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RESEARCH ARTICLE

Ultrasound Parameters of Embryo-Fetal Morphometry and Doppler Indices in the Umbilical Artery During the First Trimester of Pregnancy in Goats

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A B S T R A C T

The aim of this study was to determine the biometric parameters of the embryo/fetus and to assess the haemodynamics in the umbilical artery during the first trimester of pregnancy in 14 Boer goats. All the goats under study had twin pregnancy. Gestational vesicle diameter (GSD), crown-rump length (CRL), biparietal diameter (BPD), occipital-nasal length (ONL), trunk diameter (TD) and the placentome diameter (PD) were measured from the 30th to the 45th day of pregnancy at 5-day intervals, using a B-mode ultrasound scanner. Measurements of blood flow parameters in the umbilical artery were made using a colour Doppler combined with a pulse wave Doppler technique. During the study period, there was a significant ($P<0.05$) increase in the diameter of the gestational sac and most of the biometric parameters of the embryo/fetus. The average CRL on day 45 was almost 10 times higher than that on day 30 of pregnancy. The peak systolic velocity in the arterial vessels on day 45 of pregnancy was higher ($P<0.05$) than that from 30 to 40 days of pregnancy. On the other hand, mean pulsation index (PI) at day 45 of pregnancy was lower ($P<0.05$) than that on day 30 and day 40 of pregnancy. No end-diastolic velocity was diagnosed in any goat during the study period. All parameters showed positive ($P<0.01$) correlations with the day of pregnancy, except the PI in the arterial vessels, which showed negative ($P<0.01$) correlation with the day of pregnancy. In conclusion, changes in the parameters of embryo-fetal morphometry, placentomes and blood flow parameters of the umbilical artery may be helpful in assessing the development of the embryo and the fetus during the first trimester of pregnancy in goats.

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INTRODUCTION

Ultrasonography is a useful diagnostic imaging technique used in the reproductive management of small and large animals, as well as in humans. This technique is mainly used for the detection of early pregnancy, monitoring of embryo/foetal development and the assessment of the number and gender of fetuses (Jones and Reed, 2017). Previous studies in goats indicate that the presence of a gestational vesicle (GS) in the lumen of the uterus is a confirmation of developing embryo (Muhammad and Aziz, 2021). In goats, pregnancy lasts for about 155 days on the average and this period is divided into three trimesters. The first trimester lasts until the 49th day of pregnancy, the second from the 50th to the 100th day of pregnancy, and the third trimester begins on the 101st day of pregnancy (Karadaev *et al.*, 2018). Previous studies also showed that from the 35th day of goat's pregnancy, organs such as forestomach are visible, which are indicative of an early stage of fetal development (Garcia *et al.*, 2014).

Loss of pregnancy can occur at any time, with the highest percentage of losses occurring in the early and late stages of embryonic development and in the early stages of fetal development, including the first trimester of pregnancy (Samir *et al.*, 2016). Determining the exact causes of fetal loss or embryonic mortality in ruminants is difficult due to limitations in accurately determining the state of early pregnancy. However, studies emphasize the importance of ultrasonography in monitoring the loss of embryos and fetuses (Jones *et al.*, 2016). These studies indicate a high efficiency of detecting pregnancy loss in

ruminants, amounting to nearly 100%, which results in a small number of false negative diagnoses (Samir *et al.*, 2016; Jones *et al.,* 2016). For the ultrasonographic evaluation of the development of the fetus from the 30th day of pregnancy, parameters such as crown-rump length (CRL), biparietal diameter (BPD), orbital diameter (OD) and chest diameter (CD) are used (Karadaev *et al.,* 2018; Yazici *et al.,* 2018). Placentome diameter (PD) is also used in the ultrasound diagnosis of pregnancy (Enginler *et al.,* 2021). In addition to ultrasound diagnostics in the assessment of embryo/fetal development through B mode, much attention is paid to Doppler examinations, enabling the determination of the haemodynamics of the umbilical artery (Elmetwally *et al.,* 2016a,b; Elmetwally and Meinecke-Tillmann, 2018; Stankiewicz *et al.,* 2020).

The umbilical cord is the only link between the mother and the fetus through which respiratory gases, nutrients and metabolites can be transported. The correct structure and function of the umbilical vessels and the entire umbilical cord determine the proper development of the fetus. It is extremely important to confirm the correct number of umbilical vessels and their function in the abdominal cavity (Krzyżanowski *et al.,* 2019). Determining the pulsation index (PI) and resistance index (RI) of umbilical cord vessels is an important element of Doppler ultrasound examination and often a key element in the assessment of pregnancy development. An increased umbilical artery RI is associated with intrauterine growth retardation, congenital anomalies and other abnormalities in the course of pregnancy (Elmetwally *et al.,* 2016a,b). No lesions have been found in studies of the umbilical artery in pregnant goats in single and multiple pregnancies so far, such as end-diastolic flow (EDV) or a significant difference in PI and RI in single and multiple pregnancies (Serin *et al.*, 2010).

Most studies focus on the effectiveness of pregnancy detection, gestational age and the assessment of Doppler parameters in the second half of pregnancy (Airina *et al*., 2011; Elmetwally *et al*., 2016a,b; Gouda *et al*., 2021). The studies on the haemodynamics of umbilical vessels in the early stages of pregnancy in goats are limited. Therefore, aim of the present study was to determine the biometric parameters of the embryo, gestational vesicle and placenta, as well as to assess haemodynamics in the umbilical artery, during the first trimester of pregnancy in goats.

MATERIALS AND METHODS

Experimental animals and management: The present study was carried out on 14 Boer goats from September to December, 2021. These goats were multiparous (5-6 years old), with a balanced body weight (80 kg) and were kept on an ecological goat farm in Kołbacz (Poland: latitude 53'30 "N). They were kept on the pastures, as well as under indoor system with uniform feeding. In the grazing season, from May to October, the goats grazed on the meadow and received oats, hay and straw in the farm. In winter, from November to April, the goats stayed inside the pen, where they were fed with oats, straw and hay. The animals had constant access to water and salt licks.

The experimental goats were mated in a natural way during the natural breeding season. The stage of pregnancy was determined on the basis of the day of mating and the mating efficiency was confirmed by transrectal ultrasound (EDAN U50 ultrasound scanner, 4 MHz linear probe model V742UB) during the period from 20th to 30th day after mating. The date of conception was confirmed retrospectively on the assumption that the pregnancy lasted 148 days (Đuričić *et al.*, 2012). All the goats under study had twin pregnancy.

Ultrasound examination: The ultrasound examination was performed trans-abdominally from the 30th to the 45th day of pregnancy at 5-day intervals, using an ultrasound scanner (EDAN U50 ultrasound scanner) equipped with a sector probe with a frequency of up to 5 MHz (Model, C352UB). Before the examination, the inguinal area of the abdomen was clipped and a transmission gel was applied. The gestational vesicle diameter (GSD), crown-rump length (CRL), biparietal diameter (BPD), occipital-nasal length (ONL), trunk diameter (TD) and placentome diameter (PD) were recorded using B-mode ultrasound. The 10 largest placentomes were measured in each goat, 5 lying near the fetus and 5 lying in the uterine horns (Lekatz *et al.,* 2013) and the obtained results were then averaged. The diameter was measured at the highest position of ultrasound imaging (Stankiewicz *et al.,* 2020). In this study, the outer diameter of placentomes was measured, i.e. between the two most distal parts of the bearing (Lee *et al.,* 2005; Nwaogu *et al.,* 2010).

Measurements of blood flow parameters in the umbilical artery were made using a colour Doppler combined with a pulse wave Doppler technique. After locating the umbilical artery, blood flow measurements were taken and the following Doppler parameters were determined: peak systolic velocity (PSV), end diastolic velocity (EDV), resistance index (RI), and pulsation index (PI). The flow angle during the test was as close as possible to zero degree. The Doppler ultrasound examination of each goat took approximately 20-30 minutes. Doppler measurements were made on at least 5 continuous, regular waves of the Doppler spectrum. The ultrasound images and all relevant measurements were recorded automatically by the ultrasound scanner and saved. Measurements were not recorded during maternal and fetal movements. If any symptoms of anxiety appeared, the ultrasound examination was interrupted and repeated at a later date. No pharmacological interference was applied to the test animals, and proper welfare was ensured during the study.

Statistical analysis: Morphometric parameters are presented as mean ± standard deviation (SD), while Doppler parameters as mean±standard error of the mean (SEM). In the initial analysis, no significant differences were found between the two twin fetuses. Therefore, the results for both fetuses were averaged. The differences among means of four periods of pregnancy were analysed using the analysis of variance and the post-hoc test. Duncan's multiple range test was used to verify the significance of differences among means. The correlations between the tested parameters and days of pregnancy were calculated using the Pearson's correlation coefficient (r). Statistical analysis was performed using STATISTICA version 13.1, Stat Soft, Poland.

Fig. 1: Representative ultrasonograph showing the measurement of the crown-rump length of a goat on day 45 of pregnancy (CRL1).

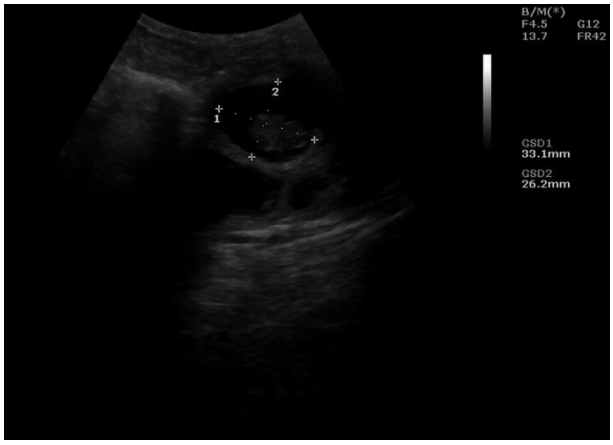

Fig. 2: Representative ultrasonograph showing the measurement of the gestational sac diameter of a goat on day 40 of pregnancy (GSD1, GSD2).

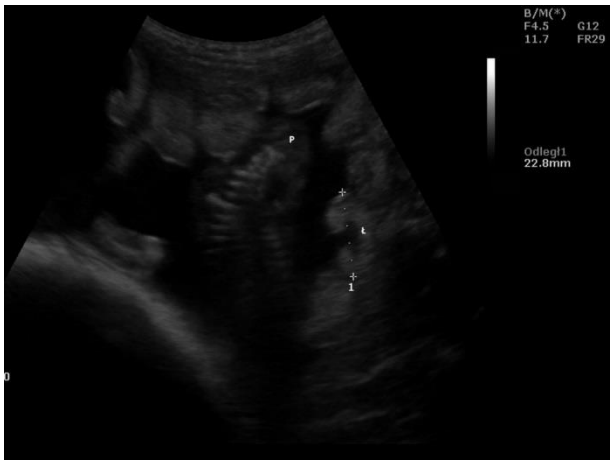

Fig. 3: Representative ultrasonograph showing the position of the fetus (P) and the assessment of placenta diameter (L) on day 45 of pregnancy in a goat.

RESULTS

Table 1 shows the mean values of the biometric parameters of the embryo/fetus, gestational sac and placentomes during the period from 30 to 45 days of pregnancy. In the analysed period, there was a significant increase in the diameter of the gestational sac and most of the examined biometric parameters of the embryo/fetus. The average CRL on day 45 was almost 10 times greater than on day 30 of pregnancy. There was also an increase in GSD and placentome diameter, but the differences in GSD between days 35 and 40 of gestation and placentome diameter between days 40 and 45 were statistically non-significant. Representative ultrasound images of the gestational vesicle, placenta and embryo are presented in Fig. 1-3.

Table 1: Biometric parameters of the embryo/fetus, embryonic vesicle and placentomes during the first trimester of pregnancy in goats (n=14)

Parameters		Days of pregnancy			
		30	35	40	45
GSD (mm)	mean±SD	21.44±1.24[a]	29.92±1.18[b]	31.47±1.58[b]	39.13±2.98[c]
	range	(19.90-24.00)	(27.20-32.10)	(29.10-34.01)	(34.90-43.60)
PD (mm)	mean±SD	8.42±0.76[a]	9.73±0.64[b]	11.07±0.81[c]	11.71±1.78[c]
	range	(7.65-10.01)	(8.95-11.00)	(9.58-12.20)	(8.49-14.01)
CRL (mm)	mean±SD	3.30±0.51[a]	17.83±2.66[b]	21.33±2.34[c]	30.94±1.61[d]
	range	(2.39-4.21)	(14.00-23.80)	(15.90-25.10)	(28.30-33.10)
BPD (mm)	mean±SD	6.13±0.13[a]	7.19±0.34[b]	8.05±0.49[c]	11.80±3.17[d]
	range	(5.92-6.30)	(6.85-7.91)	(7.20-8.51)	(8.60-16.20)
ONL (mm)	mean±SD	6.27±0.31[a]	7.21±0.69[b]	10.59±0.41[c]	18.24±1.66[d]
	range	(5.54-6.58)	(6.32-8.90)	(10.11-11.25)	(16.30-22.00)
TD (mm)	mean±SD	6.82±0.44[a]	10.05±0.61[b]	11.16±0.45[c]	14.38±2.16[d]
	range	(5.99-7.59)	(9.20-11.30)	(10.50-11.90)	(11.20-17.30)

GSD –gestational sac diameter; PD – placentome diameter; CRL –crown-rump length; BPD –biparietal diameter; ONL - occipital-nasal length; TD –trunk diameter: Mean values within a row marked with different alphabets differ significantly (P<0.05).

Table 2: Mean (±SEM) values of Doppler parameters in the umbilical artery during the first trimester of pregnancy in goats (n=14)

Parameters	Days of pregnancy			
	30	35	40	45
PSV (cm/s)	21.30±1.02[a]	22.44±0.71[a]	21.51±0.34[a]	26.65±0.93[b]
EDV (cm/s)	NO	NO	NO	NO
RI	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
PI	2.98±0.09[a]	2.83±0.10[a]	2.75±0.11[a]	2.25±0.09[b]

PSV – peak systolic velocity; EDV – end-diastolic velocity; RI – resistance index; PI – pulsatility index NO – incalculable value: Values with different letters in a row are significantly different from one another (P<0.05).

Table 3: Pearson correlation coefficients (r) between the day of pregnancy and the biometric parameters of the embryo, placentomes diameter, gestational sac, and Doppler parameters in the umbilical artery in goats during the first trimester of pregnancy (n = 14)

Parameters	Correlation coefficients
Gestational sac diameter (GSD)	0.93**
Placentome diameter (PD)	0.75**
Crown-rump length (CRL)	0.95**
Biparietal diameter (BPD)	0.75**
Occipital nasal length (ONL)	0.92**
Trunk diameter (TD)	0.90**
Peak systolic velocity (PSV) in arterial vessels	0.51**
Pulsation index (PI) of arterial vessels	-0.60**

** = Significant at P<0.01.

Table 2 shows the mean values of Doppler blood flow parameters in the umbilical artery in the first trimester of pregnancy in goats. PSV from the 30th to the 40th day of pregnancy was similar, while on the 45th day the value of this parameter was significantly higher (P<0.05). On the other hand, the mean value of pulsatility index (PI) at the end of the study period (day 45 of pregnancy) was significantly lower (P<0.05) than in the period from the day 30 to the day 40 of pregnancy. Resistance index (RI) remained the same throughout the study period. No end-diastolic velocity (EDV) could be detected in any goat during the study period (Fig. 4).

Pearson's correlation coefficients between the day of pregnancy and the biometric parameters of the embryo, the diameter of the placentomes and gestational sac, and

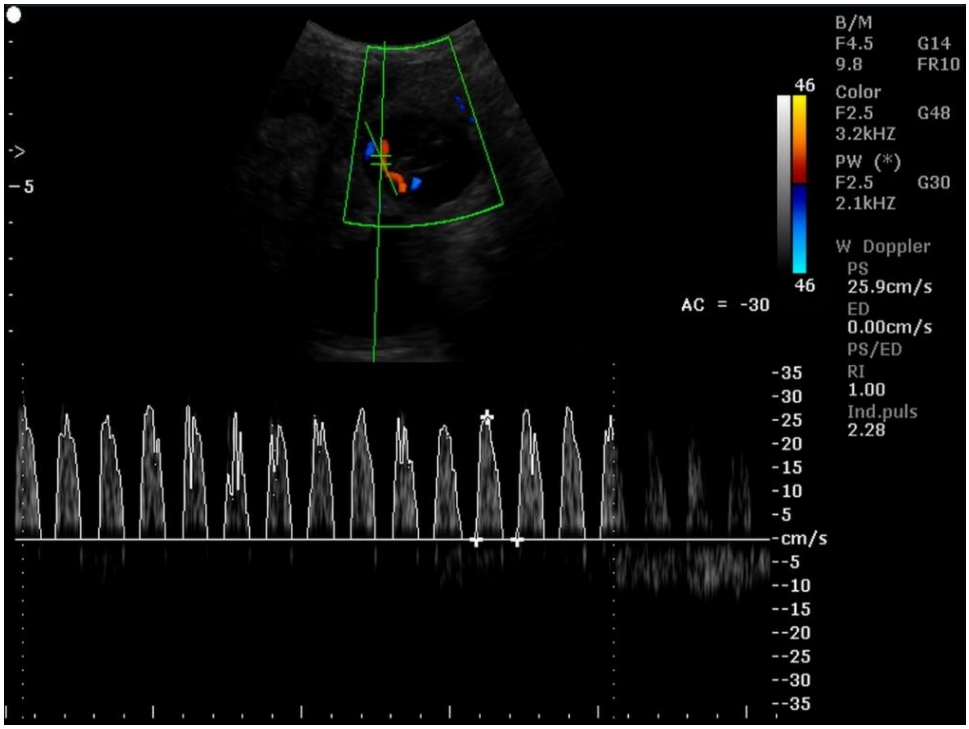

Fig. 4: Ultrasound image obtained using the pulse Doppler method showing the hemodynamic measurement of the umbilical artery on day 45 of pregnancy in a goat. PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. Puls - pulsation index.

Doppler parameters in the umbilical artery in goats during the first trimester of pregnancy are shown in Table 3. All parameters exhibited significant positive correlations with the day of pregnancy, except the pulsation index (PI) in the arterial vessels, which showed significant negative correlation with the day of pregnancy ($P<0.01$).

DISCUSSION

During an ultrasound examination, the pregnancy vesicle is visible as an anechoic spherical area with a hyperechoic embryo inside. However, type and frequency of the ultrasound head used in the examination can affect the visualization time and the measured diameter of the gestational sac (Devi *et al.*, 2019). The size of the gestational sac can be assessed using a transrectal transducer as early as 19 days after conception (Amer, 2010), while a more accurate method of assessing the gestational vesicle in goats is the transabdominal examination from the day 25 of pregnancy (Devi *et al.*, 2019). On the other hand, other authors suggest delaying the test until the 32nd day of pregnancy to avoid a false positive diagnosis resulting from early embryo loss (Jones *et al.*, 2016). In this experiment, a transabdominal transducer was used and the GSD diameter was measured from the 30th day of pregnancy and it was found that it was strongly correlated with the day of pregnancy. These results differ from those reported by Karadaev *et al.* (2016), who performed the first measurement on day 21 of pregnancy using a transrectal probe and diameter of the gestational sac was recorded as 10.3±2.7mm.

Crown-rump length is a measurement of the length of the embryo and fetus from the top of the head to the bottom of the buttock and is useful for determining growth rate and gestational age. CRL measurement in goats can be performed from days 21 to 49 of pregnancy (Karadaev *et al.*, 2018). However, some authors have shown the possibility of determining CLR of a goat fetus as early as on the 19th day of pregnancy and in the later stages of fetal development, reaching even the day 75 of pregnancy (Kuru *et al.*, 2018). In this study, the CRL was recorded from day 30 to day 45 of pregnancy and was strongly correlated with the day of pregnancy (R=0.95). Similarly, Gouda *et al.* (2021) and Kandiel *et al.* (2015) showed a high correlation between CRL and gestational age in sheep and goats, respectively. This indicates the reliability of this parameter in determining the gestational age in the first trimester of pregnancy. Interestingly, there was a 10-fold increase in CRL between 30 and 45 days of pregnancy, which was not observed by Karadaev *et al.* (2016). The differences are probably due to the individual breed characteristics of goats, type and frequency of the ultrasound probe used in the examination.

During the first trimester of pregnancy, internal organs such as the stomach, heart and lungs develop intensively. Therefore, during ultrasound examination of pregnancy in goats, the trunk diameter (TD) of the fetus is also analysed. First TD measurement can be performed from day 28 of pregnancy (Karadaev *et al.*, 2016). In this study, the abdominal or trunk diameter was defined as the diameter at the height of the stomach and liver or at the entrance of the umbilical cord to the fetus (Kandiel *et al.*, 2015) and measured from the day 30 of pregnancy. A significant increase in the TD was recorded from day 30 to day 45, which on the 45th day after insemination was 14.38±2.16 mm. However, Kandiel *et al.* (2015) reported the mean abdominal diameter of 16.32 mm in Shiba goats on day 42 of pregnancy. The differences seem to be due to the individual breed characteristics. The present study also showed a higher correlation between the day of pregnancy and TD (r=0.90) than in the case of BPD (r=0.75). Lee *et al.* (2005) also showed higher correlation between TD and

gestational age than BPD and CRL in Korean black goats. This indicates the reliability of TD in determining the gestational age in goats.

In the present study, BDP and ONL were recorded from the day 30 of pregnancy. On the other hand, Yazici *et al.* (2018) indicated the possibility of measuring the parameters of the fetal head only from the 37th day after conception. Similar observations to ours were made by Karadaev *et al.* (2016) and Nwaogu *et al.* (2010). In the present study, it was shown that ONL had higher correlation with pregnancy day (r=0.92) than BPD (r=0.75). However, Nwaogu *et al.* (2010) and Yazici *et al.* (2018) recorded relatively higher correlation between BPD (r=0.98; r=0.99) and ONL (r=0.97; r=0.99) with gestational age. These differences probably result from the characteristics of the studied breeds of goats.

In goats, the placentomes are perceived as concave structures with round shape. On the ultrasound image, depending on the imaging plane, they appear as gray image in the shape of the letter C or O (Rasheed, 2016). The previous studies show that during the first trimester of pregnancy, the mean diameter of the placentomes increases rapidly. Karadaev *et al.* (2018) indicated the mean diameter of placentomes as 9.00±1.4 mm on the 42nd day of pregnancy. These authors also suggested that the measurement of placentome diameter may be done from the day 42 of pregnancy, when they take a species-specific shape. In the present study, each placentome differed in size regardless of the age of the fetus. Similar observations were made by Karadaev *et al.* (2016; 2018). The increase in the size of the placentomes in the first half of pregnancy is mainly related to the expansion of the fetal villi and the associated network of capillaries. Rasheed (2016) showed wide differences in placentome size, depending on whether they are closer to the fetus or in the corners of the uterus. In the present study, the placentome diameter showed lower correlation with the day of pregnancy than most of the other parameters. Similar observations have been reported by Karen *et al.* (2009). According to Nwaogu *et al.* (2010), placentome diameter is the least reliable parameter in the assessment of gestational age in goats.

The changes in the size of the embryo-fetal parameters as well as the placentomes in goats in the first trimester of pregnancy reported in this study can also affect the changes in the haemodynamics of the umbilical artery. In the present study, the peak systolic velocity increased significantly in the last period (day 45) of the first trimester of pregnancy, while the end-diastolic velocity of blood flow in the umbilical artery was not recorded in any goat. Similar observations were made by Elmetwally and Meinecke-Tillmann (2018) in goats and sheep. In our view, the end-diastolic velocity of blood flow in the umbilical artery is not detected between weeks 4 and 12 of pregnancy. It should be noted that the PSV/EDV ratio was not determined due to the lack of measurement of the end-diastolic velocity during the first trimester of pregnancy. According to Stankiewicz *et al.* (2020), a significant decrease in the PSV/EDV ratio is found from the day 70 of pregnancy. Elmetwally *et al.* (2016a,b) have reported that the PSV/EDV ratio, which is one of the most important Doppler parameters, indicates about changes in end-diastolic velocity, especially in the second and third trimesters of pregnancy.

In the current study, the resistance index (RI) remained unchanged from day 30 to day 45 of pregnancy. Elmetwally *et al.*(2016a,b) indicated that the RI through week 8 of pregnancy remained unchanged at 1.00±0.00 and then dropped sharply to week 19. According to Serin *et al.* (2010), the RI increases from day 40 to day 55 of pregnancy, followed by a decrease.

Previous studies have shown that PI increases from day 39 to day 60 of pregnancy and then decreases to day 90 of pregnancy (Kumar *et al.*, 2015; Troisi *et al.* 2018). In the present study, the PI value did not change from day 30 to day 40 of pregnancy but decreased significantly at day 45. At the same time, the study also showed that the pulsation index was negatively correlated with the day of pregnancy. On the other hand, PSV showed a significant positive correlation with the day of pregnancy. Therefore, it can be assumed that along with the development of the fetus, the value of the umbilical artery blood flow pulsation index decreases in favour of an increased peak value of the systolic velocity in the first trimester of pregnancy. It is probably related to the intensive development of the fetus and the increased demand for nutrients.

Conclusions: The results of the current study showed that changes in the parameters of embryo-fetal morphometry, placentomes and blood flow parameters of the umbilical artery may be helpful in the assessment of fetal development during the first trimester of pregnancy in goats. Further research may be performed, especially with regard to any feto-placental abnormalities during pregnancy in this species.

Authors contribution: NW, TS, BB and JU conceived the idea and designed the study. NW, TS, BB and JU executed the experiment. BB, JU, NW and TS analyzed the data. All authors interpreted the data, reviewed the manuscript for important intellectual contents, and approved the final version.

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