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DETERMINATION OF GESTATIONAL AGE OF WEST AFRICAN DWARF GOATS USING SONOGRAPHIC BIOMETRY

Kenneth O. O. Anya*[1], Samuel O. Ekere[1], Leo O. Obasi[1] and Dave Ogwu[2]

[1]Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka, Enugu State, Nigeria and [2]Department of Theriogenology and Animal Production, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
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ABSTRACT

The aim of this study was to determine gestational age by utilizing sonographic measurements of crown-rump length, head length and biparietal diameter of foetuses of West African Dwarf goats. Intra-partum measurement of foetuses of 15 West African Dwarf does at known gestational ages was undertaken using trans-abdominal ultrasonography. The relationship between gestational age and measured foetal parameters was plotted as linear regressions and expressed as straight-line equations. Regression equations were established where the gestational age was the dependent variable while each of the foetal parameters was considered as the independent variables. The measurements were taken every other day starting from Day 23 to Day 59 for crown-rump length and, from Day 35 to Day 103 for head length and biparietal diameter. The results of correlation coefficients between the measured parameters and gestational age were R^2 = 0.980, 0.988 and 0.966 respectively for crown-rump length, head length and biparietal diameter. These results clearly demonstrated that foetal crown-rump length, head length and biparietal diameter were highly and positively correlated with gestational age ($p < 0.01$; R^2 = 0.980, 0.988 and 0.966, respectively) with foetal head length showing the highest correlation with gestational age. The regression (gestational) equations for prediction of gestational age of West African Dwarf goats based on these parameters were: y (days) = 0.4273crown-rump length (mm) +22.52, y (days) = 1.0221head length (mm) +22.21 and y (days) = 2.0746 biparietl diameter (mm) +20.691. These regression equations may be used to predict the gestational age of West African Dwarf goats of unknown dates of breeding as well as monitor the growth rate of foetuses with known breeding dates.

Keywords: Sonographic biometry, Gestational age, West African Dwarf Goats
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***Correspondence: E-mail:** amaefuleremy@gmail.com; **Tel:** *+2348032259467*

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INTRODUCTION

Gestational age estimation finds application in reproductive management, clinical theriogenology practice and research [1]. In Veterinary Obstetrics the computation of gestational age is predicated on knowledge of the actual date of breeding (Day 0 of gestation) [2]. In sheep and goats natural service dates are generally unobserved or unrecorded, making fertile mating difficult to determine [3,4] because of the practice of mixing the sexes together. When the date of breeding is known, gestational age estimation would be useful to monitor foetal growth but when the date of breeding is unknown, monitoring foetal development would allow for estimation of gestational age. This, in turn, would be useful for appropriate pre-natal management. [5,6]. Undetermined gestational age has been associated with adverse pregnancy outcomes such as low birth weight and peri-natal mortality [7].

Pregnancies incorrectly classified as either pre-term or post-term often result to unnecessary foetal monitoring and unwarranted interventions. This may occur in the form of induction for supposed post-term pregnancies leading to increased risk of maternal and neonatal morbidity and mortality, or in the case of pregnancies erroneously thought to be pre-term, subjection to avoidable and expensive hospitalization as well as excessive and potentially dangerous medication including tocolytic therapy [7].

In Nigeria, surgical methods such as laparo-hysterotomy, measurement of foetuses at known periods of gestation [8,9] and measurement of slaughterhouse foetuses [10,11] have been used to determine gestational age of indigenous species. These studies indicate that the entire body increases steadily in size although the rate of increase varies from one measurement to another. These studies in the indigenous breeds in which slaughterhouse specimens were measured [10,11], applied regression equations derived from exotic breeds, which may be inappropriate as there are breed variations in specific geometric parameters of foetuses. Among the limitations of the surgical method at known stages of gestation is its invasiveness.

Ultrasound has a wide range of application in clinical reproductive appraisal including the estimation of the stage of pregnancy [12]. Transrectal or transabdominal B-mode real-time ultrasonography has been used to predict gestational age by means of foetal measurements in small ruminants[13,12,,14] and has been shown to be an objective and accurate means of estimating gestational age [15]. Although reports on transrectal and transcutaneous ultrasonographic biometry in sheep and goats of several breeds abound in literature, there is scanty information on the West African Dwarf (WAD) goat. Regression equations of ultrasound-derived data for the estimation of gestational age of foetuses of several animal species also abound in literature but little or no information exists for the WAD goat. Haibel *et al*. [16] observed marked variations in foetal biometry among goat breeds, especially from the second trimester of gestation. This observation necessitated the development of a breed specific prediction chart [14]. There is therefore the need to generate regression equations of ultrasound-derived data for the determination of gestational age of WAD goats with known dates of breeding. Such data will also be used to monitor foetal growth in WAD goats with known dates of breeding as well as estimate gestational age of WAD goats of unknown breeding dates.

MATERIALS AND METHODS

Pre-Experiment Animal Management

Fifteen cyclic female WAD goats of varying parities and a buck belonging to the Small Ruminant Experimental Animal Unit of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka were used for the study. The goats were routinely checked for both endo- and ecto-parasites and treated as appropriate. They were grazed on local pastures and forages during the day and given cassava and yam peels, bambara nut chaff and waste kitchen green leafy vegetables at night. Clean fresh water was provided *ad libitum*. The does were housed in small units of threes and fours in a pen secluded from the buck.

Estrous Synchronization and Breeding

Oestrus cycle was synchronized using cloproject® (0.025% cloprostenol: a synthetic analogue of Prostaglandin $F_2\alpha$). A double-injection protocol, 11 days apart, of 250 µg/doe of cloproject® [17] was used. Following the second cloproject® injection (SCI), the does were housed in-doors, secluded from the buck and checked for signs of oestrous. Oestrus detection was done using the intact virile buck four times a day at 06.00 hr., 10.00 hr., 14.00 hr and 18.00 hr, for 20 minutes each. Any doe that showed heat by standing to be mounted was allowed to be bred and the date of breeding recorded. One breeding only was allowed per doe so that the date of breeding was considered as Day 0 of gestation [2].

Pregnancy Diagnosis

Trans-abdominal ultrasound scanning was performed every day starting from Day 15 post-breeding in all the female WAD does until pregnancy was confirmed. A doe was diagnosed pregnant when multiple fluid-filled uterine lumen, fluid- filled embryonic vesicle, whole embryo/foetus, a foetal part, foetal membrane or placentome was recognized sonographically [18,19,20,6, 21].

Sonographic Biometry

Following pregnancy detection, ultrasound foetal biometry was performed on alternate days starting from Day 23 (Date of breeding = Day 0 of gestation [2]) [25] until Day 103 of gestation. During a scanning session, 3 to 5 measurements of the foetal parameter of interest were taken and the computed mean was recorded as the dimension of the parameter for the given gestational age. A table of the measured foetal parameters against gestational age was drawn. Linear graphs (Figs. 1, 2 and 3) of Mean ± standard deviations (SD) of each foetal parameter in millimetres against gestational age in days were plotted using SPSS version 15 while the coefficients of correlation and determination (R^2) between gestational age and embryonal or foetal measurements were established by standard regression with computer program EXCEL, 2003 package data analysis. The effectiveness of the regression equations was determined by values of R^2 and the significance of the regression by the p-values.

A commercially available ultrasound scanner, SA600V® (Medison Co, Seoul, Korea) equipped with 5.0 – 8.0 MHz convex transducer was used for pregnancy diagnosis and measurement of foetal crown-rump length (CRL), head length (HL) and biparietal diameter (BPD) using in-built electronic callipers of the scanner.

Fetal Crown-Rump Length Measurement

The CRL represented the greatest length of the embryonal or foetal mass before foetal parts of the body differentiated and are discernible [14]. Following differentiation of foetal body parts, the CRL was measured from the top of the skull to the end of the sacrum [22]. The measurements were taken from the most upper part of the skull to the end of the sacrum when the foetus was fully extended. When the foetus adopted a curved posture, the measurements were taken in phases) [23], depending on the posture adopted by the foetus.

Fetal Head Length Measurement

When the head (Occipito-snout length) [24] was fully extended, head length was measured from the snout to the end of the *os occipitale* [25].

Fetal Head Diameter or Biparietal Diameter (BPD) Measurement

The BPD represents the maximum diameter of the head measured by electronic callipers [26,14]. For accurate measurement to be taken, image symmetry is essential. A symmetrical image for accurate measurement is one in which both orbits are clearly seen on the foetal skull, following which the image is frozen on the screen [6]. The criteria for measuring the BPD were that the image should have an oval shape as much as possible with closed contour of skull and the *Falx cerebri* located mid-line dividing the hemispheres into two equidistant parts and measurements were taken from the outer surface of the proximal calvarium to the inner surface of the distal calvarium [27].

RESULTS

A table each, of results of measurements of the three foetal parameters (CRL, HL and BPD) against gestational age, were made from which straight-line graphs were constructed by plotting each of the measured foetal parameters (independent variables) against gestational age (dependent variable) (Figs. 1, 2 and 3). The *p* – values and coefficient values of each parameter and the regression equations for prediction of gestational age of WAD goats based on the parameters measured. As shown in Table 1, the equations for the estimation of gestational age: y (days) = 0.4273CRL (mm) +22.52; y (days) = 1.0221HL (mm) +22.21 and y (days) = 2.0746BPD (mm) +20.691 were highly and significantly correlated with gestational age (p< 0.01; R^2 = 0.980, 0.988 and 0.966, respectively), with HL having the highest correlation (R^2 = 0.988).

Table 1. Regression Equations for the Estimation of Gestational Age and Correlation coefficients between Foetal parameters and Gestational age in WAD goats.

Measurements	Equations	Coefficient value (R^2)
Crown-rump length (CRL)	$y = 0.4273x + 22.52$	0.980 (p<0.01)
Head length (HL)	$y = 1.0221x + 22.21$	0.988 (p<0.01)
Biparietal diameter (BPD)	$y = 2.0746x + 20.691$	0.983 (p<0.01)

Fig. 1 Straight line graph of measurement of foetal crown – rump length (CRL) against gestational age in WAD goats.

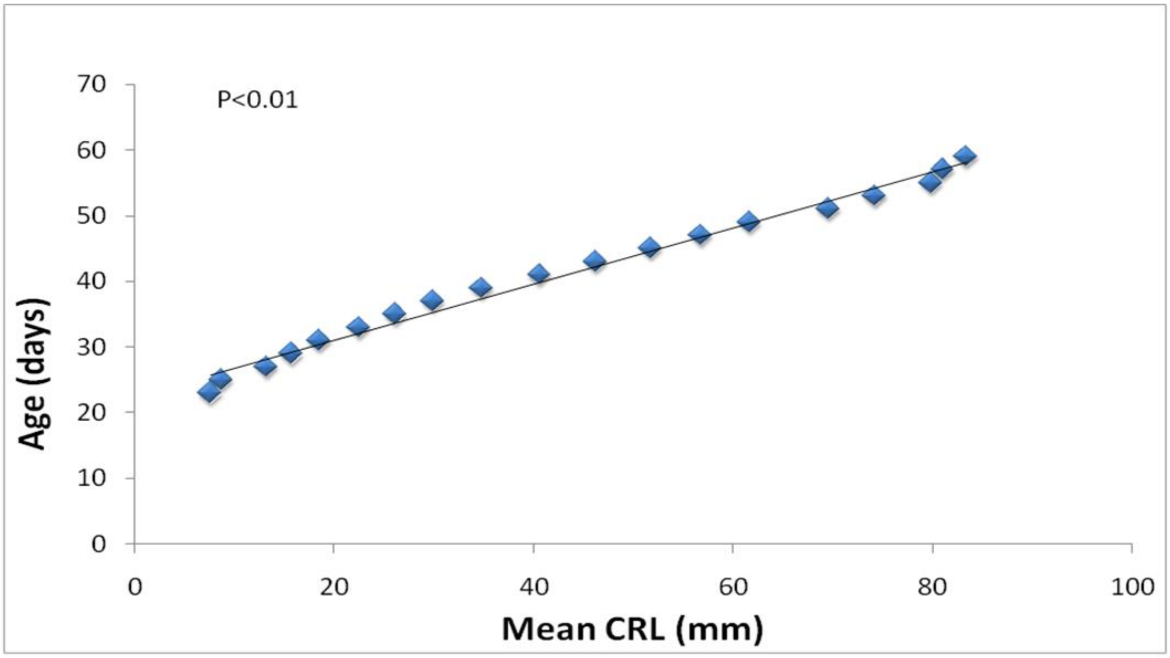


Fig. 2 Straight line graph of measuremet of foetal head length (HL) against gestational age in WAD goats

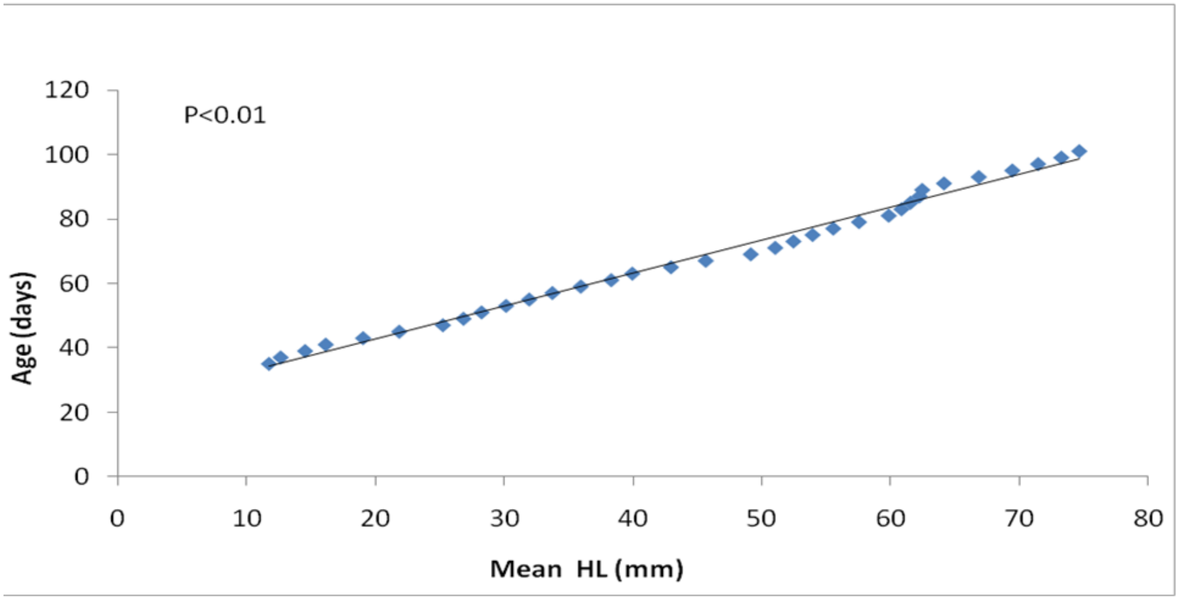
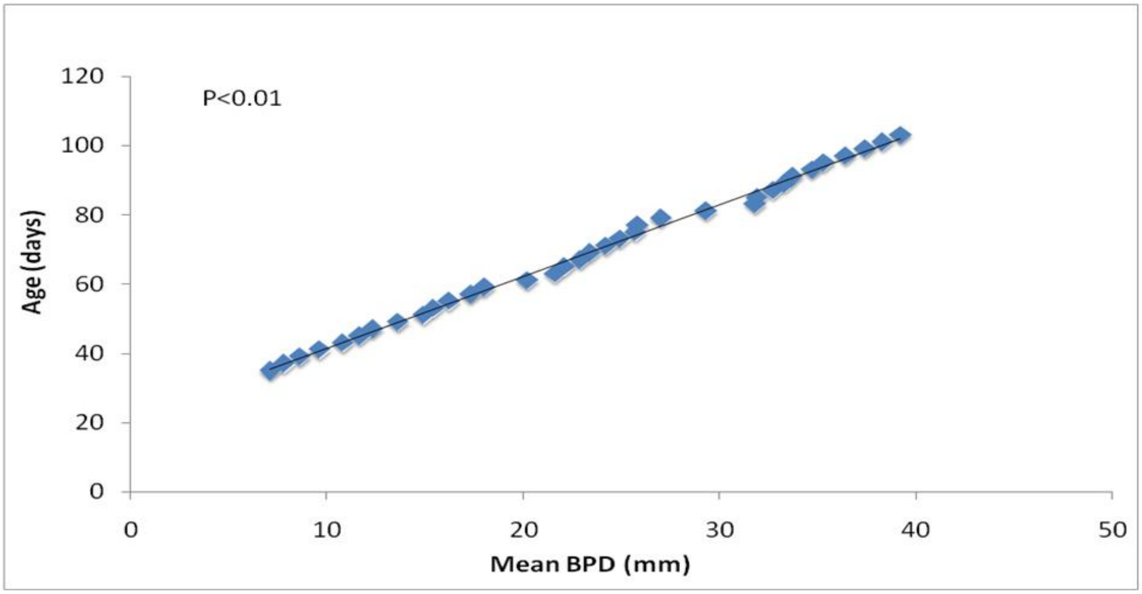


Fig. 3 Straight line graph of measurement of foetal biparietal diameter (BPD) against gestational age in WAD goats.



DISCUSSION

Repeated sonographic exposure of the pregnant does did not appear to adversely affect their pregnancies as the kids were born at the normal average gestation length of 144.4 ± 0.12 days with no apparent morphological defects. Crown rump length (CRL) measurements have been used to predict gestational age in different species [14,21]. In this study CRL was measurable in the first trimester and up to day 59 of pregnancy as with the advancement of pregnancy the foetus became too big to be wholly presented on the screen. Measurement of CRL was easily done from Day 23 to Day 59 of gestation. The CRL could not be measured beyond Day 59 of gestation, whereas the length of the foetus, 83mm, exceeded the viewing field of the screen. In the work of Karen *et al.* [14], CRL could be measured between gestational age Days 25 and 70 while Martinez *et al.* [28] measured CRL between Days 19 and 40 of gestation. The differences in the gestational ages of measurement of CRL among these studies could be due to the different ultrasound scanners used. It has been shown that sonographic measurement of CRL is limited by the viewing field and the penetration depth of the ultrasound scanner [29]. In the present study foetal biometry by CRL measurement correlated well with gestational age ($p< 0.01$; $R^2 = 0.98$). Similar high correlation ($p< 0.0001$; $R^2 = 0.94$) was reported between Days 25 and 70 in the Egyptian native goats [14] and between 19 and 40 days in Anglo-Nubian goats [28]. A slightly lower correlation ($R^2 = 0.90$) was reported in Saanen goats between the 5thand 10th week of gestation [23]. The higher correlation ($R^2 = 0.98$) between CRL and gestational age in the present work compared to the works of Abdelghafar *et al.* [23] ($R^2 = 0.90$) and ($R^2 = 0.94$) in the works of Karen *et al.*[14] and Martinez *et al.*[28] may be due to decreased intervals of successive ultrasound scanning, every other day compared to 7 days [23] and 3-5 days [28,14]. Breed differences could also be a factor responsible for some of the variations in correlation coefficients among these studies [16].

In this study, HL measured from Day 35 to 103 of gestation, accross all three trimesters of pregnancy. Head diameters provide a good index of foetal development because they show high correlations with gestational age, enabling long periods of observation [21]. The skull usually remains in a good position for observation. In small ruminants, foetal biometry by HL (occipito-nasal or occipito-snout distance) measurements have been undertaken in a few studies in available literature [25] and was shown to positively correlate with gestational age. In this study foetal head length (HL) or occipito-snout length was highly and positively correlated ($p< 0.01$; $R^2 = 0.988$) with gestational age between Days 35 and 103 of gestation. Similarly in the sheep Metodiev *et al.* [25] reported a highly positive correlation between HL and gestational age ($p< 0.001$; $R^2 = 0.85$). Similar results were reported by Haibel and Perkins [30] between Days 43 and 96 and also by Sergeev *et al*. [31] between Days 38 and 91 of gestation in ewes.

The BPD is a widely used parameter in aging human pregnancies. Reports of ultrasound-derived BPD in aging abound in available literature in various breeds of goats [16,32,6]; sheep [1,16,13] and red deer [33,34]. This is because the skull usually remains in a good position for observation and it is easy to measure BPD with a better placing of callipers on the hyperechogenic limits of the bones bordering the soft tissues [19,35]. In this study, foetal BPD was easily measured between Days 35 and 103 of gestation and was shown to be highly significantly and positively correlated ($p< 0.01$; $R^2 = 0.966$) with gestational age. A Similar high correlation was reported between BPD and gestational age in dairy goats ($R^2 = 0.9811$) [24]. In the sheep, Haibel *et al.* [25] reported that the threshold of accurate ultrasonic foetal head measurement is approximately 40 days and examinations at earlier stages of gestation may affect the clear identification of a symmetrical foetal head image. The same authors reported high correlation ($R^2 = 99.49$, 98.75 and 97.75 respectively) of BPD with gestational age between days 40 and 100 of gestation in purebred Toggenburg, purebred Nubian and Angora goats respectively. In this study of the WAD goats, however, accurate measurements could be done as from day 35 of gestation. This earlier commencement of measurement of BPD is in agreement with other works. In Egyptian native goats, Karen *et al.* [14] measured the BPD between Days 25 and 130 of gestation and reported a highly significant correlation ($p \leq 0.0001$; $R^2 = 0.956$) with gestational age. Similar results were reported by Haibel [24] in dairy goats between Days 39 and 100 and by Reichle and Haibel [32] in pygmy goats (small sized breed) between Days 36 and 102 of gestation. Santiago-Moremo *et al.* [37] measured the BPD from Day 25 onwards in a mouflon (*Ovis gmelini musimon*) and found a strong correlation with gestational age. Reichle and Haibel [32] reported that BPD was highly correlated with gestational age when measured at 2 to 3 days intervals from 36 days to 102 days in pygmy goats (R^2=0.9727). Slightly lower correlation ($R^2 = 0.91$) was reported in Saanen goats from the 6th week to end of gestation [22] and in the work of Lee *et al.* [38] in Korean black goats between 60 and 135 days of gestation, the BPD had an even lower coefficient (R^2=0.8089) of correlation with gestational age. The lower coefficient value of determination of gestational age in these latter works compared to the present study could be due to the longer intervals of ultrasound examinations (7days, 15 days and 2 days, respectively) and also because measurements extended to very near term in these other studies. It has been reported that it is increasingly difficult to measure BPD in the late stages of pregnancy [39] because at the end of the second trimester the foetal head is usually in a position which defies easy measurement of BPD [25,36]. Similar difficulties in measuring the BPD during the late stages of pregnancy were found in Ilama [40] and ungulate species [39]). Breed differences could also be a factor in accounting for the observed differences in correlation coefficients between BPD and gestational age in these various studies.

Biometry or foetal age study is a part of obstetrical ultrasound examination. It utilizes ultrasonography to take measurements of foetal anatomic structures and compare the results to expected values. Biometry is used to determine foetal age while serial biometry may be performed to assess foetal growth rate. In this study, foetal CRL, HL and BPD were measured from the same conceptuses across different developmental stages at known gestational ages. Data generated were used to construct a normogram of the relationship between CRL, HL and BPD with gestational age in WAD goats. Straight line graphs were plotted between each of the measured foetal parameters and gestational age. Regression equations were derived from the plotted data. The regression equations derived from the plotted biometric data predict gestational age of WAD goats with no records of dates of breeding by fitting measured values of foetal parameters into the derived regression equations [6]. The gestational (regression) equations generated from ultrasound foetal biometric data, y (days) = 0.4273CRL (mm) +22.52, y (days) = 1.0221HL (mm) +22.21 and y (days) = 2.0746BPD (mm) +20.691 were highly significantly correlated with gestational age ($p< 0.01$; $R^2 = 0.980$, 0.988 and 0.966, respectively). As shown, the equations had very high coefficients of determination. This is in agreement with the study of Gonzalez-Bulnes *et al.* [24] who stated that these parameters have a high correlation with foetal age. In this study, the gestational age (y days) can be predicted when the BPD (*x* mm) is given by the following equation: y=2.0746X+20.691. This equation is different from those reported for other goat breeds [14, 27, 32, 34]. Therefore, this result is in agreement with Haibel [4] who recommended a breed- specific predicting chart for each breed of goat. The equations for the prediction of gestational age using CRL and HL measurements being reported for WAD goats in this study are similarly different from those derived for other breeds of goats. In conclusion, biometry by real-time ultrasound measurement of foetal CRL, HL and BPD in this study was shown to be a useful tool for the determination of the duration of pregnancy in WAD goats. The gestational equations generated in this study can thus be used with confidence to estimate the gestational age of WAD goats of unknown date of natural mating or artificial insemination or to monitor the growth of foetuses of goats with known dates of breeding during the period Day 23 to 103 of gestation.

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