ossification centers of head of femur, patella, anterior tuberosity of tibia, lateral malleolus and tuber calcis of calcaneus appeared last at full term. Ossification centers for trochanter major, trochanter minor, proximal extremity and diaphysis of fibula, first tarsus, small metatarsus, and proximal and distal sesamoids were not detected even in at full term.

Keywords: Bones, buffalo fetuses, ossification centers, pelvic limb, radiography

1. Introduction

Growth of fetuses in size is directly proportionate to the skeletal growth. Thoracic and pelvic appendages grow outward from somatopleure as limb buds. Assessment of growth and development of the bone and teeth can be utilized as tool to estimate fetal age, as bone and teeth are quite resistant to decomposition and easily detected in fetal remain (Carneiro *et al.* 2013) [1]. Due to heavy mineralization at the site ossification, the developing bones can be easily detected in fetuses through radiography. In putrefied fetuses, the ossification centers of the bones give valuable information (Gjesdal, 1969) [3]. The study on the dynamics of ossification of the limb bones provides a great help in estimation of fetal age (Succu *et al.*, 2023) [14]. The information on time of appearance of bones of appendicular skeleton provide great help in estimation of fetal age at death. Various workers have documented the age-related chronology for first appearance of ossification centers of pelvic limb bones by radiographic and double staining studies in fetuses of domestic ruminants (Gjesdal, 1969; Richardson, 1976; Lindsay, 1969; Majeed and Ahmed, 2002; Parmar *et al.*, 2009; Chaudhary, 2017; Nissar *et al.*, 2017; Sahil and Ahmed, 2022; Succu, 1923) [2, 3, 4, 5, 6, 7, 11, 12, 14]. Very scanty information is available on the time of appearance of ossification centers of appendicular skeleton in buffalo fetuses for bones of thoracic limb (Patel *et al.*, 1996) [8] and pelvic limb (Rao *et al.*, 2012) [10]. Therefore, the present radiographic study was planned to observe the sequences of appearance of ossification centers of pelvic limb bones in buffalo fetuses.

2. Materials and Methods

The present study was carried out on 58 dead buffalo fetuses (CR 2.0 cm to full term) of earlier research work (Patel *et al.*, 1996) [8]. Age of specimens was calculated as per formulae of Soliman (1975) [13]. The pelvic limbs were separated by midsagittal cut along the pelvic symphysis and mid sacral region. Right pelvic limbs were radiographed for the latero-medial and antero-posterior views.

For all the radiographs focal film distance (FFD) was kept 90 cm with varying mAs (5 to 12) and kVp (44 to 60) depending upon thickness of the specimen. Processing of the radiographs was done on standard lines. The object film distance was kept minimum. Radiographic film was processed on the standard lines. For radiographic observation, the radiographs were arranged on series of illuminators in ascending order of fetal age. The appearance of radio-opaque area in the appropriate anatomical location was considered as criterion for presence of the ossification center.

3. Results

The comparative data of time of first appearance of ossification centers of various bones of pelvic limb in the present study and earlier reports in the fetuses of buffalo and cow are summarized in Table 1. The sequential chronological order of radiographic appearance of ossification centers observed in the present study for pelvic limb bones is given in Table 2.

3.1 Oscoxae: Oscoxae showed three chief centers of

ossification at full term.

The centers for ilium and ischium first appeared at 97 days followed by pubis at 152 days. Complete fusion of these three centers at acetabulum was not detected even at full term.

3.2 Femur: Amongst the five ossification centers for femur present at full term, centers for diaphysis first appeared at 97 days followed by distal epiphysis at 226 days and head of femur at full term. The center for trochanter major and trochanter minor were not detected even at full term.

3.3 Tibia: At full term, three ossification centers for tibia observed. The ossification center for diaphysis appeared first at 97 days, proximal epiphysis at 226 days, distal epiphysis at 250 days and anterior tuberosity of tibia at full term.

3.4 Fibula: The center for distal epiphysis of fibula, the lateral malleolus, first appeared at full term. The centers for proximal epiphysis and diaphysis were not observed even at full term.

Table 1: The comparative data of time of first appearance of ossification centers of bones of pelvic limb in buffalo fetuses in the present study and earlier reports.

Bone and ossification center	Buffalo fetuses (Age in days)		Cow fetuses (Age in days)
	Present study*	Rao (2011)** Rao <i>et al.</i> (2012)**	Lindsay (1969) [4]*
Os coxae			
Ilium	97	64	58
Ischium	97	64	58
Pubis	152	135	160
Femur			
Head of femur	Full term	252	230
Trochanter major	Not seen	Not seen	238
Diaphysis	97	59	50
Trochanter minor	Not seen	Not seen	Not seen
Distal epiphysis	226	170	163
Patella			
	Full term	216	203
Tibia			
Proximal epiphysis	226	181	188
Anterior tuberosity	Full term	216	220
Diaphysis	97	59	52
Distal epiphysis	250	186	190
Fibula			
Proximal epiphysis and diaphysis	Not seen	Not seen	52
Lateral malleolus	Full term	264	220
Tarsal bones			
Talus/ tibial tarsus	226	150	163
Calcaneus/ fibular tarsus	127	114	88
Tuber calcis	Full term	252	209
Central and fourth	226	181	190 (Central) 163 (Fourth)
Second and third	226	186	190
First	Not seen	252	Not seen
Large metatarsus (III & IV)			
Diaphysis	97	64	58
Distal epiphysis	250	216	200
Small metatarsus (II)			
	Not seen	66	Not seen
Proximal sesamoids			
	Not seen	280	238
Proximal Phalanx			
Proximal epiphysis	250	216	190
Diaphysis	97	89	68
Middle Phalanx			
Proximal epiphysis	250	216	190
Diaphysis	97	92	75
Distal Phalanx			
Body	106	66	58
Distal sesamoids			
	Not seen	280	Not seen

*= Radiographic study; **= Histological, Alizarin Red S and Radiographic study

Table 2: The sequential chronological order of radiographic appearance of ossification centers in the pelvic limb bones of buffalo fetuses.

Fetal age (days)	Ossification centers
97	Ilium Ischium Diaphysis of femur Diaphysis of tibia Diaphysis of large metatarsus Diaphysis of proximal phalanx Diaphysis of middle phalanx
106	Body of distal phalanx
127	Calcaneus
152	Pubis
226	Distal epiphysis of femur Proximal epiphysis of tibia Second and third tarsus Central and fourth tarsus
250	Distal epiphysis of tibia Distal epiphysis of large metatarsus (III & IV) Proximal epiphysis of proximal phalanx Proximal epiphysis of middle phalanx
Full term	Head of femur Patella Anterior tuberosity of tibia Lateral malleolus Tuber calcis

3.5 Tarsal bones

In the proximal row, tallus (tibial tarsus) exhibited a single ossification center at 226 days, whereas, calcaneus (fibular tarsus) demonstrated two ossification centers, one for body of calcaneus at 127 days and other for tuber calcis at full term. In the central row, both central and fourth tarsal showed separate ossification centers at 226 days. In distal row, a single common ossification center also appeared for the second and third tarsals at 226 days. Ossification center for first tarsal was not detected even at full term. The ossification centers for central and fourth tarsal not found fused with each other even at full term.

3.6 Large Metatarsus (III and IV): Each III and IV metatarsus exhibited two ossification centers, one each for diaphysis at 97 days and distal epiphysis at 250 days. The center for distal epiphysis of each metatarsus was spherical in shape at 250 days and elongated at full term. These centers did not fuse with each other and with diaphysis even at full term. Up to 162 days, centers for diaphysis of III and IV metatarsus were clearly separate visible but found fused with each other in the lower $\frac{3}{4}$ portion at 226 days. At full term, the diaphysis of III and IV metatarsus were found fused with each other throughout length except the ends, forming a single bone i.e. large metatarsus. The centers for proximal epiphysis of III and IV metatarsus were not detected during the present study. The center for the small metatarsus (II) could not be detected even at full term.

3.7 Phalanges: Both proximal and middle phalanges of III and IV digits exhibited two centers of ossification, one each for diaphysis at 97 days and proximal epiphysis at 250 days. The centers for distal epiphysis of proximal and middle phalanges were not seen at full term. The single center for distal phalanx of each III and IV digit first appeared at 106 days.

3.8 Sesamoids: A single ossification center for patella first appeared in the form of elongated oval shape at full term. The

centers for proximal and distal sesamoid bones did not appear even at full term.

4. Discussion

The chronological order of first radiographic appearance of ossification centers in the pelvic limb of buffalo fetuses was observed as ilium, ischium, diaphyses of femur, tibia, large metatarsus (III and IV), proximal phalanx, and middle phalanx at 97 days. This was followed by center of distal phalanx at 106 days, body of calcaneus at 127 days, pubis at 152 days. The centers for distal epiphysis of femur, proximal epiphysis of tibia, second and third tarsus, and central and fourth tarsus first appeared at 226 days. The ossification centers of distal epiphyses of tibia and large metatarsus; and proximal epiphyses of proximal and middle phalanges appeared at 250 days. The ossification centers of head of femur, patella, anterior tuberosity of tibia, lateral malleolus, tuber calcis were appeared last at full term (Table 2).

Ossification centers for trochanter major; trochanter minor; proximal epiphysis and diaphysis of fibula; first tarsus; small metatarsus (II); and proximal and distal sesamoids were not detected even in at full term (Table 1 and 2). In contrary, Rao (2011) and Rao *et al.* (2012) observed ossification centers for the first tarsus; proximal and distal sesamoids at 250 and 280 days, respectively in buffalo fetuses and Lindsay (1969) [4] observed ossification centers for trochanter major and proximal sesamoids at 238 days in cow fetuses. In the present study, the time of first appearance of various ossification centers of various pelvic limb bones were found later than fetuses of buffalo (Rao, 2011; Rao *et al.*, 2012) [9, 10] as these authors used histological and Alizarin Red S methods which detected mineralization very earlier than radiographic examination. In the present study on the buffalo fetuses, the ossification centers of pelvic limb bones were observed comparatively later than cow fetuses (Lindsay, 1969) [4] because of shorter gestational length in cow than in the buffalo.

The present work revealed that primary ossification centers for the diaphysis of various long bones appeared first followed by the secondary ossification centers for epiphysis and apophyseal ossification centers for tuberosities (Table 1 and 2), which corroborated previous reports on buffalo (Rao *et al.*, 2012; Patel *et al.*, 1996) [8, 10] and cattle (Lindsay, 1969) [4]. In the present investigation, most of the bones of pelvic limb bones developed from more than one ossification centers except tarsal bones and patella, however, fibular tarsal (calcaneus) developed from two centers which is in accordance to previous reports in buffalo (Rao, 2011; Rao *et al.*, 2012) [9, 10] and cattle (Lindsay, 1969) [4] (Table 1).

5. Conclusion

The sequential chronological order of ages, at which different ossification centers radiographically appeared, can be utilized as indicator to estimate age at death in buffalo fetuses. The data generated in the present investigation on fetal age associated radiographic appearance of ossification centers of various bones of pelvic limb in buffalo will provide baseline information for further breed, sex and geographic specific investigation in the buffalo fetuses.

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