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Prediction of Gestational Week in Kivircik Ewes Using Fetal Ultrasound Measurements

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Abstract: The aims of the current study were to evaluate the accuracy of fetal measurements measured by real time ultrasound to predict the gestational week during mid to late pregnancy and to establish regression equations to estimate the gestation week in singleton and twin pregnancy Kivircik ewes, separately. Data were collected from 25 Kivircik ewes (10 singleton and 15 twin pregnancy) by scanning weekly from 8-20 weeks of gestation. Fetal measurements investigated in the study were biparietal diameter, trunk diameter, fetal heart diameter, fetal heart rate and intercostal space. With the exception of fetal heart rate, all the fetal measures were significantly ($p<0.001$) ($r^2>80$) correlated with the gestational age for singleton and twin pregnancies. The highest coefficient of determination for singleton pregnancy was 0.955 for biparietal diameter, whereas the smallest coefficient of determination corresponded to the fetal heart rate ($r^2 = 0.473$). The highest coefficient of determination for twin pregnancy was 0.946 for heart diameter, the smallest coefficient of determination to the fetal heart rate ($r^2 = 0.611$). In conclusion, the gestational week of Kivircik ewes can be accurately estimated by ultrasound measuring of biparietal diameter, trunk diameter, heart diameter and intercostal space.

Key words: Gestational age, fetometry, ultrasonography, sheep, Turkey

INTRODUCTION

Lamb survival is an important factor affecting profitability in sheep production, as the greatest part of income in a sheep farming is supplied through lamb production (Ekiz *et al*., 2005). If hand mating is applied and mating date is known, management arrangements to maximize survival rates of lambs could be achieved. Furthermore, sheep breeders need accurate information about gestation age of ewes to form appropriate rations based on their nutritional needs and to dry off the lactating ewes at appropriate time (Doize *et al*., 1997). However, accurate mating date is usually unknown, because in most of the sheep flocks rams are introduced to the flock at the beginning of the breeding season without any individual record regarding mating date of ewes (Ekiz and Ozcan, 2006). On the other hand, gestation age of ewes might be estimated by monitoring fetal development when mating date is unknown (De Bulnes *et al*., 1998).

B-mode real time ultrasonography is a reliable method for diagnosing pregnancy and counting fetal numbers in small ruminants (Dawson *et al*., 1994; De Bulnes *et al*., 1998; Karen *et al*., 2009). Transrectal or transabdominal B-mode real time ultrasonography have been used to predict gestational age by means of fetal measurements in small ruminants by numerous researchers. Fetal measurements used in those studies were biparietal diameter (Aiumlamai *et al*., 1992; De Bulnes *et al*., 1998; Greenwood *et al*., 2002; Karen *et al*., 2009), trunk diameter (De Bulnes *et al*., 1998; Karen *et al*., 2009), crown-rump length (De Bulnes *et al*., 1998), fetal heart diameter (Parraguez *et al*., 2000; Karen *et al*., 2009) and metacarpus length (Greenwood *et al*., 2002). The relationship between placentome size and gestation week also investigated by Doize *et al*. (1997) and Karen *et al*. (2009). According to results of these studies, gestation week of ewes or does can be accurately estimated by fetal ultrasound measurements. In these studies, except Greenwood *et al*. (2002), only one fetal parameter was used to estimate the gestational age. Greenwood *et al*. (2002) noted that multiple regression equations developed from measurement of biparietal diameter and metacarpal bone length could provide further improvement in predictive

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capacity. The aims of the current study were to evaluate the accuracy of fetal measurements measured by real-time ultrasound in order to predict the gestational week during mid to late pregnancy and to establish regression equations to estimate the gestation week in singleton and twin pregnancy Kivircik ewes, separately.

MATERIALS AND METHODS

Animals and their breeding: This study was carried out at Faculty of Veterinary Medicine, Istanbul University, Turkey (28°S, 41°W). A total of 25 Kivircik ewes (10 singleton and 15 twin pregnancy) aged between 1.5 and 4 years were used in the study. Ewes were ear-tagged for individual identification. All of the ewes, except ewes aged 1.5 years, had lambed in the previous breeding season. Ewes had been kept indoors and concentrates were given in addition to *ad libitum* to good quality alfalfa hay and fresh water during the pre-weaning period. In the post-weaning period, the ewes were grazed at pasture during the day and good quality alfalfa hay was given indoors at night. All ewes were subjected to estrus synchronization by inserting vaginal sponges containing 30 mg of Cronolone fluorogestone acetate (Chronogest, Intervet, Turkey) for 12 days and received 400 IU pregnant mare serum gonadotrophin (PMSG, Intervet, Turkey) i.m. at sponge withdrawal. The ewes were inseminated transcervically with fresh semen 48 h after sponge withdrawal.

Ultrasound equipment: Ultrasound measurements were conducting using B-mode, Realtime Scanner (Madison SA600 V) equipped with a 5-8 MHz linear-array transducer for trans-rectal ultrasonography and a 3.5-8 MHz sector-array transducer for trans-abdominal ultrasonography. In order to asses trans-rectal ultrasonography, each ewe was placed in dorsal recumbency on a metallic cradle and scanning was done by locating into the rectum the transducer precoated carboxymethylcellulose to improve the coupling. Uterine horns were searched laterally and cranially to the urinary bladder so that the entire reproductive tract and fetus were scanned. Pregnancy status was diagnosed at 25 and 30 days of gestation and litter size was estimated at 25, 30 and 35 days of gestation.

In order to asses trans-abdominal ultrasonography, the hair in the inguinal region in both sides was clipped and in the advanced stage of pregnancy more cranial portion of the ventral abdomen needed to be shaved. A copious amount of gel was applied to the clipped area to eliminate the air spaces. Thereafter, the transducer was placed in the clipped area then slowly moved and rotated till the anaechoic urinary bladder appeared. Trans-abdominal scans were carried out from week 8 at 1 week intervals till week 20. The ewes were scanned by using a multi-frequency (3.5-8 MHz) linear or convex transducer in a lateral recumbancy. Measurements were done by manipulating the transducer until the largest section of each structure was obtained. Then, the image was frozen on the screen and its size was measured with built in electronic callipers. The measurements were done on both fetuses. When the fetus imaged the following fetal parts were measured:

Biparital Diameter (BPD): It could be determined by freezing on the screen the images of the fetal skull. It is the maximum diameter of head width at the plan of the orbit measured by the electronic caliper (Haibel, 1988).

Trunk Diameter (TD): The maximum diameter of the body of the fetus measured from the ventral aspect of the spinal column through the abomasum to the ventral aspect of the abdomen (Aiumlamai *et al*., 1992).

Fetal Heart Diameter (FHD): The maximum diameter of the body of the heart measured from dorsal to ventral.

Fetal Heart Rate (FHR): It could be measured by using the M-mode technique. After identification of the fetal heart by B-mode ultrasonography, a split screen was used to view both B and M-modes simultaneously. When an adequate M-mode imaging was obtained, the image was frozen. The time duration was determined by measuring the distance between two heart waves (either peak to peak or nadir to nadir, whichever amore distinct) on the ultrasound screen with calipers. The heart rate was automatically computed by the ultrasound scanner (Curran and Ginther, 1995).

Intercostal Space (ICS): The maximum intercostal space was measured.

Statistical analysis: In order to determine the significance of the effect of a singleton or twin pregnancy on BPD, HD and ICS, the preliminary repeated measures of ANOVA (pregnancy week as within-subject factor and pregnancy type as between-subject factor) was carried out by the GLM procedure in SPSS 13.0 programme. Since the effects of pregnancy type (singleton or twin) on these characteristics were significant in the preliminary analyses, regression analyses were performed on dataset of singleton and twin pregnancy, separately. Simple regression analyses (linear, quadratic, inverse or logarithmic) were performed to establish prediction equation to estimate the gestation week using

independent variables of BPD, HD, ICS, FHR and TD. The accuracy of the estimates was evaluated by coefficient of determination (r^2), Residual Standard Deviation (RSD) and Root Mean Square Errors (RMSE) (Kocak and Ekiz, 2008). Regression models resulting in smaller RSD and RMSE and higher r^2 were considered to be superior and were chosen for presentation of relationship between ultrasound measurements and gestational week in the figures. Subsequently, stepwise regression procedures including BPD, HD and ICS as indipendent variables were used to develop the optimized multiple regression equations in order to predict gestation week.

RESULTS AND DISCUSSION

The percentage accuracy of ultrasound in pregnancy diagnosis and determining of fetal number were 100% and 100% for positive cases. This was confirmed at delivery. An animal was considered pregnant when fluid-filled gestational sac in the uterus, heartbeats, cotyledons and/or fetal parts was recognized. Concerning fetal number, the single and twin pregnancies were 40 and 60%, respectively (Fig. 1 and 2). The relationship between ultrasound measurements and gestational week according to simple regression analyses for singleton and twin

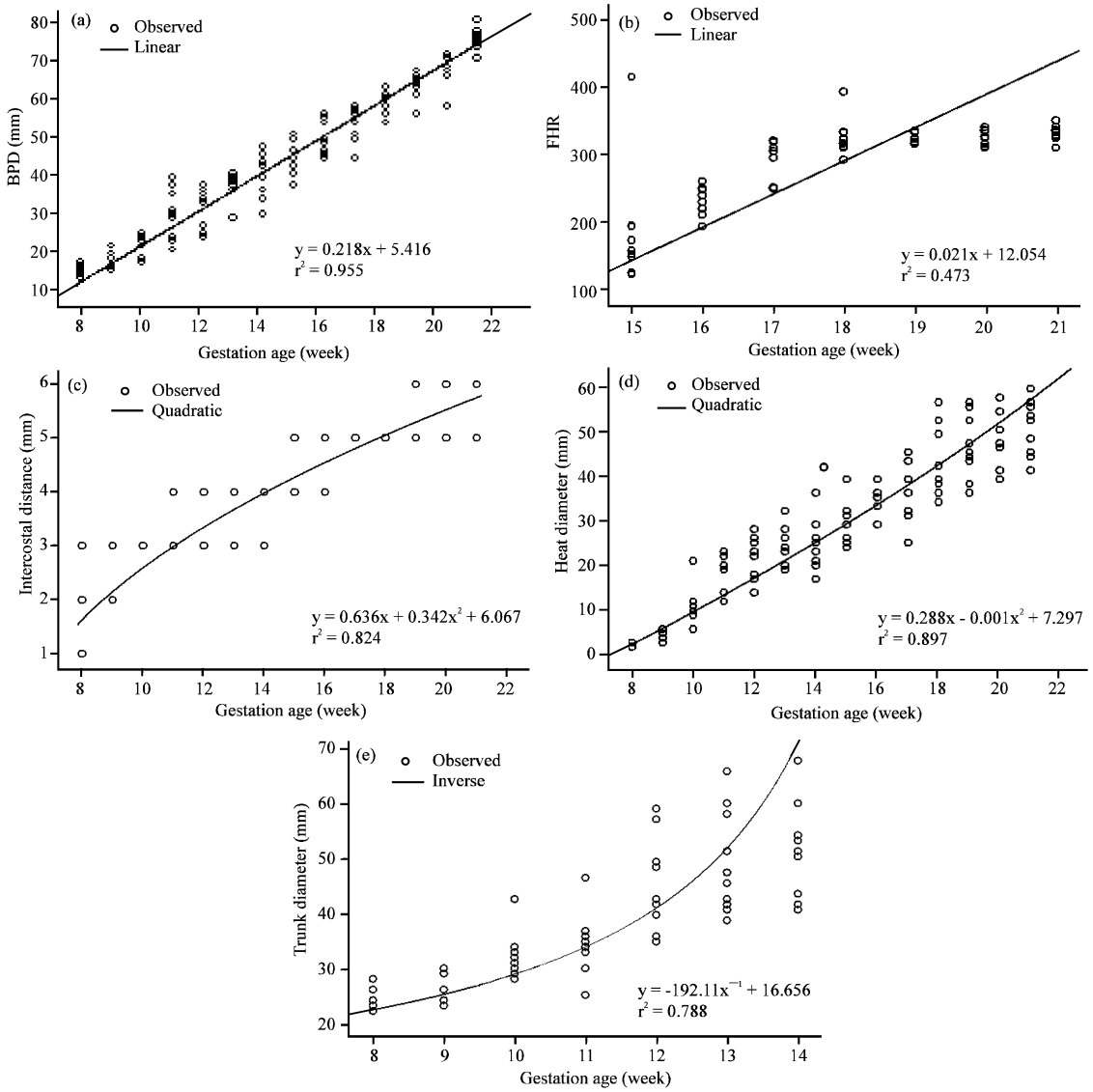

Fig. 1: Relationship between; (a) the Biparital Diameter (BPD); (b) Fetal Heart Rate (FHR); (c) Intercostal Space (ICS); (d) Heart Diameter (HD); (e) Trunk Diameter (TD) and the gestational age of Kivircik ewes which carry single fetus

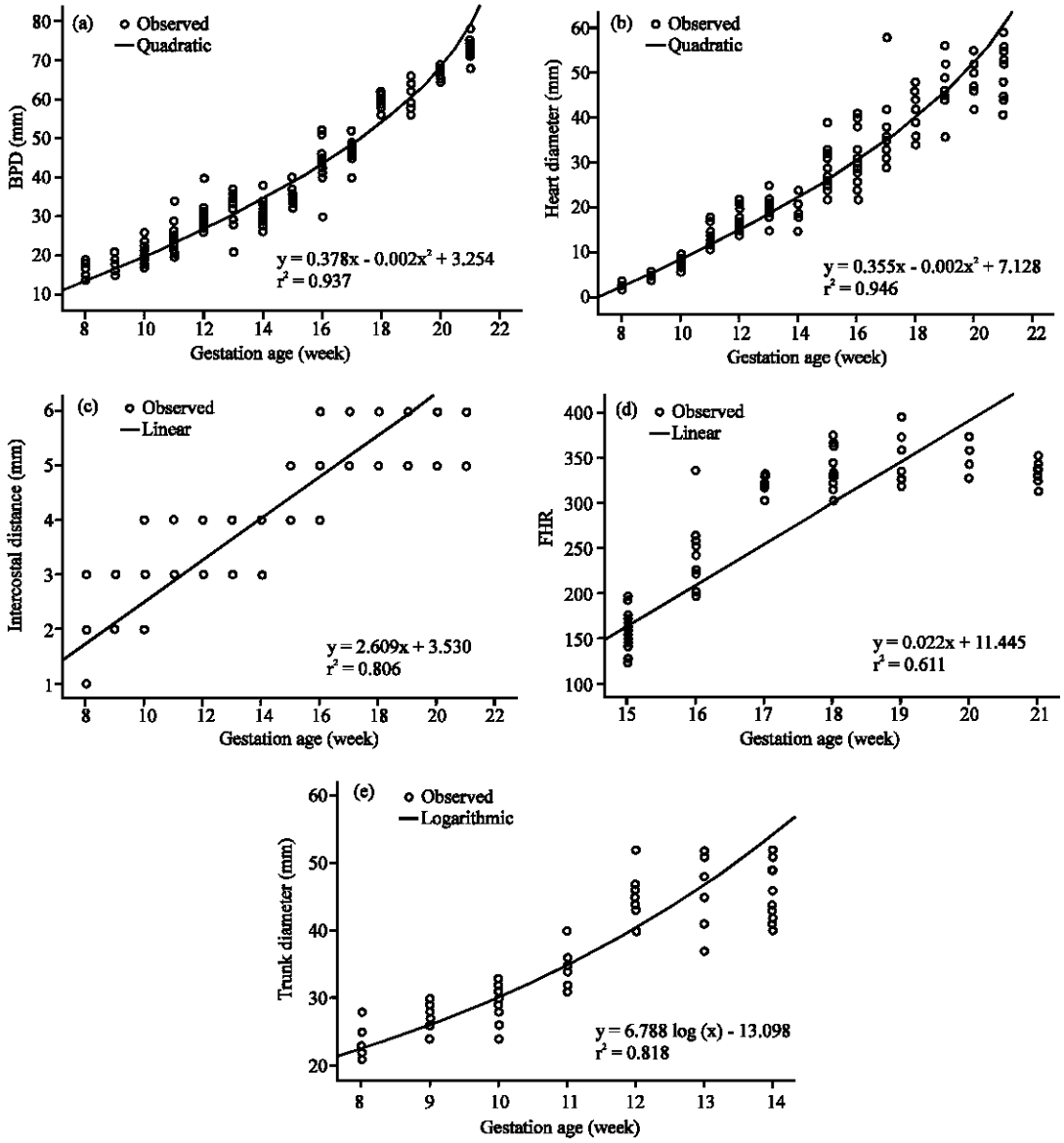

Fig. 2: Relationship between; (a) the Biparital Diameter (BPD); (b) Heart Diameter (HD); (c) Intercostal Space (ICS); (d) Fetal Heart Rate (FHR); (e) Trunk Diameter (TD) and the gestational age of Kivircik ewes which carry twin fetuses

pregnancies are shown in Fig. 1 and 2, respectively. The coeffecient of correlation (r^2) between gestational week and ultrasound measurements investigated in the current study ranged between 0.687 (FHR) and 0.977 (BPD) for singleton pregnancies ($p<0.001$) and between 0.782 (FHR) and 0.965 (HD) for twin pregnancies ($p<0.001$). Among the simple regression models for singleton pregnancies, the model based on BPD was yielded the highest r^2 (0.955) and the lowest RSD (0.864) and RMSE (0.681). However, the model including HD was the best fitted model for twin pregnancies with the highest r^2 (0.946) and the lowest RSD (0.943) and RMSE (0.696). Results of stepwise regression analyses including ultrasound measurements of BPD, HD and ICS as indipendent variables for predicting gestation week are shown in Table 1 and 2 for singleton and twin pregnancies, respectively. While linear models including BPD for single pregnancies and HD for twin pregnancies accounted for 95.5 and 93.1% of the variation in gestation week, multiple regression equations including BPD, HD and ICS provided further improvement in precision of the prediction. The inclusion of BPD, HD and ICS in the multiple regression equations increased the

Table 1: Prediction equations, coefficient of determination (r^2), Residual Standard Deviation (RSD) and Root Mean Square Errors (RMSE) values obtained from stepwise regression analyses for estimating gestation week (y) from ultrasonic measurements for single pregnancies

Variables	Equation	r^2	RSD	RMSE
BPD	y = 5.416 + (0.218×BPD)	0.955	0.864	0.681
BPD + ICS	y = 4.569 + (0.189×BPD) + (0.511×ICS)	0.959	0.825	0.635
BPD + ICS + HD	y = 4.931 + (0.154×BPD) + (0.046×HD) + (0.469×ICS)	0.961	0.796	0.585

Table 2: Prediction equations, coefficient of determination (r^2), Residual Standard Deviation (RSD) and Root Mean Square Errors (RMSE) values obtained from stepwise regression analyses for estimating gestation week (y) from ultrasonic measurements for twin pregnancies

Variables	Equation	r^2	RSD	RMSE
HD	y = 8.217 + (0.238×HD)	0.931	1.062	0.795
HD + BPD	y = 7.136 + (0.095×BPD) + (0.137×HD)	0.952	0.886	0.687
HD + BPD + ICS	y = 5.958 + (0.091×BPD) + (0.100×HD) + (0.548×ICS)	0.960	0.812	0.623

coeffecient of determination (0.6 and 2.9% for singleton and twin pregnancies) decreased the RSD (7.9 and 23.5% for singleton and twin pregnancies) and RMSE (14.1 and 21.6% for singleton and twin pregnancies).

The results of this study demonstrate that ultrasound measurement of fetal organs can be used to estimate stage of pregnancy of prolific Kivircik ewes during mid to late gestation. In the study, the accuracy for determining fetal number was 100% for singleton and twin pregnancy. Zipper *et al.* (1997) reported that the highest accuracy for determining fetal number was 89.1% at days 35-46 of gestation when examination performed in standing position using a 5 MHz linear array transducer. When the examination performed transrectally using a 7.5 MHz probe, the accuracy was reached over 80%. Also they found that as litter size increased, examinations in both groups became increasingly inaccurate in determining correct litter size. Further sources of inaccuracy, (Hertz and Zador, 1979) are the transducer resolution, the image processing and formation and the biased of echographist. Head diameters provided a good index of fetal development because they showed high correlations with gestational age, enabling long periods of observation. The skull usually remains in a good position for observation and it is easy to measure biparietal diameter with a better placing of callipers on the hiperechogenic limit of the bones bordering the soft tissues. Haibel *et al.* (1989) showed that the biparietal diameter was the most representative parameter of the gestational age during the second third of pregnancy. Kelly and Newnham (1989) consider the occipitonasal length to be a more accurate measure than the biparietal diameter, showing a linear increase until day 80. De Bulnes *et al.* (1998) reported that estimation of the gestational age is more accurate during the early pregnancy since the values obtained in later gestation are also affected by the individual characteristics of the foetus. But in the present study, for singleton pregnancy, BPD was highly significantly correlated ($p<0.001$; $r^2 = 0.955$) with the gestational age between weeks 8 and 20 of gestation. Similar results were reported by Greenwood *et al.* (2002) ($r^2 = 0.94$) in Suffolk x (Finn x Dorset) ewes between days 60 and 120 and by De Bulnes *et al.* (1998) ($r^2 = 0.96$) in Manchega ewes between days 32 and 90 of gestation. The highest correlation coefficient for twin pregnancy with time was 0.946 for heart diameter. The correlation coefficient for BPD in multiple pregnancy with time ($r^2 = 0.937$) in the study is similar to that ($r^2 = 0.94$) reported by Greenwood *et al.* (2002). Stage of pregnancy was predicted with similar reliability by BPD and FHD and the use of both measurements within a multiple regression equation improved predictive capacity. Further improvement in predictive capacity was achieved using multiple regression equations developed from measurement of BPD, FHD and ICS. Similar results were reported by Greenwood *et al.* (2002) when they used BPD and metacarpal bone length. No differences between pregnancies with single and twin fetuses were found for any of the measurements studied. This is in agreement with other studies using ultrasonography (Kelly and Newnham, 1989; Sergeev *et al.*, 1990). Postmortem studies showed that there are minor small differences between single and twin pregnancies and they are only detectable at the end of gestation (Robinson *et al.*, 1977; McDonald *et al.*, 1978).

CONCLUSION

The age of fetus in Kivircik ewes can be accurately estimated by ultrasound measuring the biparital diameter, trunk diameter, heart diameter and intercostal space. Results of the current study indicate that ultrasound measurements including BPD, HD and ICS can be good predictors of gestation week in Kivircik ewes.

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