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Radiographic assessment of growth of pelvic limb bones and age estimation in fetuses of goat (*Capra hircus*)

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Abstract
The present work was carried out on radiographs of previous study on non-descript goat fetuses, irrespective of sex and multiples. The osteometric parameters *viz*. length and width of primary ossification centers of ilium, ischium, femur, tibia and large metatarsus were recorded from radiographs and statistically analyzed to assess age associated growth and to evolve age prediction formulae in the goat fetuses. The tibia maintained its position as the longest bone of pelvic limb in all the age groups of prenatal life. The diaphyseal length of tibia showed the highest growth rate (0.89 mm/day), followed by femur (0.76 mm/day) and large metatarsus (0.62 mm/day). About 50-60% growth of full-term length and width of different bones of pelvic limb were completed in group-V (91 to 105 days). The correlation coefficients between various bone measurements and fetal age were found very high ($r > 0.9$). Among the simple linear regression equations formulated for prediction of fetal age; the length of femur and tibia gave 98 per cent predictability (R^2) indicating them as reliable bone measurements for estimation of age in goat fetuses.

Keywords: Radiography, bone, foetus, age, goat

1. Introduction
The estimation of fetal age at death in the domestic ruminants is a fundamental procedure in different circumstances including clinical, animal husbandry, biomedical research, forensic, veterolegal and archaeological context. Various authors described age estimation of fetuses at death by species specific equation derived from fetal biometry and time of appearance of various external developmental horizons (McGeady *et al.*, 2006; Parmar *et al.*, 2009a; Njaa, 2012; Rao and Ramayya, 2013; Singh, 2017; Prabhakar and Farooqui, 2022) [12, 15, 16, 18, 20, 25]. In addition, assessment of growth and development of the 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) [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) [7]. The study on the dynamics of ossification of the limb bones provides a great help in estimation of fetal age (Succu *et al.*, 2023) [23].
Various workers have reported the age-related chronology for first appearance of ossification centers of pelvic limb bones by radiographic and double staining studies in fetuses of goat (Parmar *et al.*, 2009b; Chaudhary, 2017; Chaudhary, 2019; Chaudhary, 2021a, b) [2, 3, 4, 5, 17], sheep (Richardson *et al.*, 1976; Nissar *et al.*, 2017; Sahil and Ahmed, 2022) [22, 14, 24], cattle (Gjesdal, 1969; Lindsay, 1969; Richardson *et al.*, 1990) [7, 9, 14, 21], and buffalo (Rao *et al.*, 2012) [19]. The fetal age associated assessment of growth of pelvic limb bones by gross, radiographic, double staining methods also reported in goat (Chaudhary, 2017; Chaudhary, 2019; Chaudhary, 2021ab) [2, 3, 4, 5], in sheep (Richardson *et al.*, 1976; Martin and Gracia-Gonzalez, 2015; Nissar, *et al.* 2017) [11, 14, 22], and in cattle (Gjesdal, 1969; Richardson *et al.*, 1990) [7, 21]. Gjesdal (1969) [7] evolved prediction equation to estimate bovine fetal age from cranium length. It seems that there are no reports on daily growth rate of length and width of

bone by radiographic study in goat fetuses. In view of paucity of information, the present radiographic study was aimed to assess growth pattern of pelvic limb bones to estimate fetal age in goat fetuses.

2. Materials and Methods

The present study was carried out on radiographs of earlier research work (Parmar *et al*., 2009b) [17] on 103 dead, non-descript goat fetuses with estimated age, irrespective of sex and multiples. 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). For radiographic osteometry, radio-opaque ossified part of primary ossification centers of ilium, ischium, femur, tibia and large metatarsus were measured (in cm) and recorded after its first measurable form till the full term by a digimatic vernier caliper (Mitutoyo, Japan) (Fig. 1 and 2). The maximum diaphyseal length of long bones were measured between the proximal and distal diaphyseal ends from the of latero-medial radiographic view. The length of ilium and ischium were measured from the ilio-ischial junction to the anterior margin and posterior margin of respective bone. The width of long bones was measured at the midpoint of diaphyseal length. The width of ilium and ischium was measured at the midpoint of their respective length (Fig. 1 and 2).

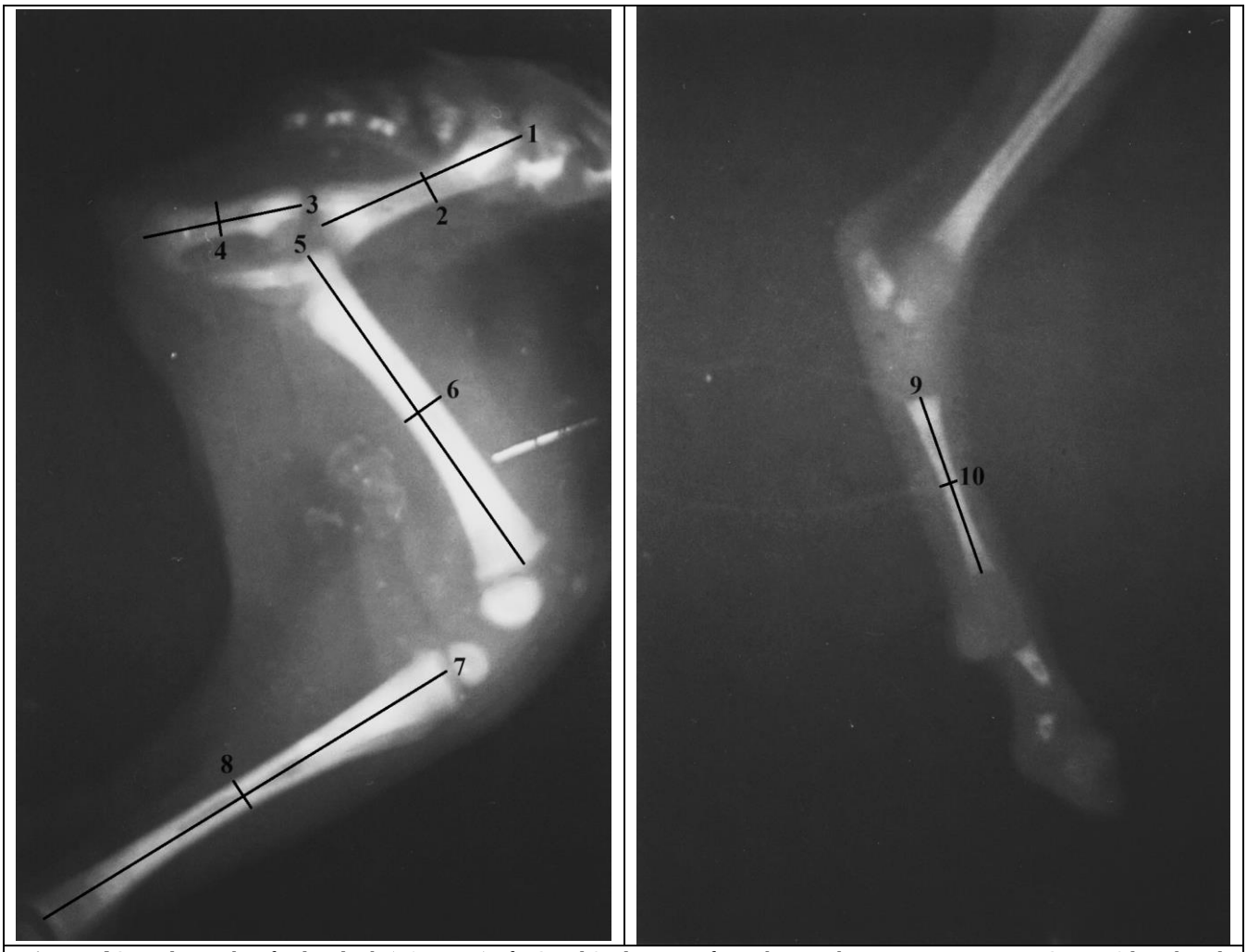

Fig 1 and 2: Radiographs of pelvic limb (L/M view) of123 and 91 days goat fetus showing bone measurements: 1, 3, 5, 7, 9 length and 2, 4, 6, 8, 10 width of ilium, ischium, femur and large metatarsus, respectively

All the recorded osteometric data were subjected to statistical analysis (Snedecor and Cochran, 1994) [26]. Per cent (%) growth achieved at different fetal age groups were calculated for each measurement considering 100 per cent growth at full term on line of Joubert (1956) [8]. The average daily growth rate for various bone measurements were calculated according to Joubert (1956) [8] and Mane *et al.* (2023) [10]. The simple linear regressions were fitted to predict the fetal age from radiographic bone measurements.

3. Results and discussion

Radiographic observation revealed that the primary ossification centers of femur, tibia, and large metatarsus were first appeared at 49 days, followed by ilium (58 days), ischium (60 days) and pubis (103 days). 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. Ischial length and width were also not measurable in Group-II. Due to overlapping shadow of ilium bone in latero-medial views, radiographic osteometry for pubis was not possible in any of fetal group. The total number of observations (n), range, mean ± S.E., C.V. (%), growth (%) and average daily growth rate, for osteometric parameters of pelvic limb bones in goat fetuses are presented in Table 1 and 2.

The tibia maintained its position as the longest bone of pelvic limb in all the age groups of prenatal life. The femur was the second longest bone; except age Group-II, where ilium length found longer than femur. Whereas, large metatarsus was the third longest; except age Group-II and III, where ilium length

found longer than large metatarsus (Table 1). Amongst the long bones of pelvic limb, femur exhibited the highest mid diaphyseal width in all age groups which was followed by width of tibia and large metatarsus (Table 2). The present radiographic study revealed that there was gradual progressive linear growth in the length and width of pelvic limb bones with increasing fetal age till the full term (Fig. 3) (Table 1 and 2).

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

Bone measurement	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)
Ilium Length	n	4	20	10	14	3	7	0.56
	Range (cm)	4.77-5.52	5.51-13.28	10.74-17.93	14.93-24.64	23.71-29.58	28.51-54.8	
	Mean ± S.E.	5.04±0.17	9.32±0.46	15.70±0.81	20.38±0.88	26.61±1.69	37.81±3.34	
	C.V. (%)	6.60	22.29	16.25	16.22	11.03	23.39	
	Growth (%)	13.33	24.65	41.52	53.90	70.38	100.00	
Ischium Length	n	-	20	10	14	3	7	0.40
	Range (cm)	-	2.15-6.99	6.67-10.98	9.84-16.72	16.19-21.1	21.51-37.57	
	Mean ± S.E.	-	4.69±0.31	9.17±0.45	13.24±0.62	18.44±1.43	26.18±2.08	
	C.V. (%)	-	29.71	15.60	17.42	13.46	21.00	
	Growth (%)	-	17.91	35.03	50.57	70.44	100.00	
Femur length	n	28	20	10	14	3	7	0.76
	Range (cm)	2.58-7.7	8.86-18.22	16.56-26.41	23.4-39.52	39.05-44.32	49.9-77.54	
	Mean ± S.E.	4.78±0.32	13.00±0.06	22.59±1.10	32.57±1.60	41.30±1.57	58.34±3.66	
	C.V. (%)	34.92	20.65	15.42	18.34	6.58	16.59	
	Growth (%)	8.19	22.28	38.72	55.83	70.79	100.00	
Tibia length	n	28	20	10	14	3	7	0.89
	Range (cm)	4.12-10.98	11.91-23.90	22.71-35.47	30.82-52.4	51.35-61.18	66.11-92.11	
	Mean ± S.E.	6.97±0.41	17.12±0.77	30.38±1.44	43.08±2.10	54.97±3.12	74.89±4.08	
	C.V. (%)	31.29	20.12	14.96	18.26	9.83	14.43	
	Growth (%)	6.31	22.86	40.57	57.52	73.40	100.00	
Metatarsus length	n	19	20	10	14	3	7	0.62
	Range (cm)	2.32-5.3	6.07-12.7	10.95-19.69	14.54-38.3	29.76-36.5	40.96-62.06	
	Mean ± S.E.	3.62±0.22	9.07±0.42	16.29±0.93	25.91±1.83	32.20±2.16	47.90±3.08	
	C.V. (%)	28.18	20.88	18.11	26.48	11.61	17.03	
	Growth (%)	7.56	18.94	34.00	54.09	67.22	100.00	

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

Bone measurement	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)
Ilium width	n	4	20	10	14	3	7	0.11
	Range (cm)	1.25-1.44	1.35-2.7	2.23-3.15	2.19-4.6	4.13-5.18	5.53-10.87	
	Mean ± S.E.	1.32±0.04	1.85±0.08	2.70±0.10	3.48±0.19	4.6±0.31	6.89±0.69	
	C.V. (%)	6.28	19.64	11.36	19.93	11.60	26.53	
	Growth (%)	19.16	26.85	39.19	50.51	66.76	100.00	
Ischium width	n	-	20	10	14	3	7	0.10
	Range (cm)	-	1.14-2.6	1.29-3.18	2.61-4.2	2.85-4.93	4.01-9.68	
	Mean ± S.E.	-	1.71±0.08	2.46±0.17	3.31±0.15	4.10±0.64	5.78±0.70	
	C.V. (%)	-	21.76	22.25	16.85	26.90	31.82	
	Growth (%)	-	29.58	42.56	57.27	70.93	100.00	
Femur width	n	19	20	10	14	3	7	0.07
	Range (cm)	1.01-1.54	1.15-2.54	2.27-3.66	3.3-5.54	4.81-6.13	6.02-8.62	
	Mean ± S.E.	1.33±0.04	1.88±0.09	2.96±0.15	4.30±0.18	5.46±0.38	7.25±0.31	
	C.V. (%)	12.94	21.28	15.63	15.97	12.09	11.22	
	Growth (%)	18.34	25.93	40.83	59.31	75.31	100.00	
Tibia width	n	19	20	10	14	3	7	0.06
	Range (cm)	0.82-1.52	1.19-2.58	1.85-3.04	2.42-5.15	4.53-5.56	5.42-7.16	
	Mean ± S.E.	1.18±0.43	1.80±0.07	2.50±0.11	3.69±0.25	4.90±0.33	6.07±0.22	
	C.V. (%)	15.91	17.21	13.33	25.52	11.75	9.62	
	Growth (%)	19.44	29.65	41.19	60.79	80.72	100.00	
Metatarsus width	n	19	20	10	14	3	7	0.06
	Range (cm)	0.3-1.15	1.07-1.92	1.51-1.98	1.79-3.91	2.96-3.33	4.44-6.13	
	Mean ± S.E.	0.80±0.05	1.42±0.06	1.76±0.05	2.63±0.18	3.10±0.12	5.06±0.22	
	C.V. (%)	24.62	17.49	8.13	25.95	6.56	11.54	
	Growth (%)	15.81	28.06	34.78	51.98	61.26	100.00	

Table 3: Coefficient of correlation (r), simple linear regression equations for prediction of fetal age and coefficient of determinations (R^2) from radiographic measurements of pelvic limb bones in goat fetuses.

Variables		Coefficient of Correlation* (r) with fetal age	Prediction equations	Predictability R^2 (%)
Age of fetus (Y)		1.000	Y = a + bx ± e	--
Versus	Length of ilium (X_1)	0.999	49.9133 + 02.1844 (X_1) ± 5.52	94
	Width of ilium (X_2)	0.933	50.0897 + 11.9929 (X_2) ± 8.14	88
	Length of ischium (X_3)	0.978	56.2569 + 02.9375 (X_3) ± 4.73	96
	Width of ischium (X_4)	0.902	51.0845 + 13.1077 (X_4) ± 9.92	81
	Length of femur (X_5)	0.988	49.0318 + 01.4569 (X_5) ± 3.66	98
	Width of femur (X_6)	0.980	43.5590 + 12.4048 (X_6) ± 4.72	96
	Length of tibia (X_7)	0.991	47.9322 + 01.1400 (X_7) ± 3.21	98
	Width of tibia (X_8)	0.961	42.7622 + 14.4890 (X_8) ± 6.68	92
	Length of large metatarsus (X_9)	0.978	53.1897 + 01.6758 (X_9) ± 4.97	96
	Width of large metatarsus (X_{10})	0.956	45.9233 + 017.9031(X_{10}) ± 7.11	91

(**Note**: All the coefficients of correlation (r) are highly significant at 1 per cent 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.)

The present findings were in close agreement with the ready reckoner tables/graphs provided for pelvic limb bone measurements and fetal age through gross, radiographic and double staining studies in goat (Chaudhary, 2017; Chaudhary *et al*., 2019; Chaudhary *et al.*, 2021ab; Majeed and Ahmed, 2002; Dammaigoro *et al.*, 2020) [2, 3, 4, 5, 6, 13]; sheep (Joubert,1956; Richardson *et al.* 1976; Martin and Garcia-Gonzalez, 2015; Nissar *et al*, 2017; Sahil and Ahmed, 2021) [8, 11, 14, 22, 24]; cattle (Gjesdal, 1969; Richardson *et al.* 1990) [7, 21].

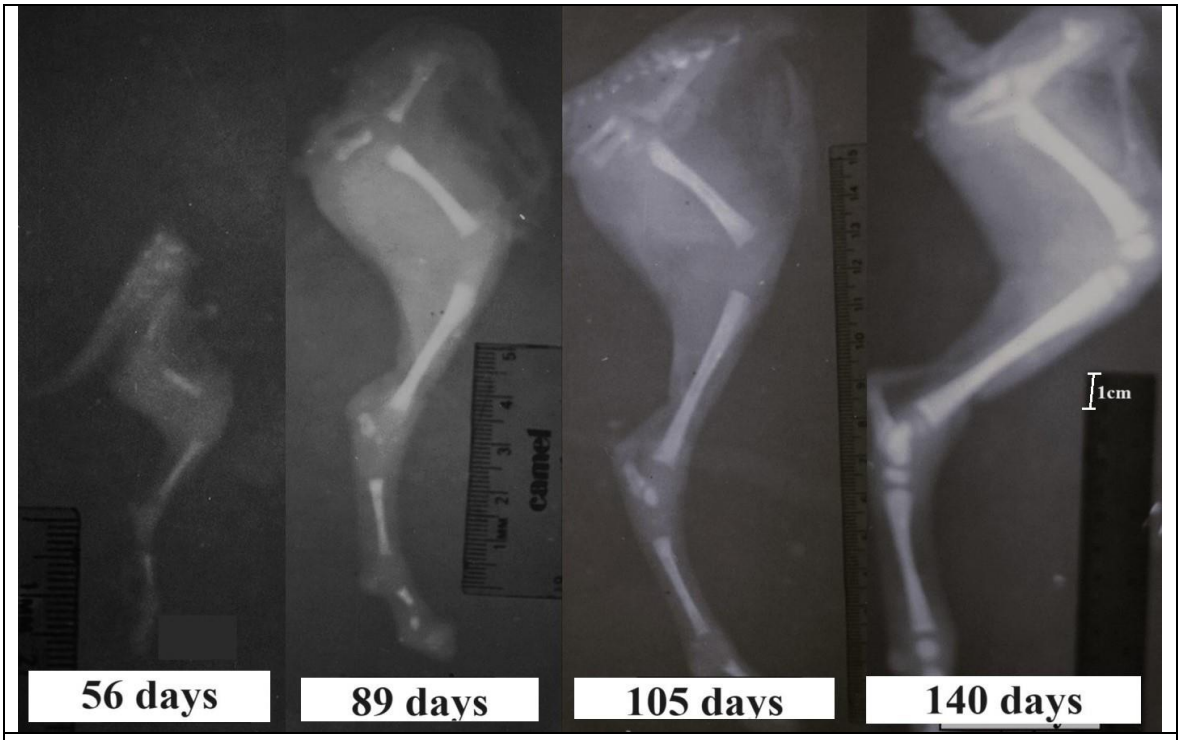

Fig 3: Radiographs of pelvic limbs (L/M view) of goat fetuses showing increasing length and width of ilium, ischium, femur, tibia and large metatarsus with advancing fetal age.

Amongst the bones of pelvic limb, the diaphyseal length of tibia showed the highest growth rate (0.89 mm/day), followed by femur (0.76 mm/day) and large metatarsus (0.62 mm/day). Whereas, the width of large metatarsus and tibia had expressed lowest growth rate (0.06 mm/day) (Table 1 and 2). The information regarding daily growth rates for the pelvic limb bones in the domestic animals was not available in the literature reviewed so far.

The correlation coefficients (r) between osteometric measurements and fetal age, simple linear regression equations for estimation of fetal age from measurements of pelvic limb bones and coefficients of determination (R^2) are given in Table 3. All correlation coefficients (r) derived between length and width of bones of pelvic limb and fetal age ($r > 0.9$) indicated very high degree of relationship (Table 3). The scatter plots with regression line for length and width of bones with age showed minimum scattering and indicated positive linear growth with advancing fetal age till full term. This suggested that bone length and width was a good indicator of age and could be utilized as a function of age. The present results were in corroboration with earlier reports on age associated correlation coefficients (r) and scatter plots of diaphyseal length of femur, tibia and large metatarsus in goat (Chaudhary, 2017; Chaudhary, 2019; Chaudhary, 2021a,b) [2, 3, 4, 5]; tibia in sheep (Richardson *et al.* 1976) [22], tibia and large metatarsus in cattle (Gjesdal, 1969; Richardson *et al.* 1990) [7, 21]. Among the simple linear regression equations formulated for prediction of fetal age, the length of

femur and tibia gave the highest (98%) predictability, followed by length of large metatarsus and ischium, and width of femur (96%). The remaining pelvic limb bone measurements expressed the predictability above 90 per cent, except the width of ilium and ischium which showed 88 and 81 per cent, respectively (Table 3). The present findings indicated that diaphyseal length of femur and tibia, amongst pelvic limb bone measurements, are reliable and best indicator for determination of age in goat fetuses. Gjesdal (1969) [7] gave prediction equation to estimate bovine fetal age from radiographic measurement of cranium. From the available literature reviewed so far, it seems that no research has previously been reported in goat fetuses on age estimation by regression equations based on radiographic osteometric data.

4. Conclusion

From the present investigation, it was concluded that there was positive linear growth in the length and width of pelvic limb bones with increasing goat fetal age. The present study revealed strong association between fetal age and growth of pelvic limb bones in length and width. The diaphyseal length of femur and tibia were found as reliable indicators (R^2 = 98 %) of age in goat fetuses. The fetal age associated radiographic osteometric data generated in the present work will provide baseline information for further breed, sex and multiples specific investigation in the goat fetuses.

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