RESEARCH ARTICLE

Radiographic Assessment of Growth of Thoracic Limb Bones and Age Estimation in Fetuses of Goat (*Capra hircus*)

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Abstract

The present study was conducted on 103 dead goat fetuses ranging from 34 days (CR 1.55 cm) to 148 days (full term, CR 42.00 cm). The length and width of primary ossification centers of scapula, humerus, radius, ulna and large metacarpus were recorded from radiographs and statistically analyzed to assess age associated growth and to evolve age prediction formulae in the goat fetuses at birth or death/abortion. Amongst the bones of thoracic limb, the diaphyseal length of ulna showed the highest growth rate (0.94 mm/day) followed by radius, humerus, scapula and large metacarpus. The correlation coefficients between various bone measurements and fetal age were very high (r= >0.9). Among the simple linear regression equations formulated for prediction of fetal age; the length of scapula, humerus and radius gave 98 % predictability (R^2) indicating them as reliable bone measurements for estimation of age in goat fetuses.

Key words: Age, Bone, Fetus, Goat, Radiography.

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Introduction

The estimation of fetal age at death in domestic ruminants is a fundamental procedure in different circumstances including clinical, animal husbandry, biomedical research, forensic, veterolegal and archaeological context; and usually carried out by species specific equation derived from fetal biometry and time of appearance of various external developmental horizons (McGeady *et al.*, 2006; Parmar *et al.*, 2009b; Rao and Ramayya, 2013; Prabhakar and Farooqui, 2022). In addition, assessment of development of bone and teeth is also 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). Due to heavy mineralization at the site of ossification, the developing bones can be easily detected in fetuses through radiography. The study of the dynamics of ossification of the limb bones provides a great help in estimation of fetal age (Succu *et al.*, 2023).

The age-related chronology for first appearance of ossification centres of appendicular skeleton by radiographic and double staining studies were reported in fetuses of goat (Parmar *et al.*, 2009a; Chaudhary, 2017), sheep (Richardson *et al.*, 1976; Ahmed, 2008), cattle (Gjesdal, 1969; Richardson *et al.*, 1990) and buffalo (Patel *et al.*, 1996; Rao *et al.,* 2013; Supriya, *et al.*, 2016). The fetal age associated assessment of bone growth by gross, radiographic, double staining methods have been also reported in goat (Chaudhary, 2017), in sheep (Richardson *et al.*, 1976; McDonald *et al.*, 1977; Ahmed, 2008; Martin and Gracia-Gonzalez, 2015; Nissar, *et al.* 2017), in cattle (Gjesdal, 1969; Richardson *et al.*, 1990) and in buffalo (Patel *et al.*, 1996). Amongst them, very few researchers have given prediction formulae for fetal age at death from limb bone measurements in sheep (Richardson, *et al.,* 1976, McDonald, *et al.*, 1977) and in cattle (Gjesdal, 1969). In view of dearth of information on age associated prenatal radiographic assessment of growth of thoracic limb bones and age estimation formulae for goat fetuses, the present study was planned.

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Materials and Methods

A total of 103 non-descript dead goat fetuses, irrespective of sex and multiples, ranging from 34 days (CR 1.55 cm) to

148 days (full term, CR 42.00 cm) were collected from local slaughter houses. The age of fetuses was predicted on the basis of CR length as per the normograph perfected by Lyngset (1971). The fetuses were divided into seven age groups at ½ lunar month interval; *viz.,* Group-I (31-45 days); II (46-60 days); III (61-75 days); IV (76-90 days); V (91-105 days); VI (106-120 days) and VII (121 to full term) according to Kadu and Kaikini (1984). Small fetuses up to 50 days age and a full-term fetus were radiographed as such in right lateral position. For rest of other fetuses right thoracic limbs were separated from body for the latero-medial and antero-posterior radiographic views. Radiographs were taken on Siemens Diagnostic X-ray machine (Model No. 5205) keeping focal film distance (FFD) 90 cm constant with varying mAs (4.0 to 8.0) and kVp (42.0 to 55.0) depending upon thickness of the specimen.

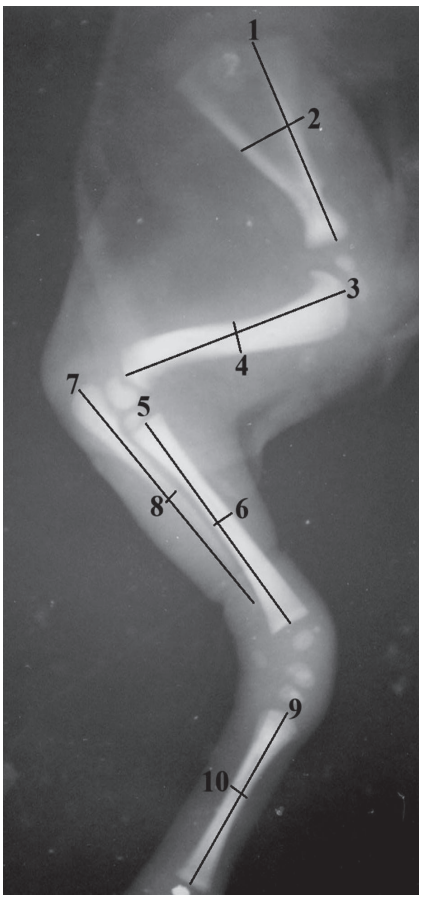

Fig. 1: Radiograph of thoracic limb (L/M view) of 117 days goat fetus showing bone measurements: 1, 3, 5, 7, 9 length and 2, 4, 6, 8, 10 width of scapula, humerus, radius, ulna and large metacarpus, respectively

The object film distance was kept minimum. Processing of radiographic film was done on the standard lines (Gillette, 1977). The measurements of radio-opaque ossified part of primary ossification center of scapula, humerus, radius, ulna and large metacarpus were recorded (in cm) after its first measurable form till the full term from the radiographs by a digimatic vernier caliper (Mitutoyo, Japan) (Fig. 1). The maximum diaphyseal lengths of long bones were measured between the proximal and distal diaphyseal ends in the latero-medial radiographic view. The length of body of the scapula was measured between vertebral border and glenoid angle at the level of spine of scapula. The width of long bones was measured at the midpoint of diaphyseal length. The width of body of the scapula was measured at the midpoint of scapular length (Fig. 1).

All the recorded data were subjected to statistical analysis (Snedecor and Cochran, 1994). Per cent growth achieved at different fetal age groups were calculated for each measurement considering 100 % growth at full term on line of Joubert (1956). The average daily growth rates for various bone measurements were calculated according to Joubert (1956) and Mane *et al.* (2023). The simple linear regressions were fitted to predict the fetal age from radiographic bone measurements.

Results and Discussion

The primary ossification centers of scapula, humerus, radius, ulna and large metacarpus first appeared between 49 to 52 days, which confirmed earlier report of Parmar *et al.* (2009a). All the bone measurements were not possible in Group-I (31-45 days) due to the absence of appearance of primary ossification center on the radiographs. Scapular width, and ulnar length and width were not measurable in Group-II & III. The total number of observations (n), range, Mean ± SE, CV (%), growth (%) and average daily growth rate, for radiographically measured ossified length and width of bones in goat fetuses are presented in Table 1 and 2. There was gradual progressive linear growth in the length and width of bones studied with increasing fetal age till the full term (Fig. 2). The ulna maintained its position as the longest bone, except age Group-II & III, where it was not radiographically measurable. Whereas, the humerus showed the highest mid-diaphyseal width in all age groups (Table 1 and 2). Several workers provided ready reckoner tables/ graphs; through gross, radiographic and double staining studies; showing increasing in length and width of thoracic limb bones with advancing fetal age in goat (Chaudhary, 2017), in sheep (Richardson *et al.* 1976; McDonald *et al*., 1977; Ahmed, 2008; Martin and Garcia-Gonzalez., 2015; Nissar *et al,* 2017), in cattle (Gjesdal, 1969; Richardson *et al.* 1990) and in buffalo (Patel *et al*., 1996), which was in close agreement with findings of the present study on radiographic assessment of growth of thoracic limb bone in goat fetuses.

Amongst the bones of thoracic limb, the diaphyseal length of ulna showed the highest growth rate (0.94 mm/day) followed by radius, humerus, scapula and large metacarpus, whereas, the width of ulna expressed the lowest growth rate (0.03 mm/day) (Table 1 and 2). More than 50 to 60 % growth in the length and width of various thoracic limb bones was achieved in the fetuses of age Group-V (91-105 days) (Table 1 and 2). The daily growth rate for the thoracic limb bone

measurements was not available in the literature reviewed so far.

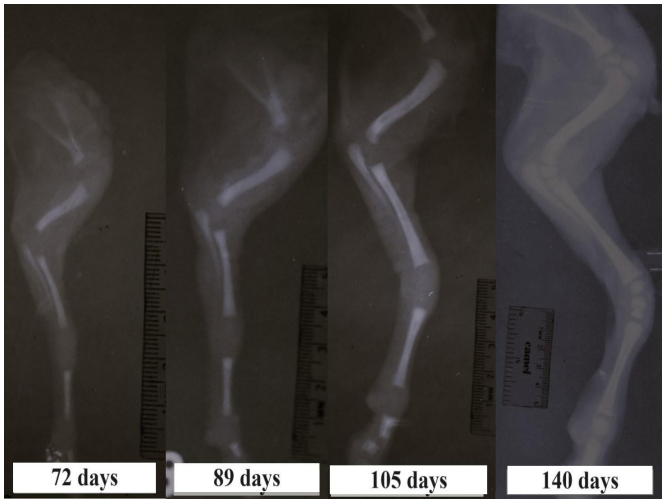

Fig. 2: Radiographs of thoracic limbs (L/M view) of goat fetuses showing increasing length and width of scapula, humerus, radius, ulna and large metacarpus with advancing fetal age

The simple linear regression equations for estimation of fetal age from measurements of thoracic limb bones, coefficients of correlation (r) and coefficients of determination (R^2) are given in Table 3. The coefficients of correlation (r) between various measurements of thoracic limb bones and fetal age were very strong (r= >0.9) (Table 3). The scatter plots with regression line for length and width of bones with age showed minimum scattering and indicated linear and positive growth with advancing fetal age till full term. This suggested that bone length and width is a good indicator of age and could be utilized as a function of age. The present study agreed with the fetal age associated correlation coefficients (r) and scatter plots for length of humerus, radius, ulna and metacarpus in goat (Chaudhary, 2017); radius in sheep (Richardson *et al.*, 1976); humerus, radius and large metacarpus in cattle (Gjesdal, 1969; Richardson *et al.*, 1990).

Among the simple linear regression equations formulated for prediction of fetal age, the length of scapula, humerus and radius gave 98 % predictability (R^2) indicating them as reliable bone measurements for determination of age in goat fetuses. Remaining measurements of thoracic limb bones gave more than 85 % predictability (R^2), except for width of scapula (R^2 = 81 %)(Table 3). Various authors formulated regression equations to predict fetal age based on length of thoracic limb bones in sheep (Richardson *et al,* 1976; McDonald *et al.*, 1977) and in cattle (Gjesdal, 1969). From the available literature reviewed so far, it seems that no research has been reported previously on age prediction equation based on radiographic osteometric data in goat fetuses.

Table 1: Mean ± SE, CV (%), growth (%) and average daily growth rate for the measurement of radiographic length of thoracic limb bones at different age group in goat fetuses

Bone measurements	Details	Group-II (n = 35) 46-60 days	Group-III (n = 20) 61-75 days	Group-VI (n = 10) 76-90 days	Group-V (n=14) 91-105 days	Group-VI (n = 3) 106-120 days	Group-VII (n = 7) 121 & above	Average daily growth rate (cm/day)
Scapula length	n	28	20	10	14	3	7	
	Range (cm)	4.64-7.96	9.54-19.06	16.88-25.77	22.52-36.87	36.39-42.38	46.33-69.27	
	Mean ± SE	5.98±0.18	14.35±0.60	21.88±0.90	30.87±1.32	38.84±1.81	52.61±3.13	0.66
	CV (%)	15.89	18.62	12.98	16.03	8.09	15.74	
	Growth (%)	11.36	27.28	41.59	58.68	73.83	100.00	
Humerus length	n	28	20	10	14	3	7	
	Range (cm)	3.25-8.44	9.69-17.79	15.67-25.8	22.42-38.16	37.96-45.65	48.62-70.41	
	Mean ± SE	5.78±0.31	13.44±0.49	21.86±1.06	31.23±1.47	41.16±2.31	56.64±3.03	0.67
	CV (%)	27.91	16.19	15.36	17.56	9.72	14.17	
	Growth (%)	10.20	23.73	38.59	55.14	72.67	100.00	
Radius length	n	28	20	10	14	3	7	
	Range (cm)	4.24-9.81	10.89-19.68	17.78-28.20	24.06-38.92	38.74-45.91	50.24-73.01	
	Mean ± SE	6.53±0.33	14.83±0.54	23.87±1.12	33.22±1.54	41.29±2.32	57.84±3.27	0.70
	CV (%)	26.85	16.14	14.87	17.33	9.71	14.95	
	Growth (%)	11.29	25.64	41.27	57.43	71.39	100.00	
Ulna length	n	-	-	10	14	3	7	
	Range (cm)	-	-	20.58-31.73	24.36-45.03	45.77-52.82	54.46-89.31	
	Mean ± SE	-	-	26.06±1.32	36.84±1.88	48.27±2.28	67.92±4.79	0.94
	CV (%)	-	-	15.96	19.14	8.18	18.65	
	Growth (%)	-	-	38.37	54.24	71.07	100.00	

Large metacarpus length	n	28	20	10	14	3	7	
	Range (cm)	1.84-5.29	6.42-12.61	12.01-19.07	16.7-36.48	28.38-35.24	38.75-60.38	
	Mean ± SE	3.24±0.22	9.33±0.38	15.89±0.78	25.12±1.63	31.01±2.13	45.86±3.08	0.60
	CV (%)	35.79	18.34	15.53	24.21	11.92	17.76	
	Growth (%)	7.06	20.34	34.65	54.78	67.62	100.00	

Table 2: Mean ± SE, CV (%), growth (%) and average daily growth rate for the measurement of radiographic width of thoracic limb bones at different age group in goat fetuses

Bone measurements	Details	Group-II (n = 35) 46-60 days	Group-III (n = 20) 61-75 days	Group-VI (n = 10) 76- 90 days	Group-V (n=14) 91-105 days	Group-VI (n = 3) 106-120 days	Group-VII (n = 7) 121 & above	Average daily growth rate (cm/day)
Scapula width	n	-	-	10	14	3	7	
	Range (cm)	-	-	5.43-9.24	8.01-11.81	10.28-12.8	11.92-16.77	
	Mean ± SE	-	-	7.50±0.37	9.54±0.35	11.23±0.79	13.93±0.70	0.14
	CV (%)	-	-	15.60	13.54	12.17	13.31	
	Growth (%)	-	-	53.73	68.49	80.62	100.00	
Humerus width	n	19	20	10	14	3	7	
	Range (cm)	0.98-1.56	1.48-2.92	2.06-3.83	3.16-5.51	4.98-6.53	6.91-8.23	
	Mean ± SE	1.33±0.04	2.15±0.08	3.19±0.19	4.47±0.21	5.65±0.46	7.47±0.19	0.07
	CV (%)	13.02	15.69	19.01	17.33	14.06	6.86	
	Growth (%)	17.80	28.78	42.70	59.84	75.64	100.00	
Radius width	n	19	20	10	14	3	7	
	Range (cm)	0.64-1.16	1.06-1.91	1.88-2.66	1.71-4.09	3.67-4.35	4.39-5.76	
	Mean ± SE	0.85±0.03	1.49±0.06	2.24±0.07	2.98±0.18	3.93±0.21	5.04±0.21	0.05
	CV (%)	16.63	18.84	10.49	22.98	9.41	10.99	
	Growth (%)	16.87	29.56	44.44	59.13	77.98	100.00	
Ulna width	n	-	-	10	14	3	7	
	Range (cm)	-	-	1.06-1.73	1.1-2.51	2.03-3.53	2.99-3.68	
	Mean ± SE	-	-	1.48±0.07	1.86±0.11	2.6±0.47	3.29±0.09	0.03
	CV (%)	-	-	15.66	22.27	31.24	7.55	
	Growth (%)	-	-	44.98	56.63	79.03	100.00	
Large metacarpus width	n	19	20	10	14	3	7	
	Range (cm)	0.5-1.14	1.09-1.91	1.32-2.17	1.51-3.49	3.12-3.60	4.23-5.4	
	Mean ± SE	0.81±0.04	1.45-0.04	1.74±0.09	2.47±0.16	3.29±0.16	4.88±0.17	0.05
	CV (%)	20.13	13.16	16.89	24.94	8.17	9.18	
	Growth (%)	16.60	29.71	35.66	50.61	67.42	100.00	

Conclusions

From the present study on dead fetuses of varying gestation in goats, it was concluded that there is linear positive growth in the length and width of thoracic limb bones with increasing fetal age. This study established strong association between fetal age and the thoracic limb bone growth in length and width. The length of scapula, humerus and radius was found as reliable indicator (R^2 = 98 %) of age in goat fetuses. The study generated the fetal age associated radiographic osteometric data which will provide baseline for further breed, sex and multiples specific investigation in the goat fetuses.

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Table 3: Simple linear regression equations for prediction of fetal age, coefficient of correlation (r) and coefficient of determinations/ predictability (R^2) from radiographic measurement of thoracic limb bones in goat fetuses

Variables		Coefficient of Correlation (r) with age	Prediction equations	Predictability R^2 (%)
Age of fetus (Y) in days		--	***Y = a + bx ± e**	--
Versus	Length of scapula (X_1)	0.989	45.1910 + 01.6711 (X_1) ± 3.62	98
	Width of scapula (X_2)	0.899	41.2310 + 06.1354 (X_2) ± 9.90	81
	Length of humerus (X_3)	0.989	47.2280 + 01.5500 (X_3) ± 3.57	98
	Width of humerus (X_4)	0.982	42.2524 + 12.1663 (X_4) ± 4.60	96
	Length of radius (X_5)	0.988	45.7474 + 01.5255 (X_5) ± 3.67	98
	Width of radius (X_6)	0.971	43.1250 + 17.5456 (X_6) ± 5.86	94
	Length of ulna (X_7)	0.975	59.6540 + 01.0439 (X_7) ± 4.26	95
	Width of ulna (X_8)	0.927	56.7062 + 21.7688 (X_8) ± 7.03	86
	Length of large metacarpus (X_9)	0.981	50.9906 + 01.8150 (X_9) ± 4.75	96
	Width of large metacarpus (X_{10})	0.949	44.8373 + 18.7705 (X_{10}) ± 7.64	90

Note: All the coefficients of correlations (r) are highly significant at 1 % level ($p<0.01$).
*Y= a+ bx ± e; where, "y" = age in days of goat fetus, as dependent variable; "a" = intercept; "b" = regression coefficient of "y" on "x"; "x" = bone measurement as independent variable; and e = random error

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