



# Doppler ultrasound measurements of the blood flow velocity in the fetal heart and aorta in Bulgarian White milk goats

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## Abstract

The aim of the present study was to determine the parameters characterising the blood flow velocity in the fetal heart and aorta in goats by Doppler ultrasonography. Twenty-four Bulgarian White milk goats, aged 4–6 years, weighing 45–51 kg were used in the study. Doppler ultrasound measurements of the blood flow velocity in fetal heart were done at the end of first, second and third trimesters of pregnancy, and in the fetal aorta – at the end of the second and third trimesters of pregnancy. Ultrasound investigations were carried out by transabdominal approach with linear and convex transducers, with frequency 8.0 MHz. Blood flow parameters that included maximum and minimum systolic velocity, end-diastolic velocity, pulsatility index, resistance index and systolic/diastolic ratio were measured by spectral Doppler ultrasonography. The results showed that the highest maximum systolic velocity in the fetal heart was observed at the end of the first pregnancy trimester. Statistically significantly ( $p < 0.05$ ) lower values of this parameter were registered in the second and third pregnancy trimesters. A similar tendency was observed for minimum systolic velocity, end-diastolic velocity and systolic/diastolic ratio. The pulsatility index and resistance index had similar values for the three studied periods. Examination of the fetal aorta showed statistically significant differences ( $p < 0.05$ ) of minimum systolic velocity, end-diastolic velocity, pulsatility and resistance index in the third, compared with the second trimester of pregnancy. The analysis of the results suggested that Doppler ultrasonography can be used to monitor fetal blood flow changes during the various stages of pregnancy.

## KEYWORDS

aorta, Doppler, fetal heart, goats, ultrasonography

## 1 | INTRODUCTION

Ultrasonography is a non-invasive, fast and accurate method for early pregnancy diagnosis (Yotov, 2020), monitoring of the embryo–fetal development (Bartlewski et al., 2000), estimation of fetal

number and sex (Karen et al., 2009; Santos et al., 2007), determination of changes in reproductive organs (Atanasov et al., 2012) and mammary gland (Fasulkov et al., 2018) in ruminants.

In goats, B-mode ultrasonography is mainly used for diagnosis of early pregnancy, monitoring of embryo–fetal development,

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determination of gestational age and estimation of fetal number and sex (Abdelghafar et al., 2011, 2012; Amer, 2010; Erdogan, 2011; Karadaev et al., 2019; Karadaev et al., 2018; Karen et al., 2014; Lee et al., 2005; Nwaogu et al., 2010; Suguna et al., 2008).

The application of Doppler ultrasonography in its three variants (colour Doppler, pulse Doppler and continuous wave Doppler) has been reported in mares (Bollwein et al., 2004), cows (Bollwein et al., 2000; Panarace et al., 2006), sheep (Panarace et al., 2008; Reed et al., 1996; Triosi et al., 2018), goats (Kumar et al., 2015; Serin et al., 2010), dogs (Alvarez-Clau & Liste, 2005; Di Salvo et al., 2006; Köster et al., 2001) and cats (Scotti et al., 2008). In the last 15 years, colour Doppler ultrasonography has been increasingly used to study blood flow in cow reproduction (Bollwein et al., 2000, 2016; Herzog & Bollwein, 2007; Miyamoto et al., 2005). This provided new information on the physiological and pathological processes in the genital organs of cows, but literature data on the use of the method in small ruminants are scarce. In cattle reproduction, colour Doppler ultrasonography was used to monitor follicular and luteal blood flow during the oestrus cycle, blood flow velocity in the vessels supplying the uterus during pregnancy and the postpartum period (Bollwein et al., 2016). This technique was applied to investigate fetal blood vessels such as *a. umbilicalis*, *v. cava caudalis*, aorta and *ductus venosus*, as well as utero-placental blood vessels (Aardema et al., 2001; Acharya et al., 2005; Bollwein et al., 2004; Mulic-Lutvica et al., 2007; Panarace et al., 2008; Reed et al., 1996; Serin et al., 2010). Doppler ultrasonography analyses the blood flow in the examined blood vessels, most often calculating the resistance index (RI) and pulsatility index (PI). RI is calculated by the formula  $SD/S$ , where S is the peak systolic velocity, D is the end-diastolic velocity, and SD is the systolic-diastolic ratio. PI is calculated by the formula  $(S-D)/A$ , where S is the peak systolic velocity, D is the end-diastolic velocity and A is the average time rate for one cardiac cycle (Blanco et al., 2011).

By Doppler ultrasound examination of pregnant goats, Serin et al. (2010) studied the cardiac activity of the fetus, pulsatility index and resistance index of the umbilical artery. The authors believed that fetal heart rate monitoring allowed for determination of gestational age, and the registered Doppler indices (PI and RI) of the umbilical artery could give useful information about fetal perfusion. By means of Doppler ultrasonography, Kumar et al. (2015) studied the haemodynamic characteristics of the umbilical cord in goats. There are no reported data from measurements of Doppler parameters characterising the blood flow velocity of the fetal heart and aorta in goats in Bulgaria.

The goal of this study was to determine the parameters characterising the blood flow velocity of the fetal heart and aorta in goats by Doppler ultrasonography.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental animals

The experiment was carried out with 24 Bulgarian White dairy goats, aged 4–6 years, weighing 45–51 kg and raised in a private goat farm

in Stara Zagora district. The animals' diet included meadow and alfalfa hay, compound pelleted feed for small ruminants and free access to water.

### 2.2 | Methods

#### 2.2.1 | Clinical examination

The clinical examination of the animals used in the studies included general health status monitoring – determination of rectal temperature, pulse rate, respiratory rate, rumen movements and cardiac activity. Only clinically healthy animals were used in the experiments.

#### 2.2.2 | Estrus synchronisation, insemination and pregnancy diagnosis

Intravaginal sponges containing 30 mg flurogestone acetate (Synro-part® 30 mg, Ceva Sante Animale, France) were used for estrus synchronisation during the beginning of the breeding season. On the 12th day, they were removed and each animal was treated intramuscularly with 500 IU serum gonadotropin (Synro-part® PMSG, Seva Sante Animale, France). The insemination was performed by mating with a fertile buck twice – after proving the standing reflex and 12 hr later. In all goats, insemination dates were recorded and the date of last mating was taken as Day 0. Ultrasonographic criteria for early pregnancy diagnosis included visualisation of an enlarged uterine lumen, the presence of anechoic amniotic fluid and embryo(s) with visible cardiac activity.

#### 2.2.3 | Ultrasonography

Ultrasonography was performed using a SonoScape S2 Vet (SonoScape, China) ultrasound machine, equipped with multifrequency (5.0–11.0 MHz) linear and convex transducers.

Ultrasound examinations were performed by the transabdominal approach with the animals in a standing position, which were restrained by an assistant. The ultrasound scans were performed in the right or left inguinal area, in the field limited dorsally by the knee fold and ventrally by the mammary gland, and in late pregnancy: in the ventral abdominal area. Previously, the hair in the inguinal area on the left and right side was trimmed, and during the second and third pregnancy trimesters, it was removed along the ventral abdominal wall. To eliminate the air spaces between the skin and the transducer and to improve the image quality, a large amount of ultrasound gel (Eco Ultra gel, Milano, Italy) was applied. The examinations were performed with linear and convex multifrequency transducers at a frequency of 8.0 MHz.

The location of the fetal heart and aorta was identified using B-mode ultrasonography. Using colour Doppler mode, the fetal heart and aorta were visualised and the direction of blood flow was determined. Using spectral Doppler ultrasonography, Doppler indices characterising blood flow velocity were measured – maximum and

minimum systolic velocity, end-diastolic velocity, pulsatility index, resistance index and systolic/diastolic ratio.

Doppler ultrasound measurements of the blood flow velocity on the fetal heart were performed at the end of first, second and third trimesters of pregnancy, while on the fetal aorta – at the end of second and third trimesters of pregnancy.

### 2.3 | Statistical analysis

The obtained results were processed using statistical software StatSoft (Statistica 7, Microsoft Corp. 1984–2000 Inc.), using the ANOVA options and non-parametric proportional comparison using Mann–Whitney for T test equivalents. The data were presented as mean and its standard deviation. The differences in the obtained values were considered statistically significant at  $p < 0.05$ .

## 3 | RESULTS

Colour Doppler ultrasonography allowed clear visualisation of the fetal heart (Figures 1a and 2a) and fetal aorta (Figures 1b and 2b) at the end of the second and third trimesters of pregnancy. Using

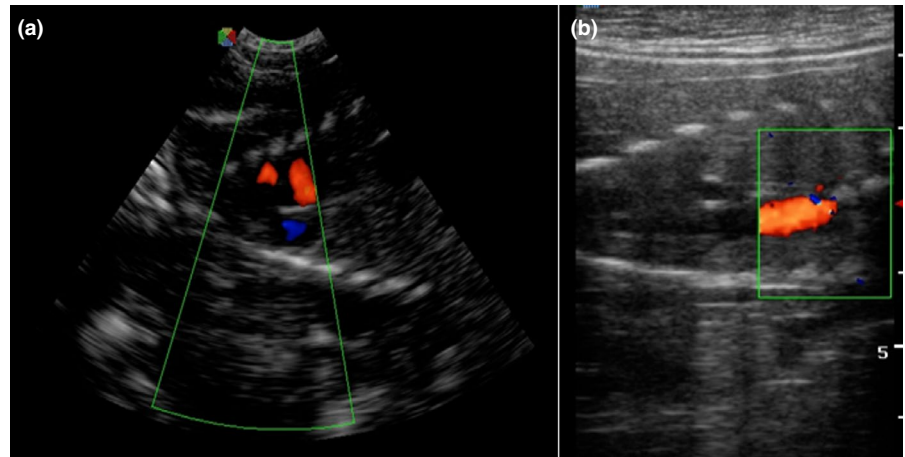
continuous wave spectral Doppler, it was possible to measure the blood flow velocity parameters of the fetal heart (Figure 3a) and fetal aorta (Figure 3b).

The obtained results showed that the highest maximum systolic velocity of the fetal heart (Table 1) was observed at the end of the first trimester of pregnancy ( $63.34 \pm 6.2$  cm/s). Statistically significantly ( $p < 0.05$ ) lower values for this parameter were registered in the second ( $31.2 \pm 9.6$  cm/s) and third ( $21.89 \pm 8.1$  cm/s) trimester of pregnancy. A similar tendency was observed for the Doppler indices minimum systolic velocity, end-diastolic velocity and systolic/diastolic ratio. The pulsatility and resistance indices were relatively similar for the three studied periods.

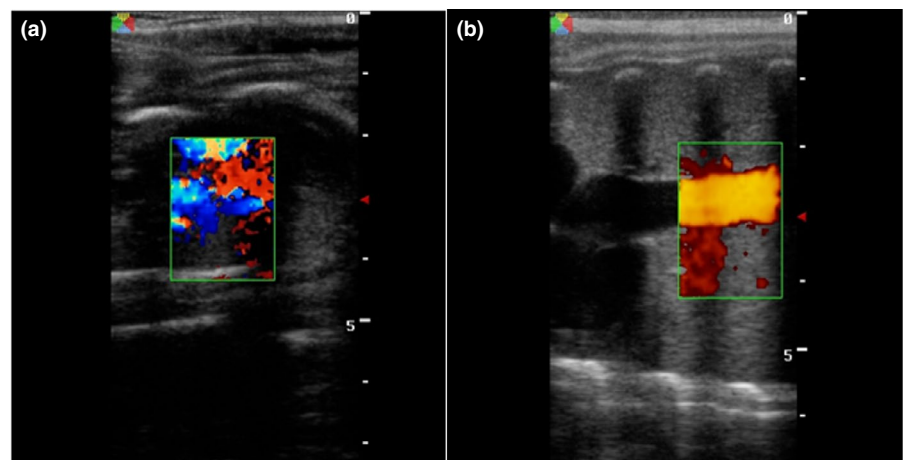
Fetal aortic Doppler ultrasound examination (Table 2) demonstrated a statistically significant difference ( $p < 0.05$ ) between the third and second trimester of pregnancy for minimum systolic velocity, end-diastolic velocity, pulsatility index and resistance index.

## 4 | DISCUSSION

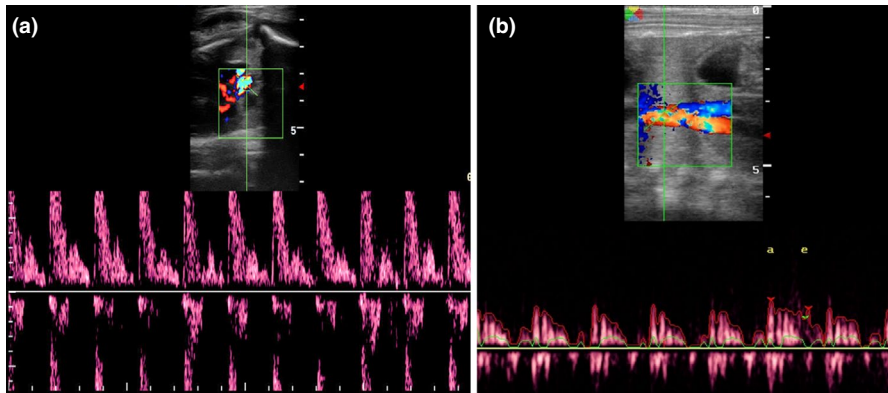
Our results showed that it was possible to determine the blood flow velocity in the fetal heart and fetal aorta during the second and third trimesters of pregnancy in goats.



**FIGURE 1** Colour Doppler ultrasonography of the fetal heart (a) and fetal aorta (b) in the 90th day of pregnancy in a goat



**FIGURE 2** Colour Doppler ultrasonography of the fetal heart (a) and fetal aorta (b) in the 133th day of pregnancy in a goat



**FIGURE 3** Continuous-wave spectral Doppler of the fetal heart (a) and aorta (b) in a goat

Doppler indices	Pregnancy trimester		
	First	Second	Third
Maximum systolic velocity (cm/s)	63.34 ± 6.2	31.2 ± 9.6*	21.89 ± 8.1*
Minimum systolic velocity (cm/s)	23.27 ± 7.3	17.33 ± 3.7	12.17 ± 4.3*
End-diastolic velocity (cm/s)	20.21 ± 2.9	11.91 ± 3.9*	8.3 ± 2.7*
Pulsatility index	1.85 ± 0.15	1.10 ± 0.16	1.8 ± 0.2
Resistance index	0.68 ± 0.04	0.62 ± 0.04	0.65 ± 0.05
Systolic/Diastolic ratio	3.13 ± 0.07	2.62 ± 0.18	1.82 ± 0.16*

\*Statistically significant differences versus first trimester of pregnancy at  $p < 0.05$ .

**TABLE 2** Doppler measurements (mean ± SD) of the parameters, characterising blood flow velocity in the fetal aorta in goats ( $n = 24$ )

Doppler indices	Pregnancy trimester	
	Second	Third
Maximum systolic velocity (cm/s)	12.62 ± 5.6	15.2 ± 2.6
Minimum systolic velocity (cm/s)	5.13 ± 1.7	12.0 ± 1.7*
End-diastolic velocity (cm/s)	6.67 ± 2.9	9.05 ± 1.9*
Pulsatility index	1.14 ± 0.16	0.68 ± 0.12*
Resistance index	0.47 ± 0.03	0.4 ± 0.1*
Systolic/Diastolic ratio	1.89 ± 0.29	1.66 ± 0.2

\*Statistically significant differences between second and third trimester of pregnancy at  $p < 0.05$ .

Studies performed during pregnancy in women and dogs have shown that decreased resistance index in the umbilical artery, fetal aorta or fetal renal arteries was associated with an unfavourable outcome (Acharya et al., 2005; Blanco et al., 2011; Coleman et al., 2000). For this reason, Doppler ultrasonography has become a routine technique in pregnant women for identifying pathological changes in the placental or fetal blood flow. Most studies on monitoring of blood flow in the umbilical artery were performed in dogs and cats (Migliano et al., 2006; Pereira et al., 2012; Scotti et al., 2008; Zambelli & Prati, 2006). The fetal aorta in cats is detectable with Doppler

sonography on the 21st day after mating (Pereira et al., 2012; Scotti et al., 2008).

There are almost no studies related to the monitoring of blood flow in the embryo-fetal heart and especially in the fetal aorta of goats. It was reported that the use of spectral Doppler made it possible to monitor the changes in the embryo-fetal blood circulation in goats (Kumar et al., 2015; Serin et al., 2010).

The movement of blood in individual vessels is usually established semi-quantitatively by the so-called Doppler indices. These indices are not a direct measurement of blood flow, but rather indicate the state of smaller vessels downstream from the vessel being analysed. As the values increase, the resistance of blood flow increases and vice versa (Dickey, 1997). The Doppler indices are relative values obtained from the maximum systolic, minimum systolic, end-diastolic velocity or time average mean frequency during a cardiac cycle.

Our results indicated that the pulsatility index of the fetal aorta was significantly ( $p < 0.05$ ) lower in the third than in the second trimester of pregnancy. Similar resistance until the 85th day of pregnancy was demonstrated by Serin et al. (2010) in the umbilical artery of goats. The blood flow velocity of the fetal heart was significantly ( $p < 0.05$ ) lower in the third compared with the first trimester of pregnancy. The opposite was observed in the fetal aorta – the blood flow velocity parameters had higher values in the third compared with the second trimester of pregnancy. This can be explained by the increased aortic size during the last trimester of pregnancy and its increased resistance, respectively. In support of this, Kumar et al. (2015) found increased peak systolic rate of the

**TABLE 1** Doppler measurements (mean ± SD) of the parameters, characterising blood flow velocity in the fetal heart in goats ( $n = 24$ )

umbilical artery in goats between the 39th and 120th day of pregnancy. Similar results were reported in dogs, cats, sheep and goats (Di Salvo et al., 2006; Elmetwally & Meinecke-Tillmann, 2012; Scotti et al., 2008).

## 5 | CONCLUSION

In conclusion, the general analysis of the results suggested that the Doppler ultrasonography could be used to monitor changes in fetal blood flow during the various stages of pregnancy. The obtained values of the parameters characterising the blood flow velocity of the fetal heart and aorta can be used as a reference for comparison with cases of abnormal fetal blood flow in goats.

## 6 | THE ETHICAL STATEMENT

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 No. 20/1.11.2012 of the Ministry of Agriculture and, Food and Forestry Food from November 1, 2012, Republic of Bulgaria.

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### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to declare.

### AUTHOR CONTRIBUTION

**Ivan Fasulkov:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft. **Manol Karadaev:** Data curation; Investigation; Methodology. **Nasko Vasilev:** Data curation; Formal analysis; Methodology; Supervision. **Kalin Hristov:** Investigation; Methodology. **Ivan Fedev:** Formal analysis; Investigation.

### PEER REVIEW

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