# Determination of gestational age by measuring defined embryonic and foetal indices with ultrasonography in Abaza and Gurcu goats

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#### Abstract

The aim of this study was to determine gestational age in Abaza and Gurcu goats by measuring certain embryonic and foetal indices with ultrasonography. A 5–7.5 MHz linear probe was used to obtain ultrasound measurements from 30 pregnant goats (10 Abaza and 20 Gurcu). Heart diameter (HD), biparietal diameter (BPD), crown-rump length (CRL), trunk diameter (TD) and placentome diameter (PD) were measured to determine gestational age. The mean of embryonic and foetal indices were calculated and linear regression was performed. Heart diameter measurements for Abaza and Gurcu goats were significantly different on days 45 (P=0.048) and 60 (P=0.019). Biparietal diameter values were significantly different on day 45 (P=0.035). Crown-rump length measurements were significantly different at days 30 (P=0.003) and 60 (P=0.002). We determined that HD and TD were the best predictors of gestational age for Abaza goats ( $R^2=0.952$ ,  $R^2=0.949$ , respectively), whereas HD and CRL were the best predictors of gestational age for Gurcu goats ( $R^2=0.933$ ,  $R^2=0.942$ , respectively). Based upon our study results, these specific indices could be applied during ultrasonographic examinations of Abaza and Gurcu goats to confirm gestational age when the day of mating is unknown.

Goat, heart diameter, crown-rump length

Abaza and Gurcu goats, which are important Turkish genetic resources, are raised locally in Northeastern Anatolia. These goats have adapted to the particularly cold climate conditions of the region. To date, very few studies have been conducted on the reproductive characteristics of these animals (Batu 1951; Yalcın 1988; Kuru et al. 2017a; 2017b).

Considering the conditions of the meadows, the exact mating day of sheep and goats are typically unknown. Thus, gestational age is not generally precisely determined in these animals. Since pregnant and non-pregnant animals require different care and feeding regimes, ultrasonographic methods have been used in the past to estimate gestational age (Haibel 1988; Haibel et al. 1989; Doize et al. 1997; Karen et al. 2009).

B-mode ultrasonography is a fast and reliable method used to check for pregnancy in small ruminants. Transrectal ultrasonography is significantly better than the transabdominal method in obtaining more accurate results during the early gestational period (days 27–30) (Ishwar 1995; Doize et al. 1997; Gürler and Kaymaz 2011; Karadaev et al. 2016).

Today, it is possible to determine gestational age in humans by measuring the biparietal diameter (BPD). Similarly, there are reported studies conducted in sheep and goats using BPD measurements (Haibel 1988; Haibel et al. 1989; Reichle and Haibel 1991; Lee et al. 2005). In addition to BPD, gestational age may also be determined by measuring the heart diameter (HD), placentome diameter (PD), crown-rump length (CRL), trunk diameter (TD), the aorta, or the umbilical cord (Martinez et al. 1998; Lee et al. 2005; Amer 2010; Kandiel et al. 2015).

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E-mail: mushapkuru@hotmail.com http://actavet.vfu.cz/ This study was designed to measure the HD, BPD, CRL, TD and PD by real-time ultrasonography in Abaza and Gurcu goats to determine gestational age based upon these embryonic and foetal measurements. In addition, differences between the foetal biometry of Abaza and Gurcu goats were determined.

#### Materials and Methods

Animals and oestrus synchronization

This study was conducted upon obtaining approval from the Kafkas University Animal Experiments Local Ethics Committee, Kars, Turkey (KAÜ-HADYEK -2015/012).

The study population consisted of 15 Abaza and 30 Gurcu goats 3–5 years of age weighing 50–60 kg. Oestrus synchronization was initiated during the breeding season. Sponges containing progesterone (60 mg, medroxyprogesterone acetate, Esponjavet\*, Hipra, Turkey) were placed intravaginally. On day 9, all goats were treated with 400 IU equine chorionic gonadotropin (IM, eCG, Folligon\*, Intervet, Turkey) and 5 mg dinoprost tromethamine (IM, Dinolytic\*, Zoetis, Turkey). The sponges were removed on day 11. Following synchronization, a teaser buck was introduced to the herd to monitor oestral activity. The goats determined to be in oestrus were mated with 9 fertility-proven bucks (4 Abaza and 5 Gurcu). The mating times were recorded for reference during future ultrasonographic examinations.

## Ultrasonographic examinations

Embryonic and foetal measurements were performed in 30 goats (10 Abaza, 20 Gurcu) diagnosed as pregnant. Measurements were performed on days 30, 45, 60, 75, 90 and 120 of gestation. Transrectal ultrasonography was used for the measurements performed on day 30, while the remaining measurements were performed using transabdominal ultrasonography (5-7.5 MHz, SonoSite Titan®, SonoSite, WA, USA). When clear images could not be obtained using transabdominal ultrasonography on day 45, transrectal ultrasonography was used. To increase the quality of ultrasound imaging and prevent artifacts, the animals were restrained during transrectal ultrasonography. Faeces were removed from the rectum using lubricant and transrectal ultrasonography was initiated with the goats in standing position. During transabdominal examinations, the animals were positioned in dorsal recumbency, and an area of 5 cm in width by 12 cm in length inferior to the breast was shaved. Ultrasound imaging of the area was performed after it was cleaned with alcohol and cotton. Ultrasound gel was used for both transrectal and transabdominal ultrasonography to facilitate obtaining clearer images.

Gestational ultrasonography measurements were taken at 30, 45, 60, 75, 90 and 120 days for HD, at 30, 45, 60, 75 and 90 days for BPD and TD, at 30, 45, 60 and 75 days for CRL, and at 45, 60, 75, 90 and 120 days for PD. On day 75, CRL could only be measured in 13 Gurcu goats due to excessive growth of the foetus (CRL was measured in 10 Abaza goats). This number was taken into consideration during calculation of the mean number and statistical assessment.

As suggested by Lee et al. (2005) and Oral et al. (2007), HD was measured by freezing the image at the heart's widest point of expansion and recording the diameter; BPD measurements were performed by freezing the image at the best angle to measure the width, as suggested by Haibel (1988) and Amer (2010); CRL measurements were performed as suggested by Kaulfuss et al. (1999) and Martinez et al. (1998); TD measurements were performed by detecting the diaphragm limit (Karen et al. 2009). For placentome measurements, once the desired image was displayed on the monitor, the image was frozen and dimensions were taken using the calipers of the ultrasound device. The average of the three largest placentomes captured in the image was recorded (Lee et al. 2005; Karen et al. 2009).

#### Statistical analysis

Statistical analysis was performed using SPSS® software (SPSS 16.0, IL, USA). Group averages (the embryonic and foetal average) were determined and linear regression was performed using the gestational age formula 'y = ax + b', where y = gestational age, a = predictors (constant), x = measured value, and b = dependent variable. Additionally, the statistical difference between the groups was calculated using Student's t-test. P < 0.05 was accepted as significant. The values obtained were presented as mean  $\pm$  standard deviation (SD).

## Results

There were 5 twin (2 Abaza and 3 Gurcu) and 25 single (8 Abaza and 17 Gurcu) births. The HD measurements for Abaza and Gurcu goats were significantly different on days 45 (P = 0.048) and 60 (P = 0.019). The BPD values were significantly different on day 45 (P = 0.035). The CRL measurements were significantly different on days 30 (P = 0.003) and 60 (P = 0.002). The TD and PD measurements were significantly different on day 75 (P = 0.041 and P = 0.005, respectively, Table 1). Based on the data,

Variables (cm)	Goat	n	Gestation Day					
			Day 30	Day 45	Day 60	Day 75	Day 90	Day 120
HD	Abaza	10	$0.3 \pm 0.1$	$0.6 \pm 0.1^{a}$	$0.8\pm0.2^{\rm a}$	$1.3 \pm 0.1$	$1.9 \pm 0.2$	$3.1 \pm 0.1$
	Gurcu	20	$0.3\pm0.1$	$0.7\pm0.1^{\rm b}$	$1.0\pm0.2^{\rm b}$	$1.3\pm0.1$	$1.9 \pm 0.4$	$3.1\pm0.2$
BPD	Abaza	10	$0.7 \pm 0.1$	$1.1\pm0.1^{\rm a}$	$1.9 \pm 0.1$	$2.9 \pm 0.6$	$3.5 \pm 0.3$	-
	Gurcu	20	$0.6 \pm 0.1$	$1.0\pm0.2^{\rm b}$	$1.9 \pm 0.3$	$2.6 \pm 0.4$	$3.6 \pm 0.3$	-
CRL	Abaza	10	$1.7\pm0.2^{\rm c}$	$3.0 \pm 0.7$	$6.5\pm0.5^{\rm c}$	$9.2\pm1.3$	-	-
	Gurcu	20	$1.6 \pm 0.3^{\rm d}$	$3.3 \pm 0.6$	$7.2\pm0.6^{\rm d}$	$9.9\pm0.9^{\scriptscriptstyle\#}$	-	-
TD	Abaza	10	$0.6 \pm 0.1$	$1.6\pm0.2$	$2.3\pm0.3$	$3.7 \pm 0.4^{\rm a}$	$4.6 \pm 0.3$	-
	Gurcu	20	$0.7 \pm 0.2$	$1.6\pm0.4$	$2.2\pm0.3$	$3.4\pm0.4^{\text{b}}$	$4.6 \pm 0.5$	-
PD	Abaza	10	-	$1.1\pm0.1$	$1.8 \pm 0.2$	$2.1\pm0.2^{\rm c}$	$2.8 \pm 0.3$	$3.4 \pm 0.2$
	Gurcu	20	_	$1.0 \pm 0.3$	$1.9 \pm 0.2$	$2.4 \pm 0.2^d$	$2.8 \pm 0.3$	$3.5 \pm 0.1$

Table 1. Embryonic and foetal measurements (mean  $\pm$  standard deviation) in Abaza and Gurcu goats performed on days 30, 45, 60, 75, 90 and 120 using ultrasound.

HD - heart diameter; BPD - biparietal diameter; CRL - crown-rump length; TD - trunk diameter; PD - placentome diameter; superscripts a and b show the P value between Abaza and Gurcu goats: P < 0.05; superscripts c and d show the P value between Abaza and Gurcu goats: P < 0.01; \*- only 13 goats were measured on day 75.

HD and TD were determined to be the best predictors of gestational age for Abaza goats ( $R^2 = 0.952$  and  $R^2 = 0.949$ , respectively), whereas HD and CRL were the best predictors of gestational age for Gurcu goats ( $R^2 = 0.933$  and  $R^2 = 0.942$ , respectively, Fig. 1).

## Discussion

Embryonic heart rate can be detected at 19–20 days post-mating (Medan et al. 2004; Padilla-Rivas et al. 2005) and measured at 22–36 days post-mating (Karen et al. 2009). It has been reported that the HD of sheep and goats can be detected at 30–40 days of gestation; however, no measurements were performed (Raja et al. 2011). The measurements in this study were taken from day 30 of gestation onwards. In the assessment performed, it was determined that embryonic or fetal HD could be used to determine gestational age as there was a high correlation between embryonic and fetal HD values and gestational age. Moreover, HD measurements were found to be the best predictors of gestational age in Abaza and Gurcu goats. These findings were comparable with the results reported by previous studies (Greenwood et al. 2002; Lee et al. 2005; Oral et al. 2007; Gunduz et al. 2010; Kandiel et al. 2015).

Biparietal diameter measurements with ultrasonography are commonly used to determine foetal age in humans (Kieler et al. 1995; Sailaja et al. 1996). In studies conducted with sheep and goats, the correlation between BPD measurements at 32–90 days of gestation and determination of gestational age was  $r \ge 0.96$  (Haibel 1988; Gonzalez de Bulnes et al. 1998; Nwaogu et al. 2010). In studies conducted with different goat breeds (Toggenburg, Nubian, Angora), a high correlation was found between BPD and gestational age ( $R^2 = 0.994$ ,  $R^2 = 0.988$ ,  $R^2 = 0.978$ , respectively) (Haibel et al. 1989). Our study also found a high correlation between BPD and gestational age at 30–90 days of gestation for Abaza and Gurcu goats. However, the obtained  $R^2$  values ( $R^2 = 0.925$  for Abaza goats,  $R^2 = 0.928$  for Gurcu goats) were lower than those previously reported, which may be due to breed differences.

Crown-rump length measurements can be easily performed at 20-50 days of gestation in small ruminants (Schrick and Inskeep 1993; Kaulfuss et al. 1999; Godfrey

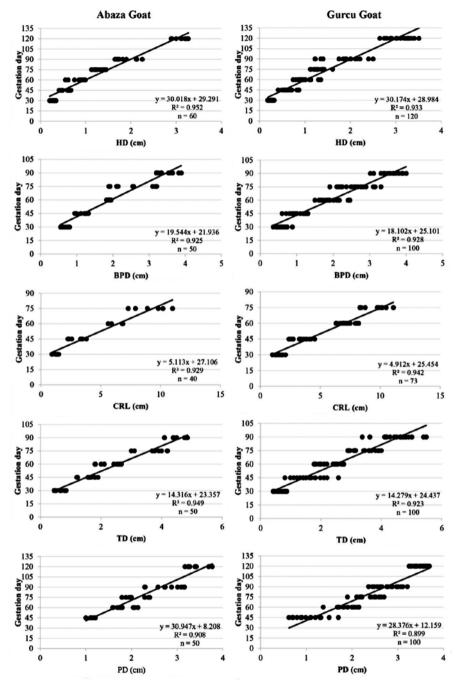


Fig 1. Regression curve indicating the correlation between embryonic and foetal heart diameter (HD, cm), biparietal diameter (BPD, cm), crown-rump length (CRL, cm), trunk diameter (TD, cm), placentome diameter (PD, cm) and gestational age (day) in Abaza and Gurcu goats; n - number of total measurements; y - gestational age; x - measured value

et al. 2010). A strong positive correlation was found between CRL and determination of gestational age in studies conducted with Saanen goats (R<sup>2</sup> = 0.90) (Abdelghafar et al. 2011). Accordingly, a high correlation has been reported between CRL in Egyptian Baladi goats at 25–70 days of gestation (Karen et al. 2009) and Anglo-Nubian goats at 19–40 days of gestation (Martinez et al. 1998). Similarly, there was a high correlation between CRL and determination of early gestational age in Abaza goats (R<sup>2</sup> = 0.929) and Gurcu goats (R<sup>2</sup> = 0.942) in our study. The CRL measurements could only be performed until day 75 of gestation as the foetus then grew too large for the display, making measurements impossible in the subsequent days. Nevertheless, CRL is the best predictor of gestational age in Gurcu goats. A study conducted by Amer (2010) reported that CRL measurements were not possible after day 89 of gestation.

In goats, gestational age reportedly could be determined by measuring embryonic and foetal TD using ultrasonography at 30–120 days (Karen et al. 2009; Kandiel et al. 2015) or 60–135 days (Lee et al. 2005) of gestation. The correlation found between TD and gestational age by Lee et al. (2005) was r = 0.887, whereas the correlation found by Karen et al. (2009) was  $R^2 = 0.962$ . In our study, there was a high correlation at days 30–90 of gestation between TD and gestational age for Abaza ( $R^2 = 0.949$ ) and Gurcu goats ( $R^2 = 0.923$ ). The data we obtained were comparable with those reported previously (Lee et al. 2005; Karen et al. 2009; Kandiel et al. 2015), and the fact that TD measurements are easier than the other foetal index measurements demonstrated that TD could be the preferred measurement for determining gestational age in goats, especially during ultrasonographic examination performed in Abaza goats.

In a study conducted by Karen et al. (2009), placentomes were detectable as small echogenic nodules as of day 28 of gestation. In our study, no exact measurement could be made on day 30; therefore, the measurements were taken on day 45. In several studies, a weak positive correlation was found between PD and gestational age in goats (Doize et al. 1997; Lee et al. 2005; Nwaogu et al. 2010). In our study, there was a high correlation (P < 0.001) between the placentome diameter and gestational age in Abaza and Gurcu goats. Our findings were similar to those of Karen et al. (2009) ( $R^2 = 0.905$ ) and Kandiel et al. (2015) ( $R^2 = 0.899$ ) and higher than those of Lee et al. (2005) (r = 0.574), Nwaogu et al. (2010) (r = 0.45) and Doize et al. (1997) ( $R^2 = 0.703$ ). These differences may have resulted from the variance in placentome sizes and goat breeds. Additionally, using the largest placentomes on the display may have increased the correlation rate with gestational age.

In conclusion, a high correlation was found between gestational ages and HD, BPD, CRL, TD and PD measured by real-time ultrasonography. Also, HD, BPD, CRL, TD and PD values were significantly different on different gestational days for Abaza and Gurcu goats. The HD and TD for Abaza goats and the HD and CRL for Gurcu goats were the best predictors of gestational age in this study. Based on our results, these indices could be beneficial in determining gestational ages of these breeds using ultrasonography when precise mating times are unknown due to field conditions.

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