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Umbilical artery doppler sonography in Saanen goat fetuses during singleton and multiple pregnancies

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Abstract

The objective of this study was to evaluate the blood flow from the umbilical artery (UA) in healthy pregnant goats. Doppler sonography examinations were performed every two weeks in Saanen goats with a singleton (n = 5) or multiple (n = 4) pregnancy from 40 to 145 days of gestation. Fetal heart rates (FHR), pulsatility index (PI), and resistance index (RI) were recorded from the mid-cord site of the free-floating umbilical cord. FHR decreased gradually as the pregnancy progressed and significantly decreased during the last two examinations of all fetuses (P < 0.05). The mean PI level was dramatically different (P < 0.05) until 85 days of gestation, after which it reached a plateau level until parturition. Similar to PI, RI decreased by 85 days of gestation (P < 0.05), and decreased again by 130s gestation. No reverse or absent end-diastolic flow were observed in fetuses during any examinations. When comparing singleton and multiple pregnancies, there were no significant differences in UA pulsatility or resistance in fetuses seen. The middle of the second trimester was observed to be a threshold stage for indices in the pattern of caprine pregnancy.

In conclusion, this work provides additional values that might be useful when evaluating singleton and multiple pregnancies, and may be evaluated in further studies regarding fetal monitoring.

Keywords: Doppler ultrasonography; Pregnancy; Umbilical artery; Goat

1. Introduction

In the last decade, transabdominal ultrasonography has become an important tool in veterinary medicine for the evaluation of the intrauterine life of the fetus [1–3]. However, the available data concerning its usefulness in the management of high-risk pregnancies that can be compromised because of a maternal and fetal disease [3] or cloned or in vitro pregnancies associated with losses throughout the pregnancy [4] are limited. As in human obstetrics, care should be taken when interpreting the results of an ultrasonographic examination of the feto-maternal vessels with Doppler ultrasonography [5].

Doppler ultrasound of the genital system provides real-time functional information such as blood velocity, blood direction, and blood type, and has important clinical gynecological implications for human and animal species [6–8]. With this noninvasive technique, most common vessels, such as the uteroplacental arteries, umbilical cord, aorta, caudal cava vein, and ductus venosus of the fetus are investigated [8–10]. For the last 10 years, some researchers have reported Doppler sonography results from mare [8,11], cow [12,13], ewe [14,15], bitch [6,16–18], and queen [19] taken during different reproductive stages.
In fetal lamb models, umbilical artery (UA) Doppler blood flow waveforms can be used to identify fetuses that might benefit from increased surveillance or planned delivery [20]. Owing to difficulties encountered with volumetric blood flow, semiquantitative indexes, such as pulsatility indices (PI) or resistance indices (RI) are often used. RIs of Doppler examinations are based on the ratio of systolic flow to some measure of diastolic flow, which indicates the flow condition downstream. They are angle independent, but are affected by the heart rate. The most frequently used are the RI, also known as the Pourcelot ratio, expressed by \((S–D)/S\), where \(S\) is the peak systolic velocity, and \(D\) is the end-diastolic velocity; the systolic/diastolic ratio (S/D); and the pulsatility index (PI), expressed by \((S–D)/velocity\), where velocity is the time-averaged maximum velocity over the cardiac cycle [21]. Recording of the vascular pulsatility and resistance from fetomaternal vessels have become a routine part of fetal surveillance. Doppler-determined UA resistance is associated with intrauterine growth retardation (IUGR), congenital anomalies and other adverse fetal outcomes [5,22,23]. Recordings of the blood flow waveform of the UA have become a routine part of fetal well-being, because it is strongly predictive of an adverse outcome of pregnancy. The measurement of an absent or reverse blood flow during diastole in the UA is a late sign of increased placental vascular resistance [5–22].

The southwest region of Turkey plays an important role in goat production, where the native Hair is the principal breed. In this region, however, goats are typically seasonal breeders. The number of the Saanen dairy breed of goats has been increasing for the last decade. New techniques are needed to evaluate fetal age, viability, and wellbeing for veterinarians who work in this area. Continuous monitoring of the FHR by Doppler ultrasound may be useful in determining the FHR variability, which is used to determine gestational age [24–28] in local breeds of goat fetuses and is an important indicator of wellbeing in fetal sheep [29]. However, there is no data available on the FHR curve in pregnancy and its possible correlation with gestational age in Saanen goats.

A multiple pregnancy differs from a singleton pregnancy with respect to the mean duration of gestation and the mean birth weight of the infants [30]. Thus, the Doppler data from the UA in multiple pregnancies may be different from that in singleton pregnancies. There are few data on the singleton and multiple pregnancies obtained in human medicine [31–32], and there are no data at all from veterinary medicine.

The aim of the current study was to assess UA Doppler indices and FHRs throughout singleton and multiple pregnancies in Saanen goats and to validate values that might be used for fetal monitoring.

### 2. Materials and methods

#### 2.1. Animals

The current study was carried out in the experimental farm of Adnan Menderes University, Çine Vocational School, located in Aydin, Turkey. Estrus was synchronized in 15 one-year old Saanen nulliparous goats, with an average weight of 27.68 kg, using intra-vaginal sponges containing 20 mg fluoroestrogen acetate. This was performed in September, during the natural breeding season of goats in the Aydin region. The intravaginal sponges were inserted into the vagina of each goat for 11 days. Forty-eight hours before sponge withdrawal, all does were injected intramuscularly with 500 IU equine chorionic gonadotropin (eCG) (Chrono-gest®- Intervet, Istanbul, Turkey) and 0.5 ml prostaglandin \(F_{2\alpha}\) (Cloprostrenol, IIiren, Intervet, Istanbul, Turkey) for stimulation of estrus and ovulation. Buck introductions were performed two days after the sponge withdrawal. Animals in estrus were allowed to mate twice during estrus. The day of estrus was designated as day 0 (day of 1st mating).

#### 2.2. Pregnancy diagnosis and Doppler ultrasound measurements

All does were examined for pregnancy detection between day 30 and day 40 after mating, using real time transrectal ultrasonography (MyLab 30- ESAOTE®, Genova, Italy) with 7.5 MHz linear transducer. Pregnancy was confirmed in nine goats with singleton (\(n = 5\)), twin (\(n = 2\)), and triplet (\(n = 2\)) pregnancies.

Transabdominal ultrasonography was performed on days 40, 55, 70, 85, 100, 115, 130, and 145 after mating. The does were scanned while in a dorsal recumbency using a (multi-frequency) 5 to 6.6 MHz convex transducer in a dorsal recumbency until 85 days of gestation. Does were then examined in lateral recumbency to avoid maternal aorta compression until 145 days of gestation. Prior to examination of the inguinal region, the hair on both sides was clipped and in the advance stage of pregnancy the more cranial portion of the ventral abdomen was also clipped. The does were not sedated. The transducer was covered with a copious amount of gel to eliminate the air.
spaces. All ultrasonographic examinations were performed by the same operator.

The UA was detected and flow velocity waveforms were obtained from the midcord site of the free floating umbilical cord (Fig. 1). First, the UA was visualized using the Color-Doppler application, and then Pulsed-Wave Doppler ultrasonography was performed. After the 70th day of pregnancy, as Pulsed Doppler examinations of the UA were possible without coloring the vessels, the Color Doppler application was not used. In the twin and triplet pregnancies, the Doppler measurements were obtained from the most caudal fetus on the right side. Recordings were obtained for at least regularly three consecutive arterial waveforms. Waveforms were disregarded during fetal and maternal movements or cardiac arrhythmias. In order to evaluate the waveform and velocity blood flow patterns of the UA, the FHR, PI, and RI were measured. In all of the examinations, the measurements were disregarded when the angle of insonation was above 20° and Doppler filter were set at 50 MHz. Due to the possible side effects (thermal and cavitation effect) of Doppler soundwave on tissues, Pulsed-Wave examinations did not exceed 30 seconds and were interrupted for a minute. Moreover, all examinations lasted between 10 and 15 minutes.

2.3. Data analysis and statistics

Average data were presented as mean ±SD. Means were compared using a paired Student’s t-test when appropriate. Analysis of variance of the Doppler indices and FHR was performed using the ANOVA method. Differences were considered statistically significant at P levels of less than 0.05.

Regression models were fitted to evaluate the relationship between the day of pregnancy and FHR, which was considered to be dependent on the day of pregnancy [33]. A 5% significance level was used.

3. Results

All parturitions occurred and without assistance between 146 and 159 days of gestation. Fifteen healthy kids, ten females and five males weighing 1.400–5.250 kg were born. There were no stillbirths or newborn deaths after delivery. Moreover, up to one-month age no kid mortality was observed.

Figure 2 presents the mean PI, RI, and FHR between 40 and 145 days of gestation in Saanen goats. The UA blood flow was characterized only by the systolic waveform until the 85th day of pregnancy. Later a diastolic waveform was also detected. The PI value increased significantly between the 40th and 55th day of pregnancy and also decreased significantly 85 days of gestation (P < 0.05). There was not alteration in PI value until the final examinations at 145 days of gestation. Although an increase of PI level was seen during the final examination, it was not found to be statistically significant.

The RI has more stable than PI values in goat fetuses. The mean RI value was limited as 0.97 during pregnancy and two significant decreases (P < 0.05) were detected on the 85th and 130th day of pregnancy. Between 130 and 145 days of gestation, no variation was observed in either pregnancy group.

The curve of FHR was characterized as descending during pregnancy, but not significantly so until last two weeks of pregnancy. In particular, from 130 to 145 days of gestation, the mean FHR value decreased to 140 ± 6.4 beats per minute (bpm) (P < 0.05). When the relationship between FHR and gestational age was evaluated, FHR values were correlated (R² = 0.77) with the gestational age.

The findings of two Doppler indices and FHR during singleton and multiple (twin and triplet) pregnancies are presented in Figures 3 and 4. Results from multiple pregnancies were obtained from the most caudal fetuses on the right side. There was no difference between the PI, RI and FHR values in singleton and multiple pregnancies’ (P > 0.05).

4. Discussion

Today Doppler examinations are used routinely to monitor high-risk pregnancies in human medicine. Sev-
eral fetal blood flow waveform evaluations have been performed in the assessment of fetal well-being, normal or abnormal placental circulations, IUGR, and perinatal death in human medicine [23,34,35] or in fetal sheep models [20,36–38]. In veterinary medicine there are only a few reports that have examined the use of blood flow waveform in the umbilical cord in sheep [14], bitch [6,18], queen [19], and mare [8] to evaluate fetal hemodynamic process. The present provided the first estimates of UA flow in healthy Saanen goat fetuses in singleton and multiple pregnancies via Pulsed-Doppler ultrasonography for fetal monitoring.

In the current study, noninvasive Doppler examination was performed during pregnancy in nulliparous healthy Saanen goats. No abnormal blood flow waveforms such as end-diastolic flow or reverse flow related to fetal distribution were observed during the study. The UA blood flow was characterized only by systolic waveform until the 85th day of pregnancy.

In the present study, the PI showed dramatic differences in the first four examinations. These variations were observed in both pregnancy groups with similar results. When comparing the normal canine and feline UA PIs with the results from the current study, a similarity was noticed among these three graphics. In canine and feline pregnancies, *a. umbilicalis* pulsatility increased (P > 0.05) between 4th and 5th week and decreased (P > 0.05) from the 5th week of gestation to parturition [6,18,19]. In the present study, similar dramatic changes were detected between the 40th and 85th

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**Fig. 2.** Pulsatility index (PI), resistance index (RI) and fetal heart rate (FHR) from the umbilical artery to the fetuses. Results are means ± S.D. of nine pregnant goats. Results with letter (a,b,c) differ from those of the previous measurements (P < 0.05).

**Fig. 3.** Pulsatility index (PI), resistance index (RI) from the umbilical artery to the fetuses. Results are means ± S.D. of five singleton (SP) and four multiple pregnant (MP) goats.

**Fig. 4.** Mean (± S.D) Fetal heart rate (FHR) values of five singleton (SP) and four multiple pregnant (MP) goats.
day of pregnancy in goats. In contrast to canine and feline pregnancies, PI values reached the plateau stage at around 85 days of gestation, and there were no more changes until parturition in goats.

In the current study, the *a. umbilicalis* resistance remained until the 85th day of pregnancy in accordance with PI values, and the first significant decrease was also obtained on the 85th day with the first diastolic phase in UA waveform. Matsas [39] reported that the fetal skeleton rapidly grows and can be visualized after 65 days of gestation in goats. Therefore there might be an increase in the nutritional requirements of fetuses while they are growing. The resistance in umbilical vessels started to decrease significantly at this stage and fetal blood flow within the umbilical cord has increased. The increase of fetal nutritional requirements may be related to these results. The subsequent significant decrease was observed on the 130th day of pregnancy (*P* < 0.05). After that, the RI curve remained at a low level until parturition.

Based on harmonious Doppler indice curves in two pregnancy groups, it is possible to outline the examinations that were performed on the 85th day of pregnancy and the threshold values for monitoring of pulsatility and resistance of UA in goat fetuses. During this stage, UA Doppler trace examination was characterized with systolic and diastolic phases and pulsatility reached a stable phase in both singleton and also multiple pregnancies. These changes were observed in previous studies in bitches [6,18] and queens [19] but a plateau stage of PI pattern was not observed.

Another finding of the present study was the relationship between the kidding dates and FHR of Saanen goat fetuses. FHR measurement was performed during evaluation of the UA blood flow waveform. According to similar studies performed in other ruminants [24,25], the FHR curve descends along with the progression of pregnancy. Karen and others [28] reported that the correlation between these parameters in Egyptian native goats was \( R^2 = -0.55 \). In the present study, the FHR value has the most correlation \( R^2 = -0.77 \) with the gestational age compared with results from similar studies [24,26].

Using Doppler ultrasonography in caprine pregnancy can be helpful for the diagnosis and prognosis in further studies that include high-risk pregnancies related to maternal disease (pregnancy toxemia, hypocalcemia) or consist of cloned or transferred embryos [4]. UA Doppler blood flow waveforms can be used to identify fetuses that might benefit from increased surveillance or planned delivery. In addition, this noninvasive technique can be used to detect any fetal growth restriction or abnormality, and congenital abnormalities.

Previously, Doppler ultrasonography was not widely used in farm fields, because of the large size of the equipment. Nowadays, hand-held, battery-powered Color or Pulsed Doppler machines are available, but due to high prices, they are still too expensive to use routinely in veterinary clinics. Prices of ultrasound machines have shown a steep decline in the last few years, so transabdominal Doppler ultrasonography is likely to be increasingly used as a diagnostic tool for the evaluation of fetus(es) in the near future.

In conclusion, the results of the present study showed that recording changes in Doppler flow indices of the UA might be useful to evaluate the fetal perfusion during pregnancy in goats. The middle of the second trimester is an important time to evaluate Doppler indices in pregnant goats because of significant decreases in both the RI and PI. After that, a plateau for PI curve was present, in contrast to the results from bitches and queens. The measuring of FHR permits the estimation of the gestation age in singleton and multiple pregnant Saanen goats. With further studies based on these data, it may be possible to estimate any abnormality in caprine pregnancy, particularly in high-risk pregnancies, cases of IUGR, discordance with twin fetuses, and other feto-plasental abnormalities as routinely used in human medicine.

**References**


