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Research Article

Doppler Ultrasonography Assessment of Uterine Artery Blood Flow throughout Late Pregnancy in Awassi Ewes

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ABSTRACT

Monitoring uterine blood flow during pregnancy allows us to gain information about fetal development. The present study was carried out to determine the changes in uterine artery blood flow after the second half of pregnancy in Awassi ewes. The study included a total of 20 ewes with a single pregnancy, separated into 4 groups according to the period of the pregnancy, as 90-105-120-135 days. By monitoring the uterine artery blood flow with Doppler ultrasound, measurements were taken of the pulsatilite index (PI), resistance index (RI), end diastolic velocity (EDV), peak systolic velocity (PSV), systolic/diastolic ratio (S/D), and time-averaged peak (TAP). The data obtained were analyzed with repeated measures ANOVA (variance analysis). Throughout the pregnancy periods, a time-related significant difference was seen between the groups in respect of the PSV, and RI values ($P<0.001$). In addition, a time-related significant difference was seen between the groups in respect of the EDV, TAP, PI, and S/D values ($P<0.01$). The PSV value was observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days ($P<0.001$). The EDV, and TAP values were observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days ($P<0.01$). The RI value was seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days ($P<0.001$). The PI, and S/D values were seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days ($P<0.01$). As a result, significantly differences were observed in the changes in the uterine artery blood flow after the second half of the pregnancy of Awassi ewes. It was concluded that as the vast majority of fetal development occurs in the second half of the pregnancy, determination of changes in Doppler ultrasound parameters will contribute to increasing hemodynamic information.

Keywords: *Doppler ultrasound, ewe, pulsatilite index, resistance index, uterine artery.*

İvesi Irkı Koyunlarda İleri Gebelik Boyunca Uterin Arter Kan Akımının Doppler Ultrasonografi ile Değerlendirilmesi

ÖZET

Gebelik sırasında uterus kan akışının izlenmesi, fetal gelişim hakkında bilgi edinmemizi sağlar. Sunulan çalışma İvesi ırkı koyunlarda gebeliğin ikinci yarımından sonra uterin arter kan akımı değişikliklerinin belirlenmesi amacıyla yapıldı. Çalışmada toplam 20 adet tekiz gebe koyun kullanıldı. Koyunlar gebeliğin dönemine göre 90-105-120 ve 135. gün olmak üzere 4 gruba ayrıldı. Doppler ultrason ile uterin arter kan akımı izlenerek pulsatilite indeks (PI), rezistans indeks (RI), diyastol sonu hız (EDV), maksimum sistolik hız (PSV), sistol/diyastol oranı (S/D) ve ortalama azami hız (TAP) ölçüldü. Elde edilen veriler tekrarlı ölçümler ANOVA (varyans analizi) ile analiz edildi. Gebelik dönemleri boyunca gruplar arasında PSV ve RI değerleri açısından zamana bağlı olarak anlamlı farklılık görüldü ($P<0.001$). Ayrıca EDV, TAP, PI ve S/D değerleri açısından da gruplar arasında zamana bağlı olarak anlamlı farklılık belirlendi ($P<0.01$). Gebeliğin 135. gününde PSV değerinin 90, 105 ve 120. günlere göre anlamlı derecede yüksek olduğu görüldü ($P<0.001$). Gebeliğin 135. gününde EDV ve TAP değerlerinin 90., 105. ve 120. günlere göre anlamlı derecede yüksek olduğu belirlendi ($P<0.01$). Gebeliğin 135. gününde RI değerinin gebeliğin 90, 105 ve 120. günlerine kıyasla daha düşük olduğu görüldü ($P<0.001$). Gebeliğin 135. gününde PI ve S/D değerlerinin gebeliğin 90, 105 ve 120. günlerine kıyasla daha düşük olduğu belirlendi ($P<0.01$). Sonuç olarak İvesi ırkı koyunlarda gebeliğin ikinci yarımından sonra uterin arter kan akımı değişikliklerinde önemli oranda farklılık gözlendi. Fetal gelişimin büyük kısmının gerçekleştiği gebeliğin ikinci yarımından sonra Doppler ultrason parametre değişimlerinin belirlenmesi ile hemodinamik bilgilerin arttırılmasına katkıda bulunacağı kanısına varıldı.

Anahtar Kelimeler: *Doppler ultrason, pulsatilite indeksi, rezistans indeksi, koyun, uterin arter.*

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Introduction

Physicians routinely use Doppler ultrasonography (USG) to monitor human pregnancies, and there has been a recent increase in studies of this by veterinarians (Herzog and Bollwein, 2007). The monitoring blood flow during pregnancy allows us to learn about fetal development. Doppler ultrasound is used to evaluate anatomic and functional vascular information such as blood flow velocity, direction, and type (Nicolaides et al., 2000). Optimal establishment of feto-maternal blood flow is necessary for the normal development of the fetus during pregnancy. Doppler ultrasound provides valuable information about physiological and pathological differences in the blood flow between the mother and fetus (Fleischer et al., 1994). Doppler USG is generally used gynaecologically to examine changes in the uterine artery (Herzog and Bollwein, 2007). Uterine artery Doppler examination performed in pregnancy makes it possible to diagnose high-risk pregnancies that threatens the health or life of the mother or fetus (Ferrell, 1991).

The increase in fetus and uterus volume in the final trimester are associated with the increased demand for nutrients and oxygen provided by the increase in uterus and fetal perfusion (Kim-Egloff et al., 2016). The uterine and placental blood flow increases during pregnancy to meet the metabolic requirements of the growing fetus (Reynolds et al., 2005). The uterine arteries primarily feed the maternal part of the placenta (Kim-Egloff et al., 2016). Uterine vascularisation is evaluated using the resistance index (RI) and pulsatile index (PI) of the uterine arteries, which are good markers of the vascular perfusion of the reproductive organs (Bollwein et al., 1998; Lemos et al., 2017). As an increase in RI is a sign of a decrease in vascular perfusion, there is a negative correlation with vascular perfusion (Dickey, 1997; Sharma et al., 2019). Abnormal vascular findings in the fetal and/or maternal structures are associated with restricted intrauterine growth, fetal stress, or signs of early embryonic death (Özkaya et al., 2007; Abdelhalim et al., 2014). The measurement of uterine blood flow in pregnant ewes from the middle of pregnancy until the last phase of the pregnancy is an important diagnostic tool to explain the results of intrauterine fetal growth restriction and evaluate fetal health during the pregnancy (Gomez et al., 2006; Papageorghiou and Leslie, 2007; Wallace et al., 2008).

The aim of this study was to determine uterine artery blood flow changes after the second half of the pregnancy of Awassi ewes. This study was conducted to contribute to increasing hemodynamic information about the physiology of ewes in the last period of pregnancy.

Materials and Methods

This study was conducted with the permission of Harran University Animal Experiments Local Ethics Committee (HRU-HADYEK) (dated 28/08/2021 and numbered 2021/007).

Animal Selection and Experimental Protocol

The study material comprised 20 Awassi ewes at the Veterinary Faculty Practice Farm of Harran University, in Eyyübiye, Şanlıurfa province. The farm records were examined to select ewes, aged 2-3 (2.25±0.05) years, each weighing mean 56.86±2.29 kg, with a body condition score ranging from 2-3 (1=Extremely weak, 5=Obese) (2.55±0.04), which had previously given birth only once, as a normal single birth with no problems following the birth. The study was conducted between August and February. The sheep were fed a mixture of hay (5.2%), clover (32.9%) and milk feed (61.9%). To ensure that all the ewes were in the same period of pregnancy, progesterone-based oestrus synchronisation was performed in the breeding season. A vaginal sponge (60 mg medroxyprogesterone acetate, Esponjavet®, Hipra Animal Health, Türkiye) containing progesterone was placed in the vagina of each ewe (day 0) to remain there for 12 days. On the 11th day, 2 ml PGF2a (Dinoprost tromethamine, Dinolytic®, Zoetis, Türkiye) was administered intramuscularly. On the 12th day, the vaginal sponge was removed and 500 IU PMSG (PMSG, Oviser®, Hipra Animal Health, Türkiye) was injected intramuscularly. For 3 days following the PMSG injection, oestrus follow-up was made with a search ram for 30 mins at 8-hour intervals, and the ewes showing oestrus were naturaly insaminated by rams that had previously been determined as fertile. The ewes were kept all together throughout the study and their nutritional and water needs were provided ad libitum. On the 35th day after the insemination, the ewes were performed transrectally examined for pregnancy as single or twin, and those with a single pregnancy were included in the study for analysis. The same ewes were used in each group at different stages of pregnancy. In the second half of the pregnancy, all the ewes underwent Doppler ultrasound examinations were performed at 15-day intervals; on the 90th day of the pregnancy (Group 1, n=20), on the 105th day (Group 2, n=20), on the 120th day (Group 3, n=20), and on the 135th day (Group 4, n=20) (Figure 1).

Doppler Ultrasound Examination

The uterine artery Doppler examination was performed transabdominally. Before the transabdominal scan, the ewes were restrained in standing position and both inguinal regions were shaved extensively towards the cranial direction. No sedative drugs were administered before and during the examinations. All the ultrasound examinations were performed by the same experienced operator using a 5-10 MHz linear transducer (Sonosite Edge II, USA). Observing the placenta-uterus border of the fetus, the artery imaged at the highest quality within the uteroplacental area was used. For the anatomic evaluation of the uterine arteries, first with selected B-mode ultrasound the bladder was identified then differentiation of the uterus tissue was made and using colour Doppler, the vessels to be measured were determined. Blood flow velocity wave forms were obtained from the proximal section (before the bifurcation) of the uterine artery

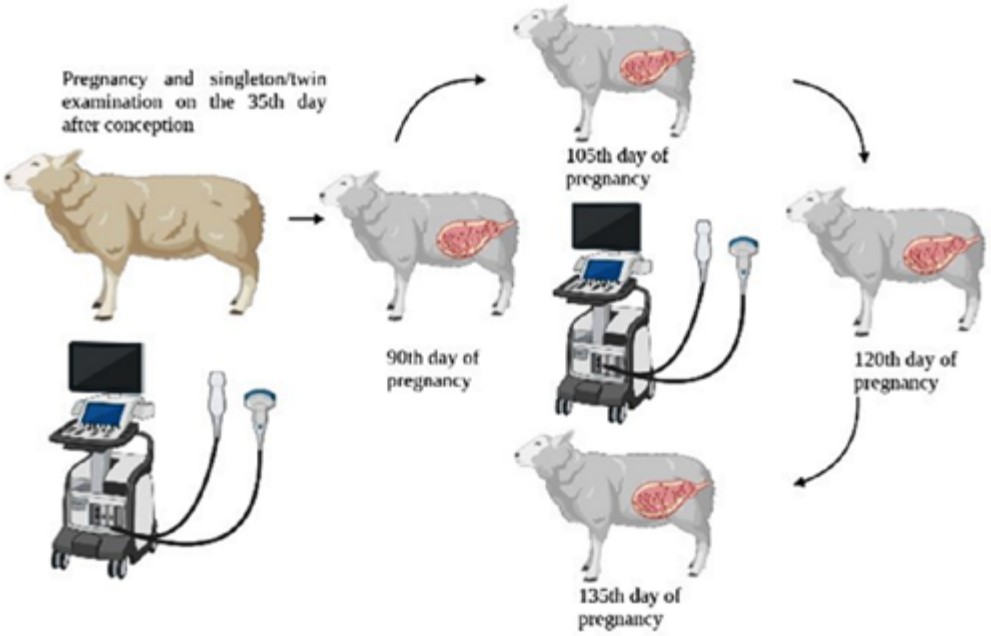

Figure 1. Study design.

after mapping the iliac vessel orientation (Elmetwally, 2016). Measurements were created using manual mode after obtaining a minimum of 3 consecutive healthy artery wave (without artefacts) images (Figure 2). In all of the examinations, the measurements were disregarded when the angle of insonation was above 20°. In the traces obtained taking Pulse Doppler USG measurements from the arteries in question, the PI, RI, EDV, PSV, S/D, and TAP values were measured. These parameters were calculated automatically by the Doppler device software using mathematical formulas or the Pourcelot index [RI: (PSV-EDV)/PSV, PI: (PSV-EDV)/mean velocity, S/D: PSV/

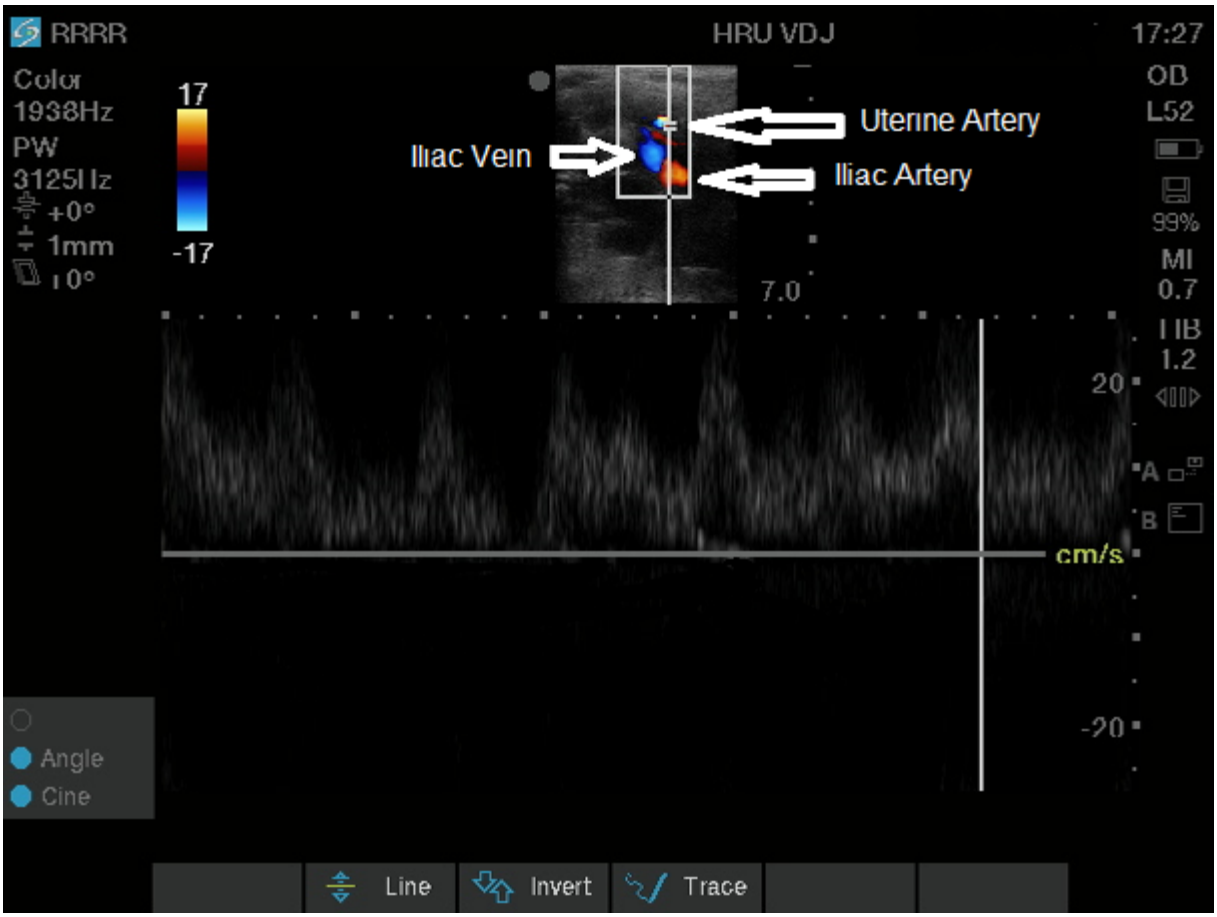

Figure 2. The Pulsed-Doppler image of uterine artery on 90th day.

EDV), TAP: PSV-EDV/PI] (Bollwein et al., 2002; Ginther and Utt, 2004). If physical movement was observed in the mother or fetus, the application was paused and the measurements were repeated after a period. To minimise the thermal and cavity effect which can form during the Doppler ultrasound examination, a pause of approximately 60 seconds was given between the 30-second measurements. All the examinations were completed in 10-15 minutes.

Statistical Analysis

Statistical analysis of the data was performed with the Statistical Package for the Social Sciences (SPSS 26.0, Chicago, IL, USA) package program. Data were examined for normal distribution using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses for variables showing normal distribution were stated as mean ± standard error of values. Time-related changes in the Doppler parameters showing normal distribution were examined using repeated measures ANOVA (variance analysis). When the sphericity assumption was not satisfied, Greenhouse-Geisser correction was performed. Variance homogeneity was determined with the Levene test. A value of P<0.05 was accepted as statistically significant.

Results

There were no pathological conditions affecting the general health of the ewes during pregnancy. Rumination was observed to decrease in the ewes a few days before birth and they remained separate from the flock during that time. These sheep were placed in separate compartments (2x2m) and the births were followed up. The births took place on 150±2 days of pregnancy (151.10±0.11), all vaginally, without assistance, and no maternal problems were encountered in the postpartum period. A total of 9 male and 11 female healthy lambs were born. The mean Doppler parameter values of the groups on days 90, 105, 120, and 135 of the pregnancy are presented in Table 1, and Figures 3 and 4. Throughout the pregnancies, a time-related significant difference was seen between the groups in respect of the PSV, and RI values (P<0.001). In addition, a time-related significant difference was seen between the groups in respect of the EDV, TAP, PI, and S/D values (P<0.01). The PSV value was observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days (P<0.001). No significant difference was determined between the PSV measurements taken on the 90th, 105th, and 120th days (P>0.05). The EDV, and TAP values were observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days (P<0.01). No significant difference was determined between the EDV and TAP measurements taken on the 90th, 105th, and 120th days (P>0.05). The RI value was seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days (P<0.001), with no significant difference between the measurements on the 90th, 105th, and 120th days (P>0.05). The PI and S/D values were seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days (P<0.01), and no significant difference was determined between the measurements taken on the 90th, 105th, and 120th days (P>0.05).

Table 1. The mean Doppler parameters value during pregnancy (Mean±Standard error).

Days	n	PSV (cm/s)	EDV (cm/s)	TAP (cm/s)	PI	RI	S/D
90th day	20	65.62 ± 1.46^{b}	25.42 ± 0.96^{b}	36.87 ± 1.11^{b}	1.06 ± 0.03^{a}	0.62 ± 0.002^{a}	2.69 ± 0.035^{a}
105th day	20	65.74 ± 1.16^{b}	25.85 ± 0.44^{b}	37.62 ± 1.21^{b}	1.05 ± 0.01^{a}	0.61 ± 0.001^{a}	2.63 ± 0.042^{a}
120th day	20	65.97 ± 1.59^{b}	25.95 ± 0.75^{b}	38.94 ± 1.23^{b}	0.96 ± 0.01^{a}	0.59 ± 0.001^{a}	2.55 ± 0.044^{a}
135th day	20	74.52 ± 1.03^{a}	31.94 ± 1.45^{a}	45.36 ± 1.06^{a}	0.92 ± 0.02^{b}	0.54 ± 0.001^{b}	2.52 ± 0.052^{b}
P value		<0.0001	0.007	0.001	0.008	<0.0001	0.007

^{a,b,c,d}:Different letters in the same column indicate a statistically significant difference. Peak systolic velocity (PSV), end diastolic velocity (EDV), and time-averaged peak (TAP), pulsatilite index (PI), resistance index (RI), and systolic/diastolic ratio (S/D).

Discussion

The results of this study present important data which will contribute to understanding the vascular physiology in the second half of pregnancy in Awassi ewes. Regular provision of nutrients to the fetus throughout the pregnancy is an important factor in the development of fetal growth (Molina-Font, 1998). Fetal nutrition depends on the size of the placenta, morphology, blood flow, and the presence of nutrients (Fowden et al., 2006). The uterine artery shows hemodynamic changes such as the volume and velocity of the blood flow to the uterus in response to the fetal requirements (Hassan et al., 2020).

In a study of ewes and goats, Elmetwally et al. (2016) showed a decrease in uterine artery RI, PI, and S/D ratio, and an increase in the TAP value until birth. Beltrame et al. (2017) conducted a study of ewes throughout pregnancy, and reported that the PI, RI, and S/D ratio decreased after the first trimester, and this decrease continued without any great changes through the other periods of the pregnancy. A corresponding continuous increase in the PSV, EDV, and TAP values was also determined throughout the pregnancy. In another study by Yilmaz et

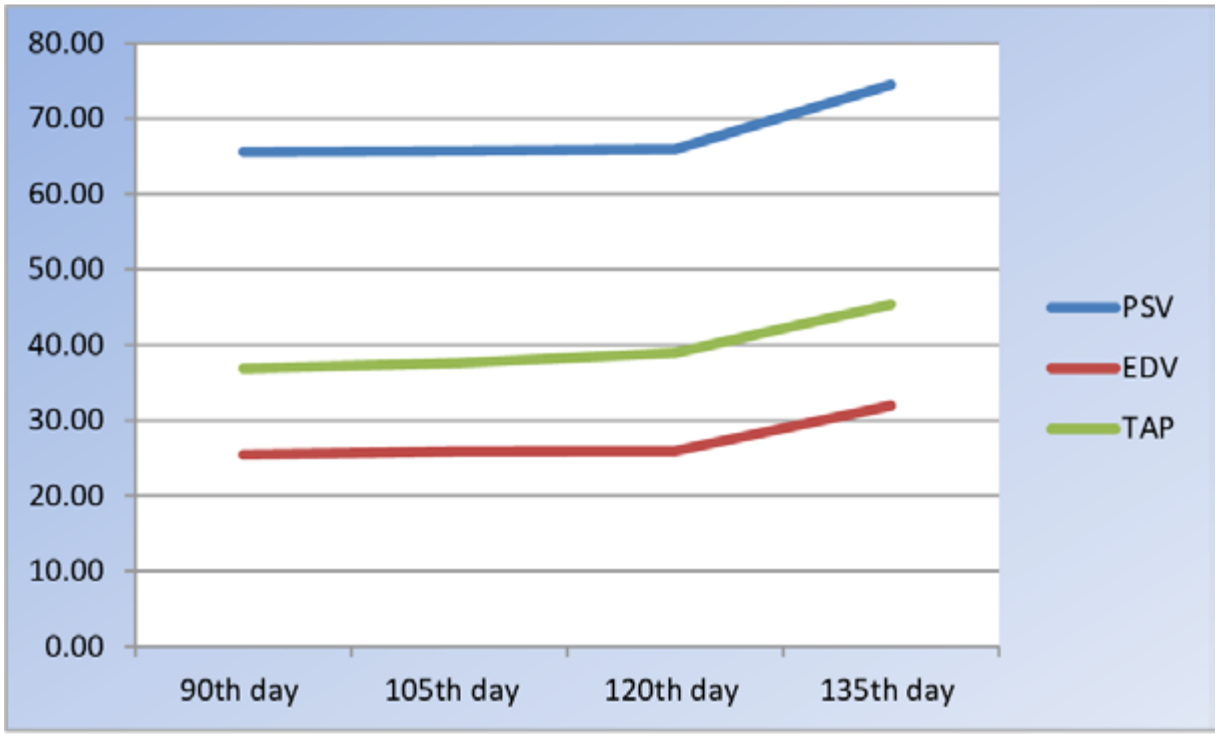

Figure 3. Peak systolic velocity (PSV), end diastolic velocity (EDV), and time-averaged peak (TAP) of the uterine artery on days 90, 105, 120 and, 135 of the pregnancies in ewes.

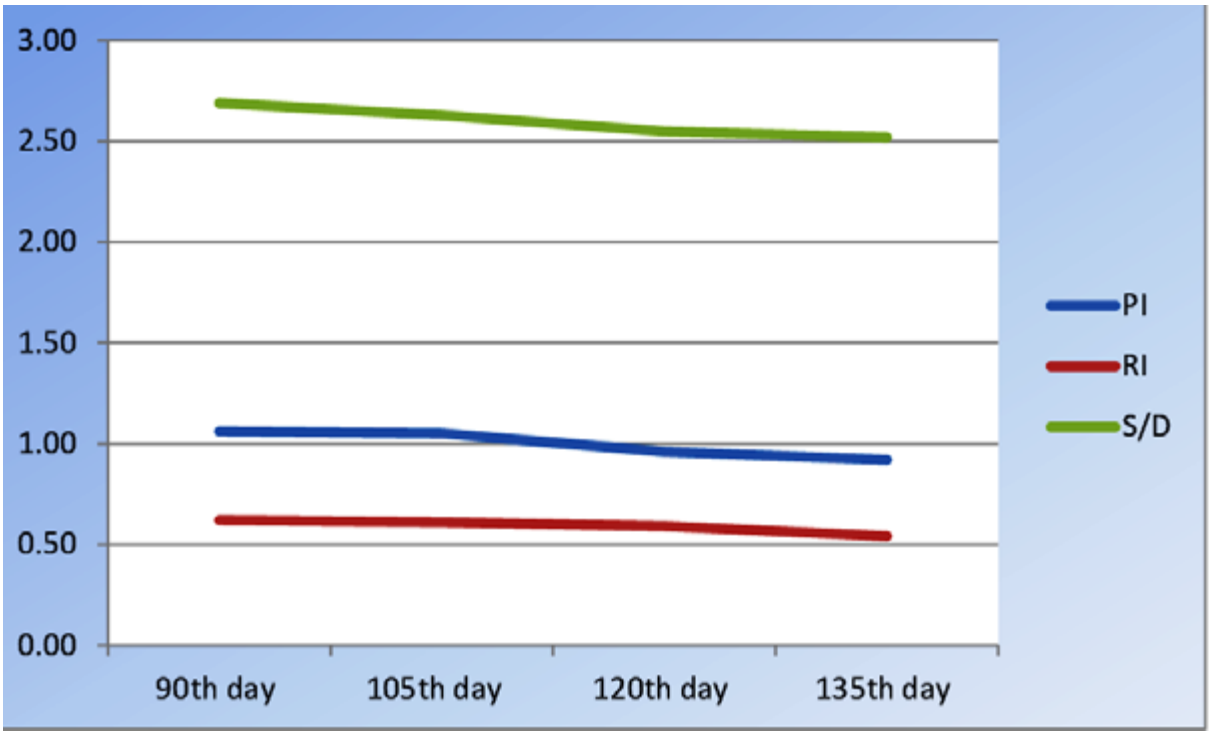

Figure 4. Pulsatilite index (PI), resistance index (RI), and systolic/diastolic ratio (S/D) of the uterine artery on days 90, 105, 120 and, 135 of the pregnancies in ewes.

al. (2017), ewes pregnancies were evaluated at 15-day intervals from the 45th day to the 135th day, and there were stated to be no significant changes in the PI and RI values. Veiga et al. (2018) examined ewes pregnancies on days 60, 90, and 120, and reported a decrease in the uterine artery PI value in parallel with the progress of the pregnancy, and an increase in the PSV, EDV, and TAP values. In an examination of pregnant ewes from the 21st day until birth, Santos et al. (2021) reported that despite an increase in the uterine artery EDV and PSV values in the last 3 weeks of the pregnancy, there was no significant changes throughout the pregnancy, and the RI value was high in the first weeks, then showed a significant fall in the last week of the pregnancy. Consistent with data in literature, our study results showed a continuous decrease in the PI, RI, and S/D ratio in the second half of the pregnancy, and a continuous increase in the PSV, EDV, and TAP values. This was thought to be due to

an increase in blood flow to the fetus as the pregnancy progressed. In our study, the continuous decrease in PI, RI and S/D ratios and the continuous increase in PSV, EDV and TAP values were thought to be due to the negative correlation between resistance and vascular perfusion. It can be interpreted that here the uterine vessels turn into a low-resistance system and increase blood flow to meet the requirements of placentation and fetal development. In our study, uterine artery PI, RI and S/D ratio showed a significant decrease as pregnancy progressed. These changes were thought to be due to the progressive development of the distal vascular bed and changes in the tone of the uterine vessels. The irregular diameter of sinusoidal capillaries on the fetal surface may reduce blood flow resistance and subsequently increase the capacity for trans-placental nutrient exchange (Hafez et al., 2010). Increases in the RI and PI show a decrease in vascular perfusion, while decreases in RI and PI are a sign of increases in vascular perfusion (Gupta et al., 2009; Elmetwally, 2016). The PI and RI values are expected to gradually decrease as the pregnancy progresses, and this shows greater fetal blood flow (Elmetwally et al., 2016). A decrease in the uterine artery PI and RI values during pregnancy and the increases in fetal nutrient demands and placental growth reflect the stage of development of the fetus and increasing diameter of the blood vessels (Rüsse, 1993; Meschia, 2011). These changes are thought to be the mechanism increasing blood flow in the uterine artery to compensate for the increased nutrient need with progression of the pregnancy, which is an important factor in the regulation of fetal growth (Molina-Font, 1998). It is also thought that these changes in vascular perfusion are due to the increased blood flow provided by changing the uterus vessels to a low-resistance system as a response to the metabolic requirements of placentation and fetal development (Herzog and Bollwein, 2007). It was observed that the average PSV, EDV, S/D ratio, PI, RI, and TAP values reported in our study were higher than those in Beltrame et al. (2017). The reason for this situation is likely to be differences in the Doppler insonation angle in the evaluation of blood flow velocity. In addition, it was thought that the location of the arterial flow may differ between studies due to branching and anastomosis in feto-maternal vascularization, making individualization and standardization difficult.

In studies of other species, very little change has been shown throughout pregnancy of the uterine artery PI value in bison (Singh et al., 2018), of the RI value in mares (Bollwein et al., 2003) and cats (Brito et al., 2010), and of both the PI and RI value in rabbits (Akkuş and Erdoğan, 2019), and although not directly related to the current study, these findings provide an idea about uterine artery Doppler follow-up. Starting from the second half of the pregnancy, there has been shown to be a steady decrease throughout pregnancy in the uterine artery PI and RI values of dogs (Nautrup, 1998; Miranda and Domingues, 2010; Blanco et al., 2011), cats (Scotti et al., 2008; Pereira et al., 2012; Blanco et al., 2014), mares (Bollwein et al., 2004; Strübing, 2011; Abdelnaby et al., 2022), cows (Bollwein et al., 2002), and rats (Mu and Adamson, 2006). In a study of pregnant cows, Panarace et al. (2006) determined a decrease in the uterine artery PI and RI values, and an increase in the TAP value as the days of pregnancy increased between days 30 and 270. Hassan et al. (2020) reported that although the uterine artery PI and RI values of pregnant cows at 1, 2, 4, 6, and 8 months were lower at the end of the pregnancy, the EDV and TAP values were higher. In the examinations of small breed dogs at 10-day intervals from the 30th to the 60th day of pregnancy, Batista et al. (2018) determined a continuous increase in PSV and EDV values and a continuous decrease in the RI value. Although not directly related to the our study, these studies of other species also provide information about uterine artery follow-up and are consistent with the current study findings. Decreased vascular resistance in the uterine artery is associated with increasing perfusion of the artery and somatic development of the fetus (Miranda and Domingues, 2010). A decrease in the indexes is a result of the loss of the musculoelastic layer of the arteries and simultaneous invasion of trophoblasts to the endometrium during placentation (Wright and Royston, 1997). Other studies have indicated that this increase in vascular resistance is a sign of retarded intrauterine growth (Owen and Ogston, 1997), maternal hypertension (Gudmundsson and Marsal, 1991), abortus (Blanco et al., 2016), and perinatal death (Dubiel et al., 2003).

Conclusion

In conclusion, the results of this study demonstrated that changes in uterine artery blood flow measured after the second half of pregnancy, when most of the fetal development occurs, were successfully evaluated using Doppler ultrasound. It was concluded that it may benefit a broader understanding of hemodynamic changes in physiologically healthy pregnancies. Our results reflect physiological uterine behavior during healthy pregnancy and therefore additional studies are needed to distinguish physiology from pathology in sheep. With further studies based on these data, it has been concluded that Doppler ultrasound can be used in the diagnosis of specific diseases by examining the uterine artery hemodynamic changes in infectious or metabolic diseases that are frequently encountered after the second half of pregnancy in ewes.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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