DOI: 10.1002/vms3.564

ORIGINAL ARTICLE

WILEY

The use of ultrasonographic measurement of the heart size and fetal heart rate variation for gestational age determination in local Bulgarian goats

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Funding information
Ministry of Education and Science, Bulgaria, Grant/Award Number: 0406-105

Abstract

Background: Determination of gestational age in goats was performed using ultrasound measurements of different fetal biometric parameters. There are no data showing quadratic and exponential dependence between fetal heart parameters and gestational age.

Objectives: The objective of the present study was to test the significance of the defined indicators (fetal heart rate, longitudinal and transverse fetal heart axes) for determining gestational age in local Bulgarian goats.

Methods: A total of 24 pregnant local Bulgarian goats, aged between 2 and 5 years, body weight 42–50 kg were included in the study. Ultrasonographic examinations were performed weekly on gestational days 21, 28, 35, 42 and 49. After gestational day 49 until parturition, scans were performed biweekly. The data from the measurements were subjected to linear ($y = a + bx$), quadratic ($y = ax^2 + bx + c$) and exponential ($y = ax^n$) correlation. **Results:** Average fetal heart rate values decreased with pregnancy progression. The coefficient of determination (R^2) and standard error of estimate (SEE) of the heart rate indicator were 0.72, 0.75, 0.58 and 15.1, 14.4, 19.2 days, respectively, for the three correlations. Longitudinal diameter (long axis) had the following values for $R^2 = 0.94$, 0.96, 0.96 and for SEE = 7.0, 5.5, 5.5 days, for the linear, quadratic and exponential correlations, respectively, while the values of the fetal heart transverse diameter (short axis) were higher than those of the external one ($R^2 = 0.95$, 0.97, 0.97). Simultaneously, SEE is lower (SEE = 6.1, 4.9, 5.0 days) compared to that found for the long heart fetal axis.

Conclusions: For precise estimation of gestational age, use of longitudinal (long) and transverse (short) axes is recommended. Fetal heart rate is not an exact indicator but can be used as a reference for gestational age along with changes in the heart size and echogenicity.

KEYWORDS
gestational age, goats, heart, ultrasonography

1 | INTRODUCTION

The development of ultrasonography allows for a detailed study of pregnancy and assists the precise management of the reproductive process in goats (Erdogan, 2012). Ultrasonographic imaging of the heartbeat is an important marker for confirmation of pregnancy and embryo vitality assessment. According to many researchers (Chandolia & George, 2011; Martinez et al., 1998; Medan et al., 2004; Padilla-Rivas et al., 2005), 2 days after visualization of the gestational sac, heartbeat can be registered. By means of the transabdominal technique some researchers observed heart pulsations on gestational days 27–35 (Dawson et al., 1994; Hesselink & Taverne, 1994; Padilla-Rivas et al., 2005). Amer (2010) also noted heart pulsations on day 22.9 ± 0.7 (3.5 days after transrectal visualization of the gestational sac) with transducer frequency 6.0 MHz, while with 3.5 MHz frequency and transabdominal access the heart pulsation was established for the first time on gestational day 27.0 ± 0.6. Suguna et al. (2008) consider that heart pulsations can be found on day 21 for the first time (in one of six examined animals), and heart rate can be read and recorded on day 28 via transrectal access. Similar data were reported by Hesselink and Taverne (1994) and Padilla-Rivas et al. (2005), who registered heart rate on gestational day 33 and 35, respectively, via transabdominal access.

In a previous study of ours (Karadaev et al., 2016), fetal heart rate was measured for the first time on gestational day 35 with an average value of 212.5 (±6.1) bpm. Although fetal structures with high correlation coefficient ($R\check{s} \geq 0.90$) are most frequently used to determine gestational age – embryo/fetus length (crown-rump length CRL), trunk diameter (TD), and biparietal diameter (BPD) (Abdelghafar et al., 2011; Karadaev et al., 2018), some authors (Serin et al., 2010) assume that monitoring fetal heart rate can be used to determine gestational age in Saanen goats and they reported correlation coefficient $R\check{s} = 0.77$. Kahn (1989) established that fetal heart in cattle decreased with progression of pregnancy. A similar tendency in goats was reported by Martinez et al. (1998). Later, Karen et al. (2009) also registered reduced fetal heart rate in Egyptian local goats, but they found lower correlation of that parameter to gestational age ($R\check{s} = 0.55$).

Fetal heart size also relates to gestational age and can be used to determine the latter when mating date is unknown (Raja Ili Airina, 2011). Oral et al. (2007) reported higher correlation between fetal heart diameter and gestational age in sheep between days 40 and 100 of pregnancy. In goats, Lee et al. (2005) found that of all indicators measured, longitudinal and transverse heart axes had the most significant correlation to gestational age $R^2 = 0.9168$ and $R^2 = 0.8819$, respectively, $p < 0.001$.

All authors in the available literature use linear equation to determine gestational age. In none of the cited studies, we have not found data showing quadratic and exponential dependence between fetal heart parameters and gestational age.

The objective of the present study was to test the significance of the defined indicators (fetal heart rate, longitudinal and transverse fetal heart axes) for determining gestational age in local Bulgarian goats.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

The experiment was approved by the institutional Animal Ethics Committee of our faculty. A prospective observational study was conducted on 24 clinically healthy pregnant local Bulgarian goats, aged between 2 and 5 years, body weight 42–50 kg. They were fed grain fodder, hay, drinking water and stone salt. All animals in the experiment were clinically healthy and preventionally dehelmintized.

For estrus synchronization in goats, intravaginal sponges (Syncropart, Seva Sante Animale) were used. On day 12, the sponges were removed and the animals were injected intramuscularly with 500 IU PMSG (Folligon, MSD Animal Health). Goats were mated with fertile buck twice after demonstrating the passive phase and again 12 h later. The day of second mating was recorded and counted as day 0 of pregnancy.

2.2 | Sonographic examination

Sonographic examinations were done by SonoScape A5Vet (SonoScape, China) and linear transducer with frequency 7.5 MHz. The ultrasonography was done by two sonographers – one to conduct the examination and the other to perform the measurements. The examination was accomplished via transrectal (up to gestational day 35) and transabdominal (after gestational day 35) access with standing animal (Yotov, 2020). For better contact between the skin and the transducer, sonographic gel Aqua Ultra Clear (Ultragel, Hungary) was used. Sonographic criteria for early pregnancy diagnostics were the visualization of enlarged uterine lumen filled with an echogenic amniotic liquid and hyperechogenic embryo. All goats of the present study had twin pregnancy.

After proving the pregnancy, weekly ultrasound scans were performed on all goats – on gestational days 21, 28, 35, 42 and 49. After gestational day 49 until day 133, scans were performed biweekly. Fetal heart rate was measured following the methods of Curran and Ginther (1995) and Karen et al. (2009). The fetal heart was visualized in B-mode, afterwards the ultrasonic device was set to B-M-mode so that both B- and M-modes could be simultaneously visualized. The time for examination and measurements was up to 3 min. When an appropriate image was visualized in M-mode, the image was frozen. Fetal heart rate was calculated automatically by the device software measuring the distance between two heart waves (from crest to crest or from trough to trough depending on which part of the wave was more pronounced). Measurement of the longitudinal (long) and transverse (short) heart axes was done after simultaneous visualization of fetal heart atria and ventricles in transverse axial projection during diastole and no movement of the fetus (Lee et al., 2005). In order to avoid variations in measurements of the fetal heart axes, heart scans were performed always at an exact angle (Raja Ili Airina, 2011).

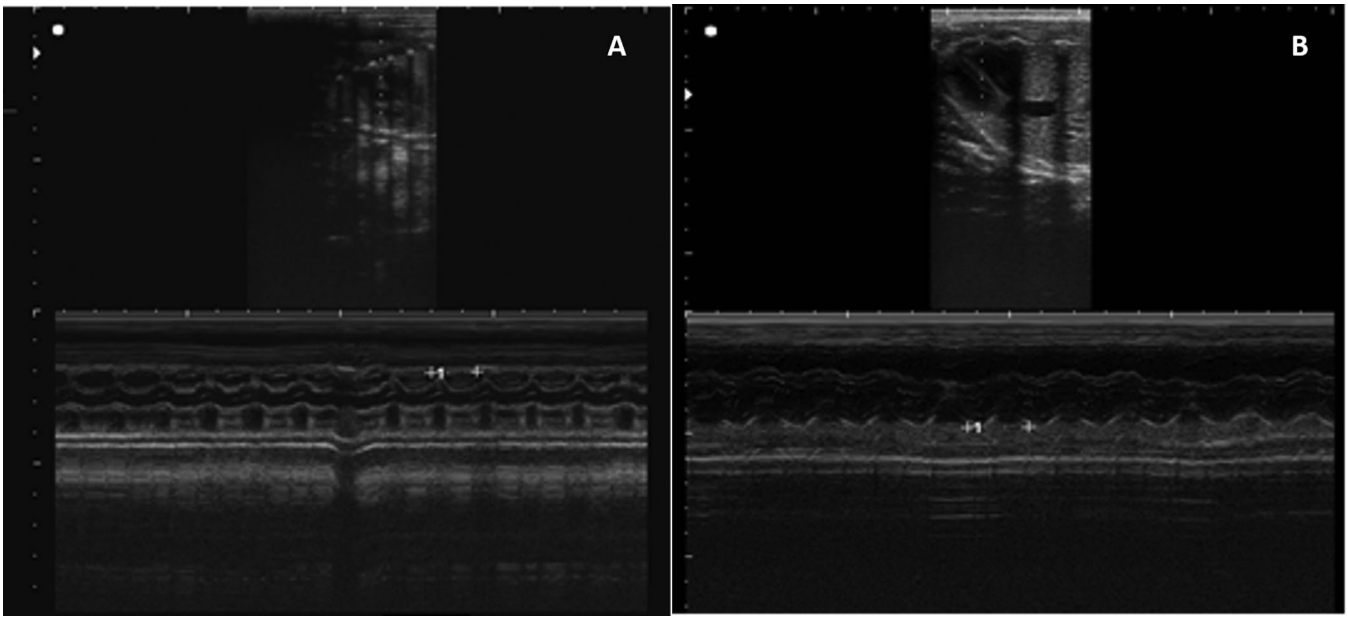

FIGURE 1 B and M-mode ultrasonographic visualization and measurement of fetal heart rate on gestational day 91 (a) and 133 (b) in a goat fetus

2.3 | Statistical analysis

To establish the correlation between gestational age and the measured fetal heart sonographic parameters and to obtain regression equations, the statistics module in Microsoft Excel was used. The following correlations were employed:

$$-\text{linear}(y = a + bx);\ \text{quadratic}(y = ax^2 + bx + c);\ \text{exponential}(y = ax^n), \quad (1)$$

where y is gestational age in days, x is value of the respective biometric indicator (mm), n is exponential index and a, b and c are regression coefficients.

Coefficient of determination R^2 and standard error of estimate (SEE) were calculated for evaluation of the equations.

Coefficient of determination R^2 provides the relative measure of the percentage of the dependent variable variance that the model explains. Higher coefficients of determination values indicate that the data points are closer to the fitted values.

Graphic design of scans was made in Office 2016 (Microsoft, Corp.). Figures were processed in Microsoft Office Excel 2016 (Microsoft Corp.) and ultrasound images in Microsoft Power Point 2016 (Microsoft Corp.).

3 | RESULTS

Our study of the goats revealed fetal cardiac activity on gestational day 28. During that period, the heart was visualized as an echogenic, hardly noticeable, rhythmically pulsating structure, not distinguished echogenically from the surrounding embryonic mass. Fetal heart activity was visualized in B-mode as rhythm fibrillation on gestational day 28. Heartbeats per minute could be measured in B-M-mode of the sonographic device for the first time on gestational day 35. The initially measured average heart rate values were 214.04 ± 5.07 bpm. Till the end of the second trimester, fetal heart rate lowered slightly and on gestational day 91 the average values were 204.86 ± 4.49 bpm (Figure 1a). From the beginning of the third trimester (day 105), fetal pulse lowered sharply and on gestational day 133 we registered average fetal pulse values 158.13 ± 8.14 bpm (Figure 1b).

TABLE 1 Equations, coefficient of determination (R^2) and standard error of estimate (SEE) in linear, quadratic and exponential correlation between gestational age and fetal heart indicators in local goats ($n = 24$)

Biometric indicator	Equation (formula)	R^2	SEE
Fetal heart rate, bpm	$Y = -1.4676x + 369.55$	0.7226	15.1
	$Y = -0.0169x^2 + 4.867x - 214.35$	0.7538	14.4
	$Y = 3 \times 10^9 x^{-3.333}$	0.5883	19.2
Long heart axis, mm	$Y = 1.9015x + 45.719$	0.9354	7.0
	$Y = -0.0255x^2 + 3.2749x + 32.643$	0.9612	5.5
	$Y = 21.802x^{0.466}$	0.9638	5.5
Short heart axis, mm	$Y = 2.9138x + 41.808$	0.9514	6.1
	$Y = -0.0459x^2 + 4.6018x + 30.419$	0.9692	4.9
	$Y = 23.533x^{0.4933}$	0.9701	5.0

The coefficient of determination (R^2) and SEE were 0.72, 0.75, 0.58 (Table 1) and 15.1, 14.4, 19.2 days for the three correlations, respectively (Figure 2).

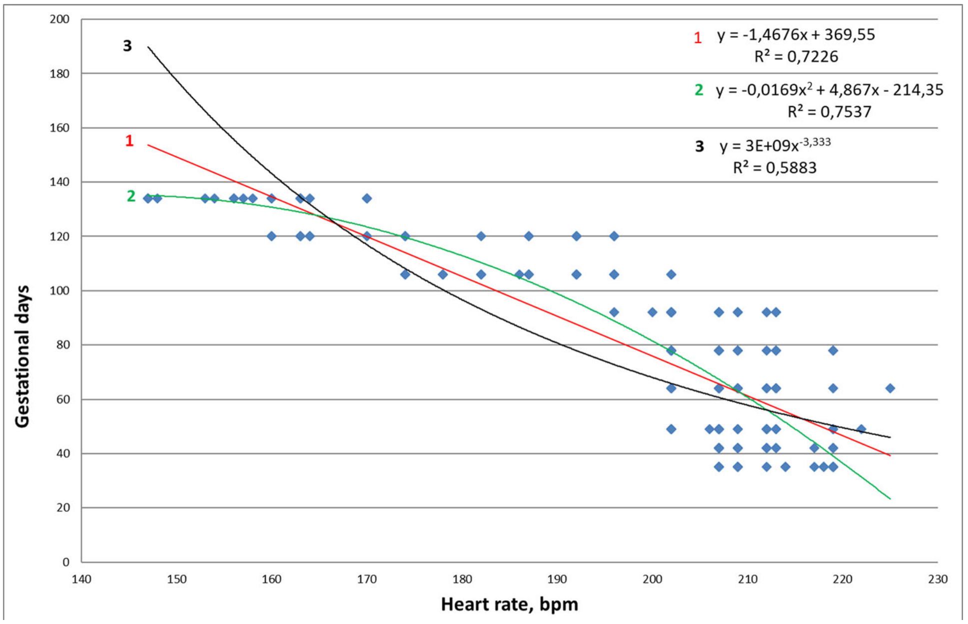

FIGURE 2 Sample scatterplots showing the linear (1), quadratic (2) and exponential (3) correlation between fetal heart rate and gestational age of the goat fetus

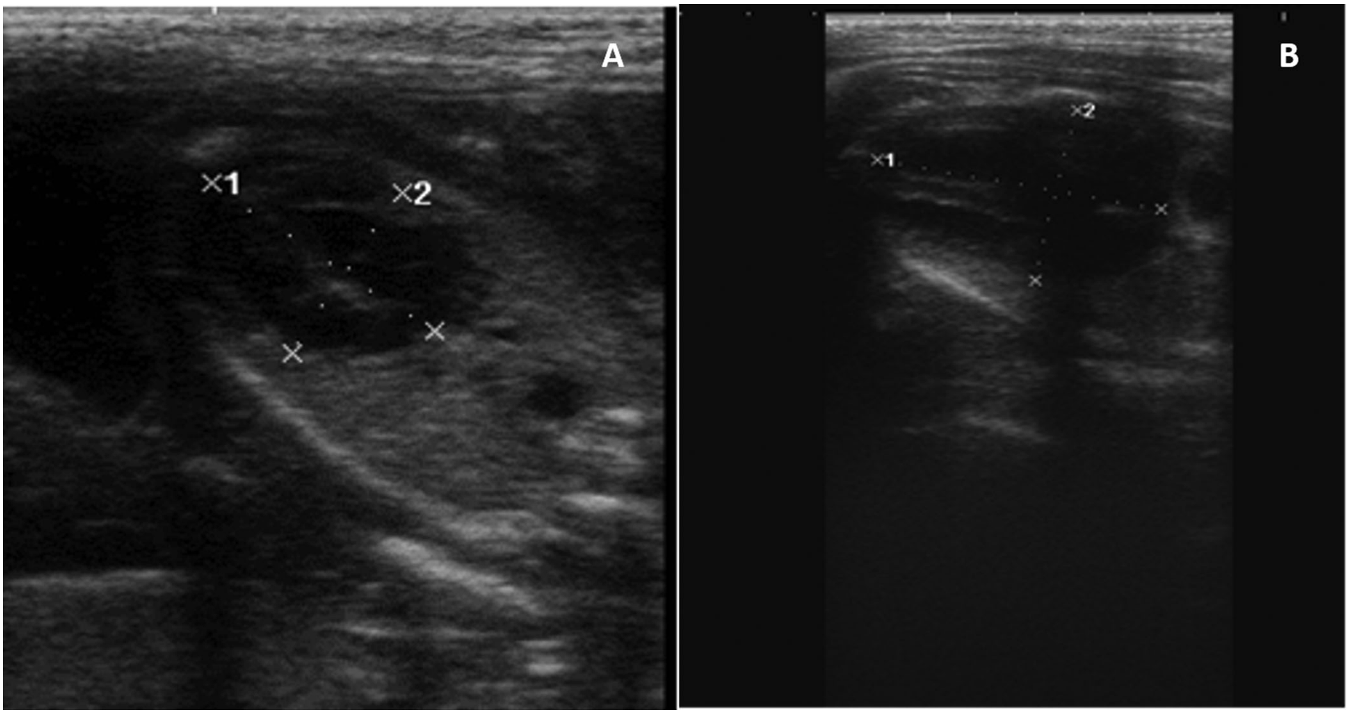

FIGURE 3 B-mode ultrasonographic exam for visualization and measurement of long (1) and short (2) fetal heart axis ongestational day 91 (a) and 133 (b) in a goat fetus

Fetal heart visualization with distinct echogenicity from the surrounding fetal tissues was registered for the first time at the end of the first trimester (day 49).

The established average fetal heart size was 6.1 ± 1.05 mm for the long and 4.5 ± 0.67 mm for the short heart axis at the pregnancy day 49. Fetal heart development with progress of pregnancy was accompanied by change of echogenicity. From hypoechogenic during the second trimester (day 91), it changed into an echogenic with hypoechogenic contour towards the end of the studied period (day 133) (Figure 3).

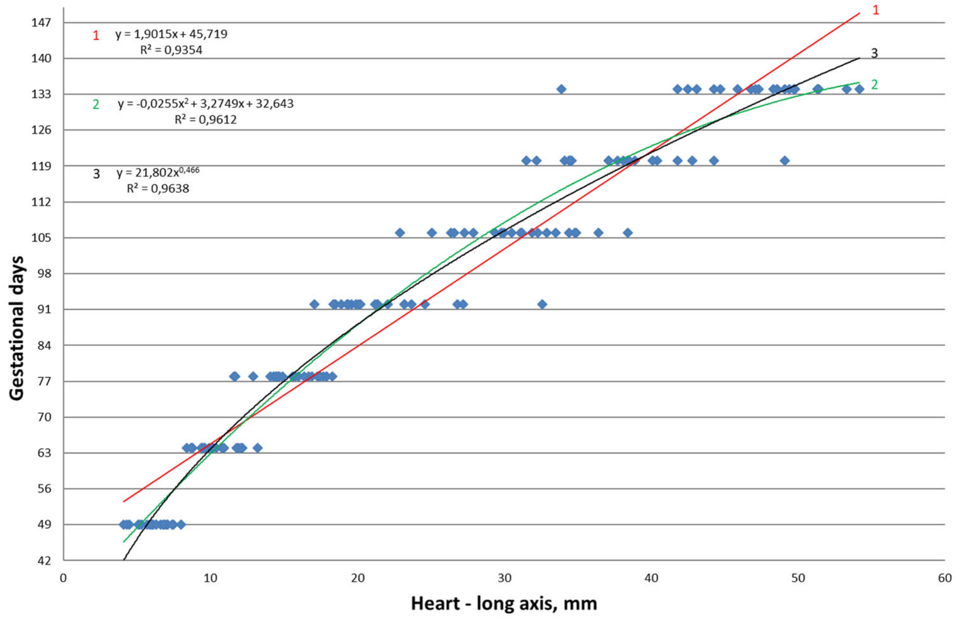

FIGURE 4 Sample scatterplots showing the linear (1), quadratic (2) and exponential (3) correlation between long axis of the fetal heart ultrasonographic measures and gestational age of the goat fetus

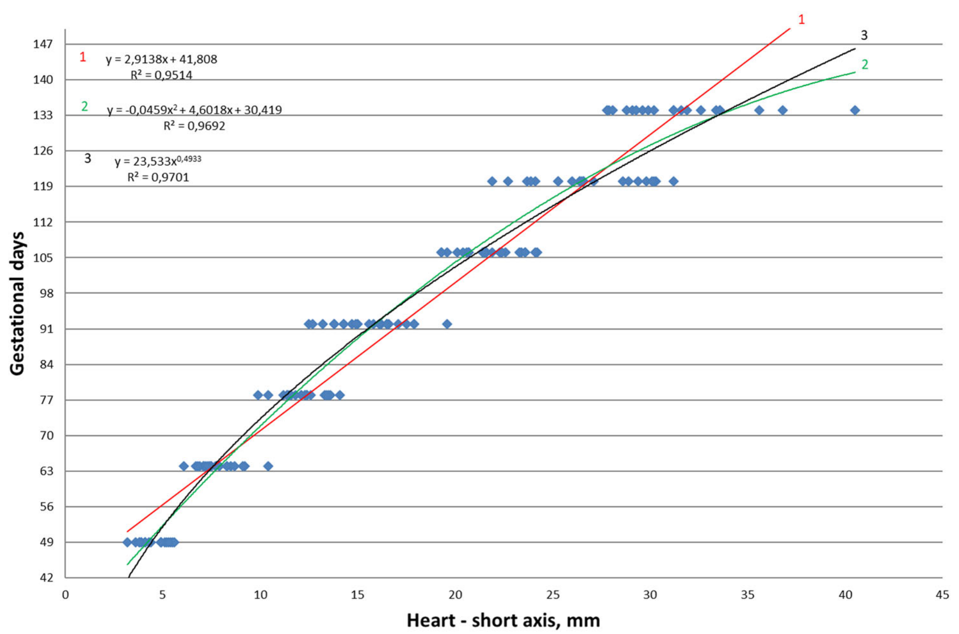

FIGURE 5 Sample scatterplots showing the linear (1), quadratic (2) and exponential (3) correlation between short axis of the fetal heart ultrasonographic measures and gestational age of the goat fetus

Longitudinal diameter (long axis) had values of $R^2 = 0.94, 0.96, 0.96$ and SEE = 7.0, 5.5, 5.5 days for the linear, quadratic and exponential correlations, respectively (Figure 4).

The coefficient of determination for the transverse fetal heart diameter (short axis) was higher than that of the external one ($-R^2 = 0.95$, 0.97, 0.97). At the same time, the SEE was lower (SEE = 6.1, 4.9, 5.0 days) compared to that of the long fetal heart axis (Figure 5).

4 | DISCUSSION

Registered fetal heart pulse values in all three measurements showed significant drop (186.77 ± 8.78, 176.95 ± 13.13, 158.13 ± 8.14) in the last trimester, which could in practice be used as a parturition precursor. Fetal heart rate reduction with progress of pregnancy in goats was reported by other researchers as well (Karen et al., 2009; Martinez et al., 1998). Although fetal heart rate is an indicator that could be measured and monitored during a long period of pregnancy, we think that it is not an appropriate criterion for exact determination of gestational age in local goats, especially concerning the first and second trimester. This statement of ours results from the fact that differences in fetal heart rate in different animals in the same gestational period were significant. For example, the lowest value measured on gestational day 77 in the present study was 202 bpm, while the highest one was 219 bpm. Certainly, these differences could be caused by errors in measurement since correct recording of cardiac pulse requires both the mother and the fetus to be at full rest. Each movement of the fetus or the mother during recording results in change in the echocardiogram, which in turn requires second measurement. This takes more time and adds more stress to the mother. It is known that stress during pregnancy has a negative effect, hypothetically it could affect fetal cardiac activity as well (Amer, 2010; Serin et al., 2010). All these facts and hypotheses are sustained by the low coefficient of determination (*Rš* = 0.72, 0.75, 0.58), established in the present study. The results published by Karen et al. (2009) and Serin et al. (2010) correspond to our data, and they also found low coefficient of determination, *Rš* = 0.55 and *Rš* = 0.77, respectively. Low correlation between fetal heart rate and gestational age is accompanied by relatively high (14–19 days) SEE. The above findings give us reason to consider that ultrasound imaging of the heart rhythm is an extremely important indicator for confirmation of pregnancy and embryo/fetus vitality assessment. Reduction of heart pulsations during the last trimester accompanied by changes in the size and echogenicity of the cardiac muscle could be an indicator of the gestational phase. However, the use of fetal heart rate for determining gestational age is not a precise indicator.

Although we were able to visualize heart and cardiac activity on gestational day 28, correct ultrasound measurement of the long and short axes of the fetal heart in local goats was possible for the first time as early as gestational day 49. The reason behind that is the similar echogenicity of the cardiac structure and the embryonic/fetal tissues around it during the first trimester. For cardiac axes to be accurately measured, it is necessary to freeze the image on the screen and to distinguish sonographically the cardiac muscle from the remaining fetal parts. In support of this, Raja Ili Airina et al. (2011) studied sonographically fetal heart structure and reported that fetal heart had a specific shape after gestational week 8. The authors traced changes in heart echogenicity and reported that with progress of pregnancy echogenicity altered and it was visualized as an echogenic structure up to gestational week 4, afterwards echogenicity decreased and it was seen as a greyish-white, grey, greyish-black and black structure in gestational weeks 4–8, 8–12, 12–16 and 16–21, respectively. This change in echogenicity, according to the authors, is due to the increased amount of blood in the fetal heart and with progress of pregnancy it is visualized as a hypo- or an echogenic structure (Raja Ili Airina et al., 2011). We registered similar changes in fetal heart echogenicity with the difference that monitoring of the size started in gestational week 7. At that time, specific shape and echogenicity were observed, different from that of the surrounding fetal tissues (Raja Ili Airina et al., 2011). Our results, similar to Lee et al. (2005), showed significant correlation of gestational age with the longitudinal and transverse fetal heart diameter. However, in our study correlation is higher in the transverse (short) axis (*Rš* = 0.95, 0.97, 0.97) and lower in the longitudinal (long) heart axis (*Rš* = 0.93, 0.96, 0.96). In the report of Lee et al. (2005), it is the opposite, longitudinal diameter has higher correlation (*Rš* = 0.91) to gestational age than the transverse one (*Rš* = 0.88). According to the authors, the lower correlation in the transversal diameter (short axis) results from variations in size during systole and diastole of the fetal heart muscle. In our study, these variations were minimized due to the option of the sonographic device to analyze retrospectively the saved image. This option makes it possible to measure the short heart axis always in diastole, that is, when the transverse diameter is the greatest. This is why we consider the transverse diameter as a more reliable indicator for determination of fetal age. This is supported by the smaller SEE in the short (5–6 days) rather than that in the long heart axis (5.5–7 days). Nevertheless, determining the size of both heart axes combined with change in cardiac muscle echogenicity allows for more precise determination of the gestational phase.

5 | CONCLUSION

In conclusion, the cardiac fetal ultrasonographic morphological analyses, like transverse and longitudinal heart axes measurements, show to be a precise indicator for age gestational determination in the local Bulgarian goats. The average fetal heart rate values decreased within pregnancy progression. The measurement of fetal heart rate values isolated is recommended to forecast gestational age when the mating date is unknown and has to be used with caution.

ETHICAL APPROVAL

The experiment was approved by the Animal Ethics Committee to the Faculty of Veterinary Medicine, Trakia University - Stara Zagora, in compliance with the minimum requirements for protection and welfare of experimental animals according to Ordinance Number 20/1.11.2012 of the Ministry of Agriculture and, Food and Forestry, Republic of Bulgaria.

ACKNOWLEDGEMENTS

This research was funded by National scientific program "Reproductive biotechnologies in breeding in Bulgaria" – REPROBIOTECH (Number 0406-105), Ministry of Education and Science, Bulgaria.

AUTHOR CONTRIBUTIONS

Conceptualization, formal analysis, investigation and methodology: Manol Karadaev. *Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization and writing-original draft*: Ivan Fasulkov. *Conceptualization, formal analysis, funding acquisition, project administration and supervision*: Nasko Vasilev. *Data curation, formal analysis and software*: Stefka Atanasova.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.564.

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How to cite this article: Karadaev, M., Fasulkov, I., Vasilev, N., & Atanasova, S. (2021). The use of ultrasonographic measurement of the heart size and fetal heart rate variation for gestational age determination in local Bulgarian goats. *Veterinary Medicine and Science*, *7*, 1736–1742. https://doi.org/10.1002/vms3.564