

## Estimation of gestational age using ultrasonography in Baluchi sheep

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### Abstract

The aim of this study was to evaluate the correlation between some fetal parameters measured by ultrasonography and fetal age for developing a formula which could be used for estimation of fetal age in Baluchi sheep. Placentome diameter (PD), gestation Sac diameter (GSD), biparietal diameter (BPD), straight crown-rump length (CRL), femoral length (FL), occipito-snout diameter (OSD), thoracic diameter (TD) and fetal heart rate (FHR) were measured by transrectal and/or transabdominal ultrasonography. All ewes were synchronized using progesterone sponge for 6 days. On the first day, they received PGF2 and on the last day received 400 IU of equine serum gonadotrophin (eCG). All of the parameters revealed significant correlation by increasing fetal age. Except for PD, for other values linear regression curve was illustrated. BPD (between 36 - 96 days) (R2 = 0.961), CRL (R2 = 0.935), FL (R2 = 0.950), OSD (R2 = 0.981), TD (R2 = 0.975) showed high correlation with fetal age ( $p < 0.001$ ). Moderate correlation was calculated for FHR ( $p < 0.001$ , R2 = 0.883). Low correlations were assessed by measurement of GSD ( $p = 0.018$ , R2 = 0.318), BPD (between 96-138 days;  $p = 0.038$ , R2 = 0.29) and PD represented the significant non-linear correlation with age ( $p < 0.001$ ), maximum correlation was assessed by measurement of mean placentome wall diameter. In conclusion, OSD was recommended as the best parameter for estimating of gestational age between days 36 - 109 in Baluchi sheep because of the lowest residuals, the highest correlation coefficient and wide period of availability for imaging in gestation.

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### Introduction

Knowing the gestational age would be useful to determine appropriate gestational age for caesarean section and induction of parturition<sup>1</sup> and to plan an appropriate vaccination schedule for protection of pregnant ewes and their offspring especially against life-threatening diseases causing neonatal mortality.<sup>2</sup> Furthermore, essential information such as number of fetuses, culling of non-pregnant females can be provided for effective herd management by early pregnancy detection.<sup>1</sup>

Many methods have been described to diagnose pregnancy in sheep and goat<sup>3</sup>, among them B-mode ultrasonography is a simple, reliable and non-invasive imaging technique with safety for operator and patients. Determination of pregnancy status including viability, number, age and sex of the fetus can be considered by ultrasonography in farm animals.<sup>3,4</sup> It is commonly introduced in small ruminant to improve management of

pregnant ewes and monitor the fetuses during pregnancy.<sup>3</sup> The first record of using ultrasonography for gestational evaluation in sheep was in the late 1960's.<sup>5,6</sup> In 1971, the technique was validated for gestational evaluation and determination of the fetal viability.<sup>7</sup>

Estimation of fetal age by measuring the fetal parameters in sheep using ultrasonography has been discussed in previous researches.<sup>1,8-26</sup> Furthermore, due to variations in breed and nutrition among different management systems, it is important for each breed to be assessed for the information to be relevant and applicable to the target population of sheep and goats.<sup>24</sup> Equitation prediction of parameters measured by ultrasonography is introduced for different breeds of sheep.<sup>8,9,12-14,19,20,24,26</sup>

Baluchi sheep is one the most numerous breeds in Iran and plays an important role in meat, and wool production.<sup>27</sup> No study was reported on the ultrasonographic fetometry in this breed, therefore, the objective of this work was to perform some fetal measurements to estimate the

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gestation age using transrectal ultrasonography between days 15-70 and transabdominal ultrasonography between days 70-140 in this breed.

## Materials and methods

**Animals.** In breeding season twelve pregnant Baluchi sheep with history of at least one parity, aged 1.50 - 3.50 years old and weighting 43.00 - 59.00 Kg were recruited in this study. All animals were housed under standard condition, fed on pasture, alfalfa and standard ewe concentrate (containing barley, soybean and corn) three times a day and access to clean water was *ad libitum*.

**Experimental design.** All methods were in compliance with the guide for the care and use of agricultural animals in agricultural research and teaching in Ferdowsi University of Mashhad, which has been critically evaluated and approved on 6th Jan 2015. Nineteen ewes were synchronized with intramuscular injection of a synthetic analogue of prostaglandin F2 $\alpha$  15.00 mg per ewe (Vetalyse, Aburaihan, Iran) followed by administration of intravaginal progesterone sponge (Hipra, Girona, Spain) for 6 days. After removal of sponges, eCG (400 IU per ewe; Hipra) was injected intramuscularly. Forty-eight hours later two rams were introduced to ewes for 4 days and serum progesterone level was measured every day from two days before for 6 days. Twelve pregnant ewes were kept in private area separated from herd in early to mid-pregnancy and in herd at late pregnancy. The day represented decreasing the serum progesterone level below of 1.00 ng mL<sup>-1</sup> was considered as day 0 of pregnancy. All animals were subjected to ultrasonography examination from day 17 on a 3 - 5 days interval until day 68 and continued on days 68, 82, 96, 110 and 138 using real time B-mode scanner (DP 6600-vet; Mindray, Shenzhen, China) equipped with a transrectal linear probe, frequency 7.50 MHz and a transabdominal convex probe, frequency 3.50 MHz. The embryonic vesicle observation was considered as definitive diagnosis of pregnancy using transrectal examination. Ewes were investigated until day 70 transrectally and thereafter it was continued transabdominally. Animal preparation included clipping and shaving the ventral aspect of abdomen for transabdominal examination and the ewes were kept off-feed 15 hr before ultrasonography. Standing position and sometimes dorsal recumbency on special designed table was considered during examination. For transrectal ultrasonography, well-lubricated transducer with stiffened handle gently moved into rectum. In transabdominal ultrasonography, sufficient amount of gel was applied on clipped area and ultrasonography was carried out.

Measurement of the maternal heart rate was performed before ultrasonography by stethoscope in non-stressed

condition from day 48 - 50 to the end of the study. The following parameters were measured on fixed images by in-built caliper as an indicator for estimation of gestational age. For increasing the accuracy, all fetometric measurements were performed at least three times by one skilled operator and the mean values of the data were set for statistical analysis.

a) Embryonic vesicle or gestational sac diameter (GSD): Internal diameter of the GS was measured on the early stage when was imaged as a circular structure on days 17 to 21 (Fig. 1A).

b) Biparietal diameter (BPD): It is was the maximum diameter of fetal skull on the transverse plane when flax cereberi was imaged centrally (Fig. 1B).

c) Crown-rump length (CRL): It was taken as the greatest straight length of the fetal mass between occipital bone and rump. The CRL was measured from days 19-55 transrectally and continued until days 81-83 transabdominally (Fig. 1C).

d) Placental diameter (PD): When it could be imaged as a C-shaped in cross section or circular structure in longitudinal plane, mean and maximum diameters of placentome and its wall were measured from days 18-20 to the end of pregnancy (the Last scan on day 138). For accurate judgment measurement was done on at least 5 big placentome (Fig. 1D).

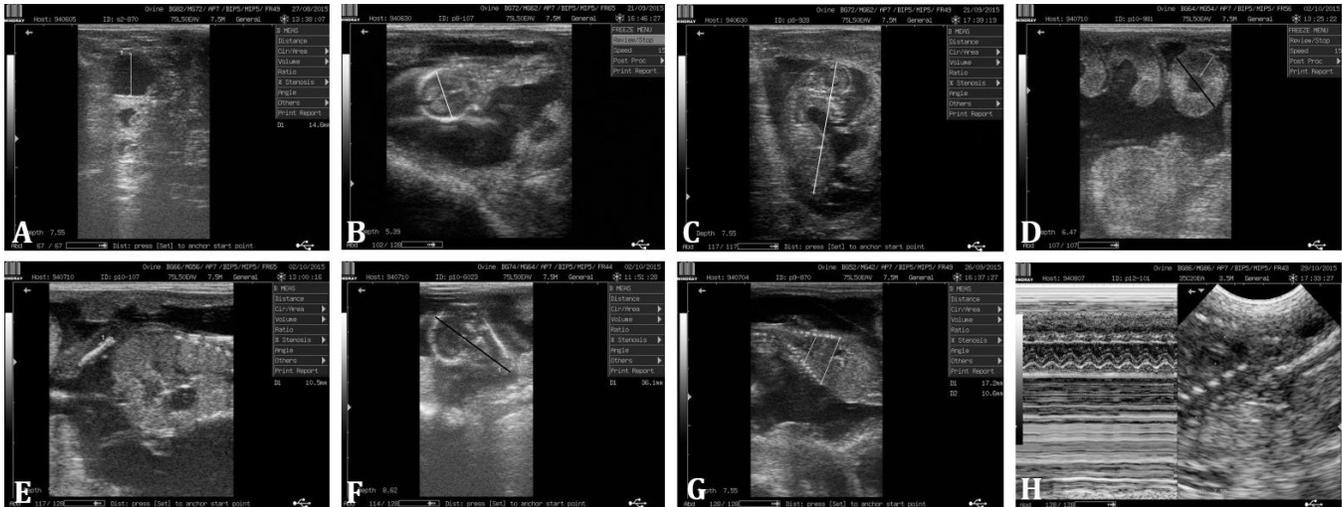
e) Femoral length (FL): Maximum length of the femoral bone was measured as echoic structure on longitudinal image on days 49-109. Fetal pelvic and urinary bladder were considered as a landmark for imaging of femoral bone (Fig. 1E).

f) Occipito-snout diameter (OSD): It was the maximum length between rostral aspect of the nose and occipital bone on true dorsal plane on days 36-109 (Fig. 1F).

g) Thoracic diameter (TD): After imaging the dorsal plane of the thoracic cavity, maximum internal diameter of the thoracic cavity was measured on central part and last rib junction level on days 36-138 and 36-109, respectively (Fig. 1G).

h) Fetal heart rate (FHR): Using M-mode technique, FHR was measured on days 48-50 to end of pregnancy (the last scan on day 138), (Fig. 1H).

**Statistical analysis.** The correlations of gestational age and fetometric parameters were determined by linear regression. The relationship between placental diameter and fetal age as well as fetal heart rate and maternal heart rate were analyzed by Spearman Correlation Test. The SPSS Software (version 21.0; IBM Corp., Armonk, USA) was applied and  $p < 0.05$  was considered significant with 95.00% confidence interval. For data expressed as linear regression, equitation for estimation of gestational age was set. Mean and maximum difference between observed values of the dependent variables and predicted value so-called residuals were calculated for each parameter.



**Fig. 1.** Ultrasound images representing fetal biometry parameters, **A)** Gestation sac diameter (GSD), **B)** Biparietal diameter (BPD), **C)** Crown-rump length (CRL). **D)** Placental diameter (PD): Placental diameter (black line). Placental wall diameter (white line). **E)** Femoral length (FL). **F)** Occipito-snout diameter (OSD). **G)** Thoracic diameter (TD): The last rib (line 1), central (line 2) **H)** Fetal heart rate (FHR).

**Results**

Nineteen synchronized estrus ewes were mating naturally and 12 pregnant ewes were selected for this study. Observing the embryonic vesicle was considered as a definitive diagnosis of pregnancy using transrectal ultrasonography. Table 1 illustrates equation prediction of gestational age, *p* value, correlation coefficient (R2) and residuals (mean and max) data in parameters with significant linear regression. Linear regression model for relationship of the ultrasonography fetometry measurements and gestation age shown in Figure 2.

**Gestational sac diameter.** Growth of GS showed an increase with the progress of gestational age significantly (*p* = 0.018). It was detected on days 17 to 21 and linear regression pattern with equation  $y = 14.665 + (0.2707 \times GSD)$  with low correlation coefficient (R2 = 0.318) was calculated (Fig. 2A).

**Biparietal diameter.** Recorded BPD showed a linear regression with gestation age. Two different regression lines were analyzed on days 36 - 96 and 96 - 138. A gestation equation for BPD was described as  $y = 18.37 + (1.90 \times BPD)$  with high value of the

determined coefficient (R2 = 0.961) on days 36-96 (Fig. 2B) and  $y = 24.19 + (2.05 \times BPD)$  with a low R2 = 0.290 on days 96 - 138.

**Straight crown-rump length.** On days 21-55, development of CRL was significantly progressed with increase in fetal age (*p* = 0.01) and followed linear regression equation  $y = 21.537 + (0.3722 \times CRL)$  with correlation factor 0.935. Linear regression plot model is shown on Fig. 2C.

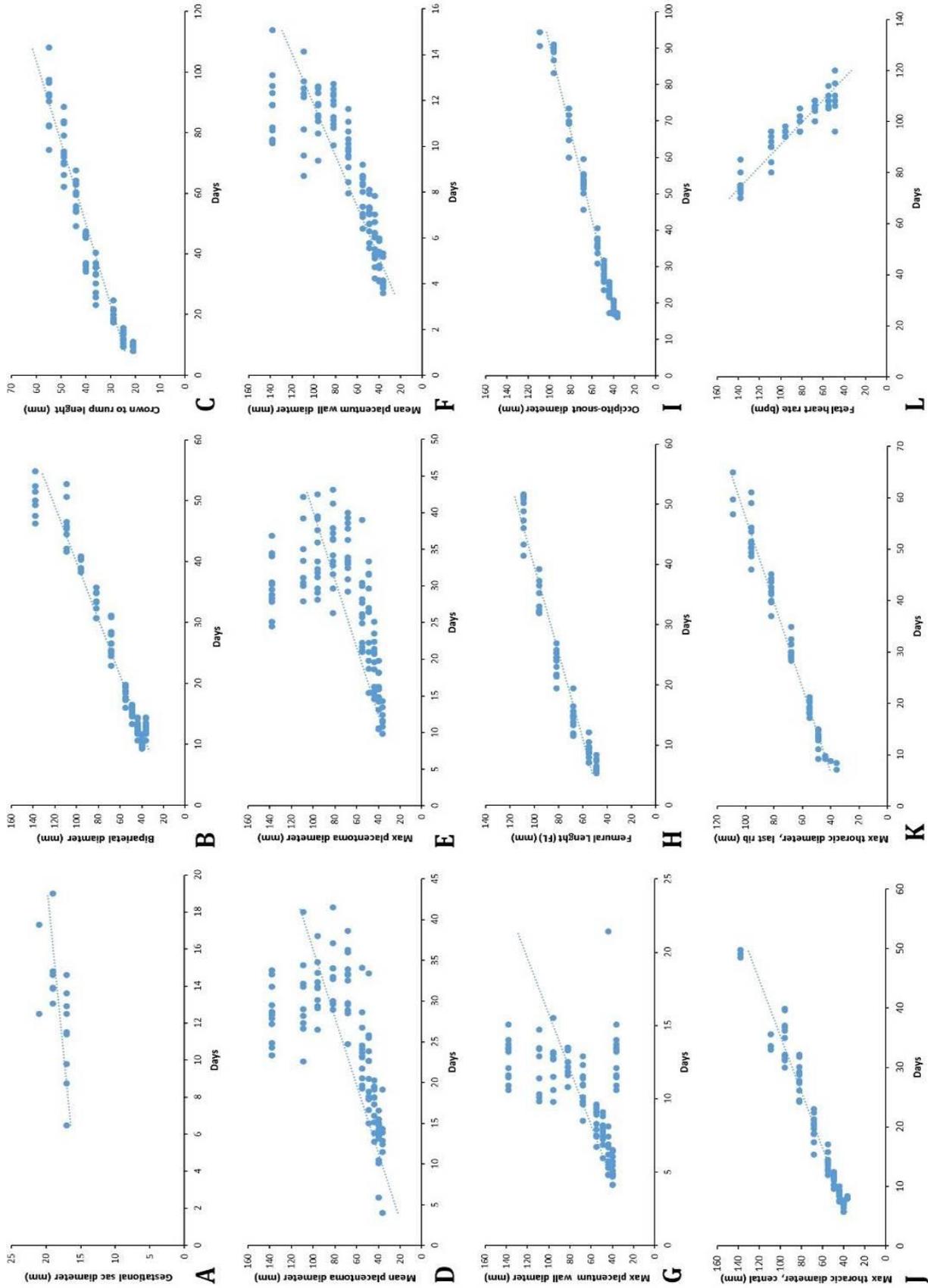
**Placental diameter.** It could be imaged on days 36 to end of pregnancy (the last scan on day 138). Placental development showed significant correlation with gestational age without linear pattern. Mean and maximum placental diameters (*p* < 0.001, R2 = 0.75 and *p* < 0.001, R2 = 0.73, respectively) (Figs. 2D and 2E) and diameter of placental wall (*p* < 0.001, R2 = 0.91 and *p* < 0.001, R2 = 0.51, respectively) (Fig 2F, G) were significantly increased by progressing of fetal age.

**Femoral length.** Relationship between FL and gestation age (Fig 2H) represented linear regression (*p* = 0.001) with high correlation coefficient (R2 = 0.950) on days 49-109. A gestational equation for FL was considered as  $y = 45.072 + (1.387 \times FL)$ .

**Table 1.** Relationship between ultrasonographic fetal measurements and gestational age of Baluchi sheep.

Fetometric parameter	Regression equation	Correlation Coefficient (R <sup>2</sup> )	Residuals (day)		Examination Period (day)	p-value
			Mean	Maximum		
Gestational sac diameter	Y = 14.66 + (0.27 × X)	0.318	0.88	2.95	17 - 21	0.018
Biparietal diameter(day 36 - 96)	Y = 18.37 + (1.90 × X)	0.961	2.84	9.70	36 - 96	0.001
Biparietal diameter(day 96 - 138)	Y = 24.19 + (2.05 × X)	0.290	10.25	23.00	96 - 138	0.038
Crown-rump length	Y = 21.53 + (0.37 × X)	0.935	2.22	6.00	21 - 55	0.001
Femoral length	Y = 45.07 + (1.38 × X)	0.950	4.11	10.00	49 - 109	0.001
Occipito-snout diameter	Y = 24.79 + (0.82 × X)	0.981	1.94	9.30	36 - 109	0.001
Thoracic diameter (central level)	Y = 24.93 + (2.12 × X)	0.958	3.65	13.80	36 - 109	0.001
Thoracic diameter (junction of last rib)	Y = 31.96 + (1.21 × X)	0.975	2.32	9.80	36 - 138	0.001
Fetal heart rate	Y = 310.04 + (-2.30 × X)	0.883	7.87	380	49 - 138	0.001

X = Fetal parameter (mm), Y = Gestational age (day).



**Fig. 2.** Linear regression model and relationship of the ultrasound fetometric measurements (mm) and gestational age. **A)** Gestational sac diameter (GSD). **B)** Biparietal diameter (BPD). **C)** Crown-rump length (CRL). **D and E)** Placental diameter (mean and maximum). **F and G)** Placental wall diameter (mean and maximum). **H)** Femoral length (FL). **I)** Occipito-snout diameter (OSD). **J)** Thoracic diameter, central. **K)** Thoracic diameter, the last rib. **L)** Fetal heart rate (FHR).

**Occipito-snout diameter.** Significant correlation was observed between OSD and fetal age on days 36 to 109 with linear regression pattern. High value of correlation coefficient ( $R^2 = 0.981$ ) of the regression equation  $y = 24.79 + (0.8271 \times OSD)$  was measured (Fig 2I).

**Thoracic diameter.** It was measured on two levels: Central (TDC) and the last rib junction (TDR) on days 36-109 and 36-138, respectively. Positive significant correlation with gestational age was observed between fetal age and TDC ( $p = 0.001$ ,  $R^2 = 0.958$ ) and TDR ( $p = 0.001$ ,  $R^2 = 0.975$ ). The derived growth equation was  $y = 24.933 + (2.1279 \times TDC)$  and  $y = 31.961 + (1.2103 \times TDR)$ , respectively (Figs. 2J and 2K).

**Fetal heart rate.** Negative significant correlation between HR and fetal age was observed ( $p = 0.001$ ) with  $R^2 = 0.883$  on days 48-50 to the end of pregnancy. Regression line was equated with  $y = 310.04 + (-2.305 \times FHR)$ , (Fig 2L).

## Discussion

Pregnancy diagnosis and estimation of gestational age (GA) in sheep are necessary for reproduction management and maintenance of high level of efficacy.<sup>15</sup> Several parameters were measured in previous studies for estimation of gestation age and in the present study we measured GSD, BPD, CRL, PD, FL, OSD, and FHR. All of the parameters showed a significant gradual progress with increasing of fetal age. Except for PD, the linear regression curve was represented in all of the values.

Gestational sac (GS) was imaged as a first definitive ultrasonographic finding for pregnancy diagnosis.<sup>28</sup> It was first detected on day 17 by transrectal ultrasonography in this study, others identified GS on days 24 - 27,<sup>8</sup> 18,<sup>23</sup> 18-19,<sup>28</sup> 20,<sup>29</sup> and 22,<sup>30</sup> although accuracy was improved by day 26<sup>30,31</sup>. In the current study, diameter of the GS was imaged on days 17-21 and showed significant correlation with GA ( $p < 0.01$ ) similar to previous studies.<sup>8,9,19,21</sup> The  $R^2$  value derived from the prediction equations verified a low correlation coefficient ( $R^2 = 0.318$ ) in this study indicating GS diameter was a less relevant value for estimation of gestational age. Moderate correlation was reported by de Bulnes *et al.* ( $R^2 = 0.76$ ) between 3rd and 5th week of gestation<sup>9</sup> and Correia Santos *et al.* between 3rd and 7th week ( $R^2 = 0.689$ )<sup>21</sup>, however, high value ( $R^2 = 0.926$  and  $0.924$ , respectively) was reported by Petrujkić *et al.*<sup>19</sup> and Ali and Hayder<sup>8</sup>. These differences might be attributed to different time of scanning and/or different scanning plan and/or breed differences.

de Bulnes *et al.*,<sup>9</sup> Greenwood *et al.*,<sup>12</sup> Santiago-Moreno *et al.*,<sup>20</sup> Metodiev *et al.*<sup>32</sup> and Correia Santos *et al.*<sup>21</sup> reported that BPD showed high reliable value to estimate the gestational age in sheep, with determination coefficients ( $R^2$ ) of 0.95, 0.95, 0.96, 0.83 and 0.96 at gestation week 7 - 17, 5 - 16, 5 - 13, 6 - 9 and 4 - 17,

respectively. Similar results were also obtained by other authors.<sup>14,25</sup> In the current study, the BPD had the high correlation coefficient for the gestational age ( $R^2 = 0.961$ ) on days 36-96, however, it was not the highest and low correlation coefficient ( $R^2 = 0.290$ ) was calculated on days 96 - 138, as well.

With regard to the occipito-snout diameter (OSD), significant correlation with gestation age and the highest correlation coefficient ( $R^2 = 0.981$ ) was achieved in the current study. This high level of  $R^2$  was corroborated with other studies.<sup>9,10,18-20</sup> Accuracy of OSD was more than BPD in the present study that was compatible with other works.<sup>18</sup> Because of the highest correlation coefficient, low residuals and wide period of availability for imaging, OSD was recommended as the best parameter for estimation in gestational age on days 36-109 in Baluchi sheep.

In the current study, CRL was measured on days 21-55 and showed high coefficient with increasing of the fetal age ( $R^2 = 0.935$ ) that was consistent with de Bulnes *et al.* ( $R^2 = 0.94$ )<sup>9</sup> and Santiago-Moreno *et al.* ( $R^2 = 0.95$ )<sup>20</sup>. It was considered less accurate in Correia Santos *et al.* ( $R^2 = 0.804$ )<sup>21</sup> and Ali ( $R^2 = 0.876$ )<sup>8</sup>. Moderate correlation ( $R^2 = 0.56$ ) was reported by Godfrey *et al.*<sup>11</sup>

Placentome can be frequently detected in ultrasonography examination, however, with progression of the pregnancy imaging of the larger placentomes is difficult that is confirmed the previous published data.<sup>9,21</sup> In the current study, for precise evaluation of the possible relationship of the placental diameter (PD) and fetal growing, mean and maximum diameter of the placentome as well as mean and maximum diameter of placental wall were measured on days 18-138 and significant correlation with gestation age with different determination coefficient ( $R^2 = 0.75$ ,  $0.73$ ,  $0.91$  and  $0.51$ , respectively) was identified. Although, high  $R^2$  was calculated for mean diameter of the placental wall, linear regression model was not determined in none of them. Several authors declared significant correlation of placental diameter and GA.<sup>1,9,21,32,33</sup> Correia Santos *et al.* measured PD between the 3rd and 8th week and moderate reliability ( $R^2 = 0.716$ ) to estimate the GA.<sup>21</sup> de Bulnes *et al.* reported a determination coefficient of 0.76 on the 3rd and 7th week of gestation.<sup>9</sup> A low correlation coefficient ( $R^2 = 0.156$ ) was represented by Doizé *et al.* between the 5<sup>th</sup> and 13<sup>th</sup> weeks and variations of the number and size of the placentomes within the uterus, difference of the number and weight of the placentomes between two horns, variation of placentome development in relationship to season and variation of the placentome size related to its location were considered as causes of poor correlation between gestational age and size of placentome.<sup>1</sup> High significant regression and correlation of PD and gestation age was not reported by Ali and Hayder in Ossimi sheep.<sup>8</sup>

Low correlation coefficient ( $R^2 = 0.435$ ) of FL and gestation age on days 50 - 130 was reported by Barbera

et al.<sup>34</sup> de Bulnes et al., Santiago-Moreno et al. and Correia Santos et al. showed moderate correlation of FL with growing fetus ( $R^2 = 0.780$  and  $0.89$ ,  $0.89$  respectively).<sup>9,20,21</sup> This parameter was imaged within days 49-109 in the current study and revealed high correlation coefficient ( $R^2 = 0.950$ ). It must be noted that there was improper visualization of this structure because of fetal movement and caused some imaging difficulty.

In the current study for precise evaluation of the thoracic diameter in estimation of the gestation age, two level of thoracic cavity was measured as described. Significant correlation of TD and GA with high correlation coefficient in both level, central and last rib, ( $R^2 = 0.958$  and  $0.975$ , respectively) were obtained on days 36-138 and 36 - 109, respectively. These findings were in agreement with previous studies.<sup>8,9,20-22</sup>

For fetal heart rate (FHR), there was a moderate negative correlation with GA ( $R^2 = 0.883$ ) in the current study. Godfrey et al. reported linear regression curve with low correlation coefficient ( $R^2 = 0.17$ ) for FHR and GA on days 35 - 140.<sup>11</sup> Correia Santos et al represented a moderate correlation with GA ( $r = -0.56$ ).<sup>21</sup>

In conclusion, several parameters (GSD, BPD, OSD, CRL, TD, PD, FL, and FHR) was evaluated in the current study for estimation of gestation age. All of the values represented significant correlation with increasing of fetal age and showed linear regression curve except for PD. The OSD and TD in wide range of gestation period were imaged. Low residuals were calculated in GSD and OSD. High correlation coefficient was identified for OSD, TD, BPD (on day 36 - 96), FL, CRL and mean diameter of placentome. It was concluded that the most accurate prediction of gestational age in Baluchi sheep was provided by occipito-snout diameter of the fetus because of the lowest residuals, the highest correlation coefficient and wide period of availability for imaging in gestation.

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### Conflict of interest

The authors have any conflict of interest to declare.

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