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Ruminants

Changes in Doppler Ultrasonography and Echotexture Parameters in Cows During the Last 10 Days of Pregnancy

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ABSTRACT

Objectives: This study aimed to investigate changes in Doppler sonographic measurements of uterine artery blood flow to the placenta, as well as variations in placentome echotexture/perfusion and myometrial thickness in cows during the last 10 days of pregnancy.

Methods: The study utilized eight pregnant Brown Swiss cows. In assessing the echotexture of the placentome and myometrium, the mean grey value (MGV) was measured, along with the placentome area and myometrium thickness (MT). For placentome perfusion analysis and spectral analysis of the uterine artery, parameters, including pulse rate (PR), resistance index (RI), blood flow velocity (BFVe), blood flow volume (BFVo) and diameter (DM), were recorded.

Results: During the last 10 days of pregnancy, the placentome area and MT exhibited a declining trend from the start of sampling until delivery ($p = 0.005$). Both placentome and myometrium MGV showed an increasing trend over the study period ($p < 0.001$). Placentome perfusion decreased significantly 3 days prior to delivery ($p < 0.05$). A significant difference was observed in BFVe/DM in the uterine arteries between pregnant and non-pregnant horns. RI ($p = 0.005$) and PR ($p = 0.009$) values changed significantly over time.

Conclusions: As a result, differences in ultrasonographic findings were observed during the last 10 days of pregnancy in cows. It can be concluded that changes, particularly in the final 2 days, may be associated with placental maturation mechanisms.

1 | Introduction

The placenta is a structure composed of trophoblastic cells, foetal membranes, placentomes, amniotic and allantoic fluids (Peter 2013). In cows, the allantochorionic membrane and endometrium are modified at specialized contact points called placentomes. This structure, which consists of maternal caruncles and foetal cotyledons, is the organ where respiratory gases, nutrients and waste products are exchanged. The growth and development of placentomes are essential for foetal growth and development (Laven and Peters 2001).

Increasing blood flow to the uterus during the last trimester of pregnancy helps to improve uterine perfusion, which in turn meets the foetus' increasing need for nutrients and oxygen (Ferrell and Ford 1980). Placental perfusion adapts during pregnancy to the changing demands of the growing foetus, which is crucial for foetal development (Ferrell 1991). Uterine arteries

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have historically been examined for blood flow using invasive techniques; these arteries are crucial to uterine perfusion (Ford and Chenault 1981; Waite et al. 1990). However, these techniques have been replaced by colour/power Doppler sonography (Bollwein, Baumgartner, and Stolla 2002). Blood flow volume (BFVo), vascular resistance, pulse waves and variations in blood flow are all measured via non-invasive Doppler sonography (Dickey 1997). This imaging method is used in human medicine for the diagnosis and monitoring of pregnancies with pathologies (Goldenberg and Cliver 1997). It can also be used in humans to assess both foetal and placental circulation in advanced pregnancy to facilitate the diagnosis and monitoring of important conditions, such as foetal growth restriction, foetal anaemia and preeclampsia (Mone, McAuliffe, and Ong 2015). However, in veterinary medicine, this method is less developed, and the evaluation of uterine blood flow in large animals has not been as widely implemented as it has in human medicine. Studies conducted in the late stages of pregnancy have generally investigated how drug administration affects pregnancy (Kim-Egloff et al. 2016; Waldvogel and Bleul 2014).

Doppler sonography has also been used to study uterine perfusion in cows during normal pregnancy (Bollwein, Baumgartner, and Stolla 2002; Herzog et al. 2011; Panarace et al. 2006). Because the uterine artery provides the major portion of blood supply to the bovine uterus, changes in blood flow in these vessels reflect changes in uterine perfusion (Vollmerhaus 1964). These changes can also be revealed in detail with the Spectral mode of Doppler. Studies have also been conducted to monitor placental blood flow throughout pregnancy in large animal species, particularly mares (Bollwein et al. 2004). The other mode of Doppler is Color Doppler sonography describes blood flow based on the frequency shift of a flow volume, whereas power-mode Doppler sonography displays the strength of the Doppler signal in colour by determining all moving particles in the blood, which allows recording of blood flow independent of blood flow velocity (BFVe) and direction. This is crucial for the quantitative and semiquantitative assessment of blood flow in tissues with low BFVe and numerous blood vessels (Bude and Rubin 1996) such as placentomes. Nowadays, using computer-assisted image analysis instead of traditional subjective image analysis allows for a more objective evaluation of the tissue being examined (Bader et al. 2000). Primarily utilized in human medicine as part of evidence-based practice, this methodology has also been adapted for the reproduction of farm animals (Demiṙ, Kaçar, and Polat 2022; Schmauder et al. 2008; Cengiz et al. 2014). The ultrasonographic appearance or image pattern of an organ is referred to as 'echotexture'. Computer-assisted ultrasonographic image processing of B-mode ultrasonography (USG) has been used in recent years to evaluation of female reproductive organs, including the corpus luteum (Davies et al. 2006), ovarian follicles (Vassena et al. 2003), uterus (Ginther 2014) and placentome (Demiṙ, Kaçar, and Polat 2022). Ultrasonographic images consist of pixels numerically represented in grey tones (0–255) according to their brightness intensities (Tom, Pierson, and Adams 1998). In the evaluation of the tissue echo image, a mathematical matrix is created using these numerical values and the average pixel value is analysed (Singh, Adams, and Pierson 2003).

Sonographic evaluation of the placenta has become an important part of routine foetal monitoring. Placental thickness is a technique utilized for diagnosing pregnancy abnormalities (Jauniaux, Ramsay, and Campbell 1994; Tongsong and Boonyanurak 2004). Through such monitoring techniques, the thickness of the myometrium can be determined by transrectal sonographic measurement of the uterus and placenta (Renaudin, Troedsson, and Gillis 1999). The thickness of the placenta can help diagnose gestational age in animals and humans (Afrakhteh et al. 2013; Zoller et al. 2019; Campos et al. 2017). Studies have shown a positive correlation between gestational age and placental thickness, especially in mares and cows (Campos et al. 2017; Maldonado et al. 2012; Kimura et al. 2018). In addition, myometrial contraction activity is critically important for optimal reproductive performance; it is necessary for the transport of sperm in the oviduct and the movement of the embryo within the uterus as well as playing a significant role during delivery (Eytan 1999). Coordinated contractions assist in the expulsion of the foetus, placenta and lochia, and in the involution of the uterus (Kündig et al. 1990). The disruption of the physiological mechanism of myometrial contraction is the source of many obstetric (Noakes, Parkinson, and England 2001) and reproductive (Slama, Vaillancourt, and Goff 1991) disorders that directly affect fertility.

Towards the end of pregnancy, both the placenta and the foetus undergo a profound process of maturation (Ferrell and Ford 1980). The maturation process of the placenta is crucial for the occurrence of a normal delivery. This hypothesis was formulated to describe the sonographic characteristics of this physiological process occurring in cows in the last days of pregnancy. The aim of this study is to investigate changes in uterine artery Doppler sonographic measurements providing blood flow to the placenta, as well as differences in placental echotexture/perfusion and myometrial thickness during the last 10 days of pregnancy in cows. In addition, the study will evaluate the correlations between B-mode and Doppler ultrasonographic parameters to examine their interrelationships.

2 | Materials and Methods

2.1 | Animals

In the presented study, eight clinically healthy Brown Swiss cows, aged between 3 and 7 years (with an average age of 3.40 ± 0.45 years), each having calved at least once and body condition scores ranging from 3.25 to 3.75, at Day 270 of gestation were utilized. Throughout the study period, cows were managed and fed under standard conditions, receiving a mixed ration consisting of pasture grass, wheat straw and cattle-concentrated feed.

2.2 | Experimental Design

After the oestrus synchronization protocol, the artificially inseminated cows were subjected to ultrasound examination (Draminski, iScan, Poland) on Day 28, and the cows determined to be pregnant were included in the study. Imaging was tracked over a period of time, and the last 10 days of data were used, starting 1 day before the cows were born. Examinations were conducted at the same time each day (i.e., between 07:30 and 12:00), with each cow's total imaging time limited to a maximum of 15 min. A single

operator conducted the imaging. Prior to the procedure, the cows were placed in separate paddocks and allowed to rest in a calm environment for at least 30 min.

2.3 | B-Mod and Doppler USG

B mode and Doppler ultrasonographic images were obtained for the investigation using a Doppler USG (ESAOTE brand Mylab DeltaVet model, Esaote Biomedica, Genova, Italy) and an automatic multifrequency (5–10 MHz) linear probe that belongs to this device. In order to USG standardization, the following initial image parameters were recorded before to the first examination: frequency 7.5 MHz, image width 5 cm (maximum) and focus 2.5 cm. All examinations were performed using these standard settings.

According to Bollwein, Baumgartner, and Stolla (2002), the uterine artery's (arteria uterina media) anatomical position was established. The insonation angle was maintained between 20° and 60° for every blood flow analysis. Doppler USG images were recorded in a way that at least three consecutive wave currents could be observed in high quality. The uterine artery's diameter (DM) was measured using three different B-mode images, each of which had a perfect circular structure free of artefact in the transverse section. The average of these three measurements was used for the computations.

After imaging the pregnant horn, myometrium images were obtained from the interplacentomal regions in the dorsal part of the uterus. Images from three different regions were selected, and the average of the three images was taken. If there were differences in thickness between different parts of the myometrium, the thickest part was used. Uteroplacental roughness and distinguishability of the uterus and placenta, that is, if images were obtained where a clear boundary line between these tissues could be seen, were recorded. Placentome images were taken from the dorsal curvature of the uterus, the same region of the posterior placental location. Sampling was performed by selecting at least three different placentomes consecutively in the horizontal plane from the dorsal line of the horn. First, B-mode imaging was used to determine the angle and image clarity. Then, colour Doppler mode images with the best blood flow imaging were selected. It was ensured that similarly sized placentomes in the region were imaged. At least three different placentomes were selected, and at least three sagittal images were captured from each placentome using both B-mode and colour Doppler modes. Calculations were made on the average of these three measurements.

2.4 | Image Analyses

ImageJ software (NIH, USA—Image Processing and Analysis Java) was used for the analysis of B-mode images. PixelFlux software program (Chameleon Software, Münster, Germany) was employed for calculating placental perfusion values and haemodynamic parameters of the uterine artery in Doppler images (Figure 1A,B,E,F).

Four regions of interest (ROIs) with square areas of 225 (15 × 15) pixels were chosen on the placenta after the B-mode ultrasound images were examined with ImageJ software (Figure 1C,D). When choosing ROIs, great care was taken to make sure they were all contained within the myometrium. For each sample, the arithmetic mean of ROI values was calculated from three different myometrium images obtained, resulting in a single value for mean grey value (MGV), which was then transferred to the software program for statistical analysis.

The PixelFlux software program was used to determine pulse rate (PR), resistance index (RI), BFVe and BFVo via the pulse wave Doppler image taken from the uterine artery. B-Mode image was also used to determine placentom area (PA), DM and myometrium thickness (MT). After determining the spectrums to be measured in the Pixel Flux program, the desired data were obtained (Figure 1E,F).

2.5 | Statistical Analysis

The sample size ($n = 8$) was determined on the basis of an effect size of 1.87 reported in a previous study (Demiṙ, Kaçar, and Polat 2022), with a power of 95% and a significance level of 5%. G*Power program (Version 3.1.9.7, Germany) was used for power analysis.

The suitability of the data for normal distribution was assessed using the Shapiro–Wilk test, homogeneity of variances was examined using the Levene test and sphericity assumption was verified using the Mauchly test. Changes in placentome area, MT, myometrium MGV, placental MGV and placental perfusion over sampling time were determined using repeated measures analysis of variance (ANOVA). Bonferroni correction was applied for pairwise comparisons between days. For the analysis of BFVe, BFVo, RI, PR and DM measurements obtained from pregnant and non-pregnant horns, two-way ANOVA was employed to assess the group effect (pregnant horn vs. non-pregnant horn), time effect and group × time interactions statistically. Tukey's multiple comparison test was used for pairwise comparisons within groups across days and between groups on sampling days. Pearson correlation coefficient was used to evaluate relationships between parameters. A significance level of $p < 0.05$ was adopted for all statistical analyses. The data were presented as mean ± standard error of mean (SEM). GraphPad Prism (Version 8.0, GraphPad Software Inc., San Diego, CA, USA) and SPSS 26 (SPSS Inc., Chicago, IL, USA) software packages were utilized for data analysis.

3 | Results

3.1 | B-Mod/Echotexture

The placentome area showed a decreasing trend from the beginning of sampling until delivery ($p = 0.005$). Placentome area (cm^2) showed statistically significant differences over time between prepartum days 1–6 ($p = 0.049$), 8 ($p = 0.009$) and 10 ($p = 0.015$). The placentome area was lowest on Day 1 prepartum (4.205 cm^2) compared to Days 6, 8 and 10 (Figure 2A). In terms of MT, a significant decrease was observed between the first 2 sampling days (prepartum days 9 and 10) and the last 2 sampling days (prepartum days 1 and 2) ($p = 0.009$). MT, particularly, exhibited a significant decrease in the last 2 prepartum days (Figure 2B).

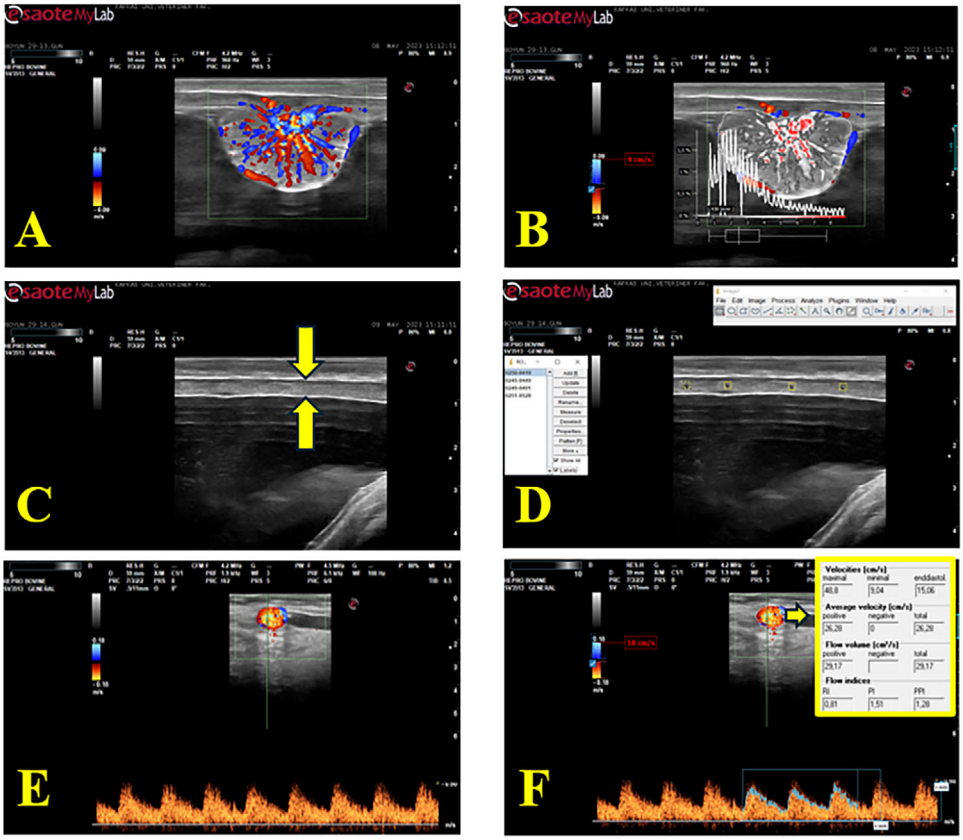


FIGURE 1 | (A and B) Colour Doppler ultrasound image and image analysis; (C and D) formation of the ROI set for myometrium B-Mode image and echotexture analysis; (E and F) spectral data obtained using the PixelFlux program.

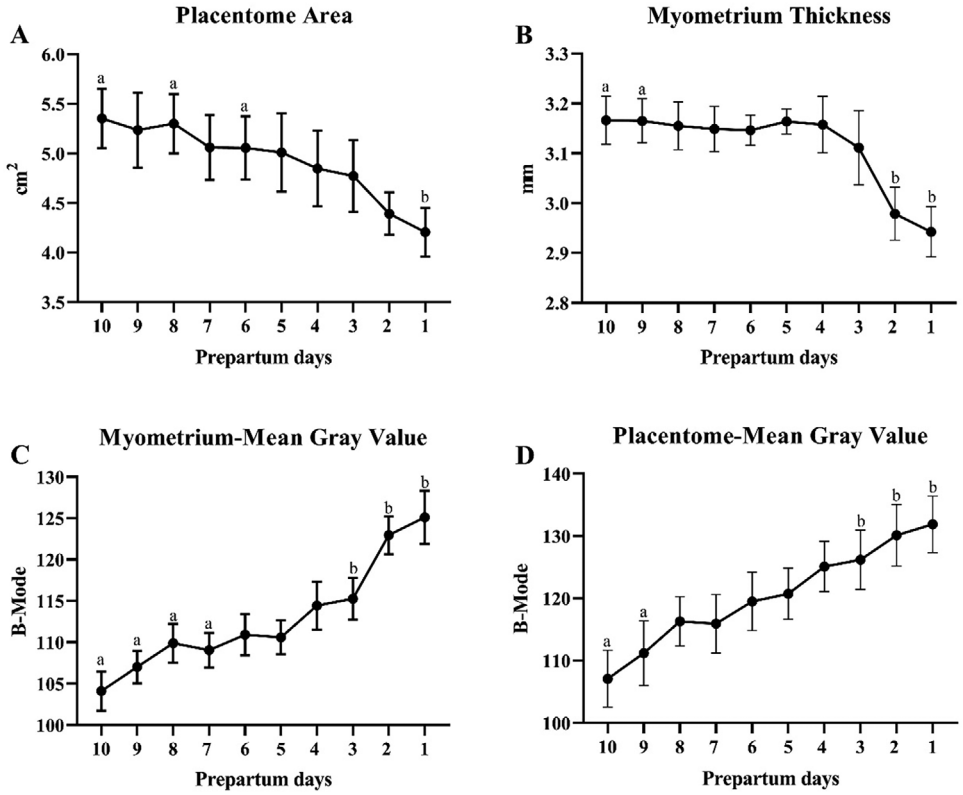


FIGURE 2 | (A and B) Changes in placental area and myometrial thickness during the last 10 days prepartum; (C and D) changes in the mean grey values of myometrium and placenta over time. [a,b]Denote statistical differences between days ($p < 0.05$).

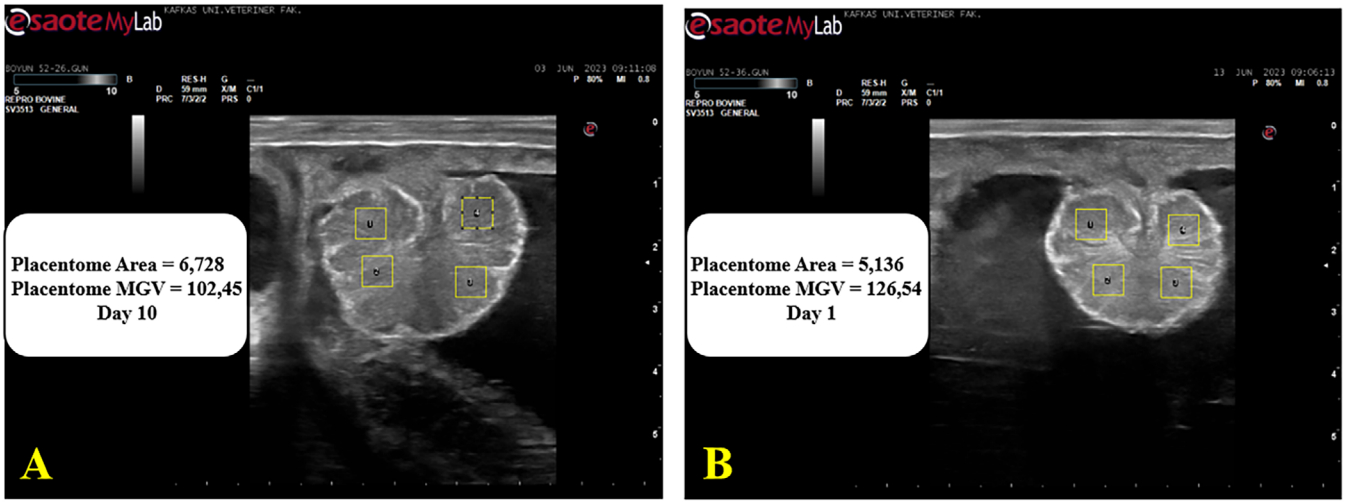


FIGURE 3 | Changes in the area and MGV of the same placentome at prepartum 10 (A) and 1 days (B).

The myometrium MGV showed an increasing trend throughout the study period, which was statistically significant ($p < 0.001$). Especially, the last 3 days prepartum were significantly different from prepartum days 7–10 ($p < 0.05$) (Figure 2C).

Placentome MGV exhibited similar increasing trends as myometrium MGV throughout the study period. Placentome MGV increased as parturition approached, and the measurements taken on these days were statistically significant in multiple comparisons ($p < 0.001$). Especially, the difference between prepartum days 10 and 9 and the last 3 days was statistically significant ($p < 0.05$). Placentome MGV was determined as 107.1 on prepartum day 10 and 131.9 on prepartum day 1 ($p < 0.05$) (Figure 2D).

According to the image analysis conducted on prepartum days 10 and 1, the area belonging to the same placentome decreased (Figure 3A), whereas the placentome MGV increased (Figure 3B).

3.2 | Doppler

Placental perfusion exhibited a decreasing trend 3 days before parturition. Notably, the difference in placental perfusion between Days 3 and 1 prepartum was statistically significant ($p = 0.008$) (Figure 4A).

In this study, there was a group effect on uterine artery BFVe ($p < 0.001$). The uterine artery BFVe between pregnant and non-pregnant horns was statistically significant on prepartum days 9 ($p < 0.05$), 7 ($p < 0.001$), 3 ($p < 0.05$), 2 ($p < 0.001$) and 1 ($p < 0.001$). However, there was no statistically significant time effect on uterine artery BFVe (Figure 4B).

The uterine artery BFVo in pregnant and non-pregnant horns was not statistically significant throughout the study period ($p > 0.05$) (Figure 4C).

The RI in non-pregnant horns was numerically higher than in pregnant horns throughout the study period, but there was no statistically significant difference between the two groups ($p < 0.05$). Additionally, there was a time effect on RI ($p = 0.005$) (Figure 4D).

The uterine artery PR of both horns increased significantly throughout the study period, with a time effect ($p = 0.009$). PR on prepartum days 1 and 2 was higher than on prepartum days 8–10 ($p < 0.05$) (Figure 4E).

There was a group effect on uterine DM between pregnant and non-pregnant horns ($p = 0.02$), but there was no statistically significant time effect ($p < 0.05$). Specifically, the difference between the two groups was statistically significant after prepartum day 7 (Figure 4F).

3.3 | The Pearson Correlation Coefficient Between Doppler Findings

The correlation coefficients and significance levels between Doppler findings are provided in Table 1. Placental perfusion correlated with pregnant uterine artery RI ($r = -0.362$, $p < 0.01$) and DM ($r = 0.343$, $p < 0.01$). Similarly, there was a significant correlation between non-pregnant uterine artery RI ($r = -0.323$, $p < 0.01$) and DM ($r = 0.540$, $p < 0.01$) of non-pregnant horns and placental perfusion (Table 1).

Pregnant horn BFVe showed a strong positive correlation with pregnant uterine artery BFVo ($r = 0.818$, $p < 0.01$) and DM ($r = 0.391$, $p < 0.01$) (Table 1).

3.4 | The Pearson Correlation Coefficients Between Variables Determined by B-Mod

The Pearson correlation coefficients between the variables determined by B-Mod are given in Table 2. There were correlations between placental area and myometrial thickness ($r = 0.622$, $p < 0.01$), myometrial MGV ($r = -0.430$, $p < 0.01$), placental MGV ($r = -0.498$, $p < 0.01$) and placental perfusion ($r = 0.351$, $p < 0.01$) (Table 2).

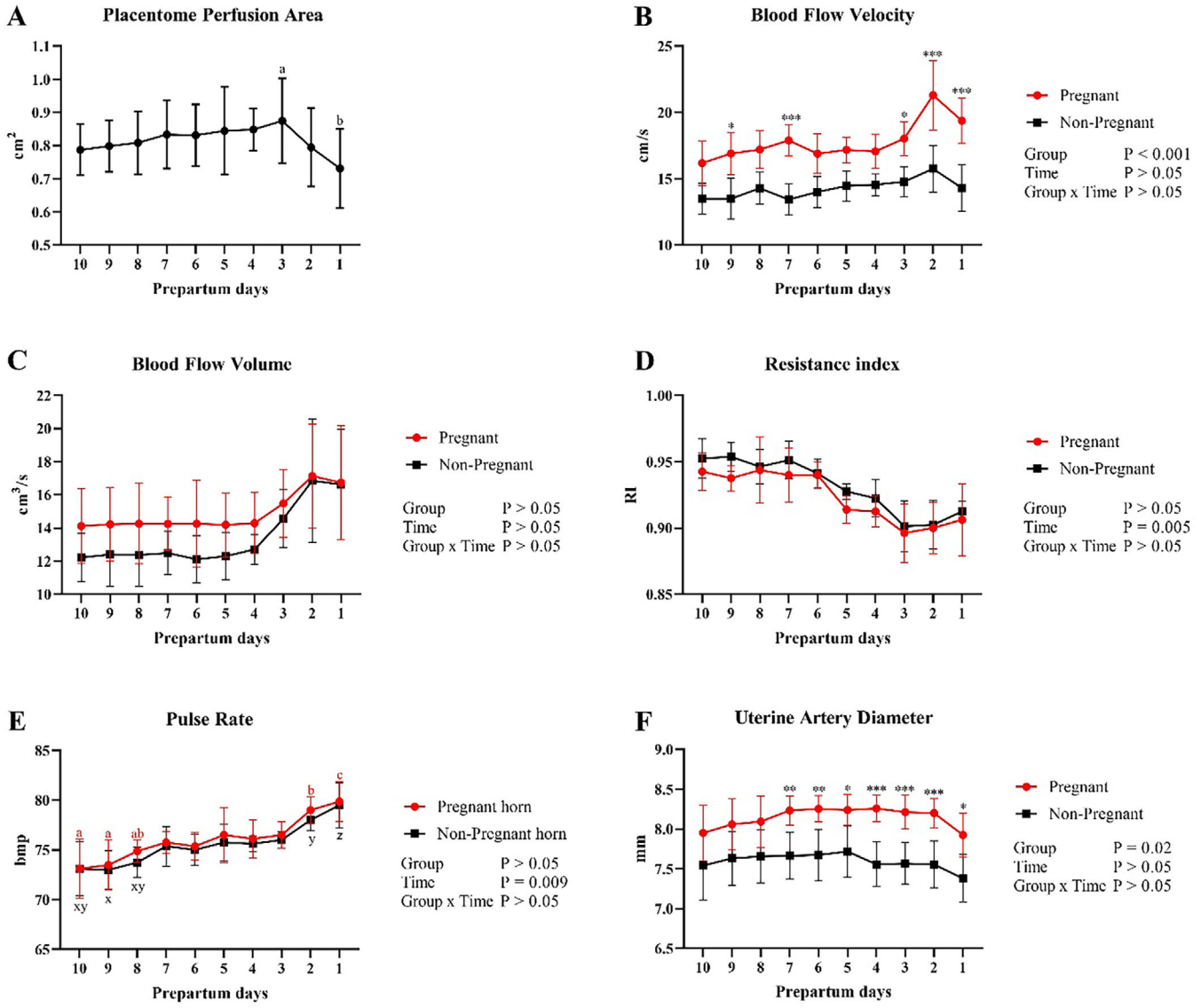


FIGURE 4 | (A) Changes in placental perfusion during the prepartum period; (B–F) changes in BFVe, BFVo, RI, PR and DM of uterine arteries belonging to pregnant and non-pregnant horns. BFVe, blood flow velocity; BFVo, blood flow volume; RI, resistance index; PR, pulse rate; DM, uterine artery diameter. [a–c]Statistical differences in pulse rate within days in pregnant horns. [x–z]Statistical differences in pulse rate within days in non-pregnant horns. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

4 | Discussion

Transabdominal B-Mode/Doppler USG is commonly used in women for the diagnosis of foetal/placental developmental anomalies and placental lesions (Fadl et al. 2017). However, in veterinary medicine, particularly in farm animals, studies evaluating placental perfusion during pregnancy are quite limited and typically involve intermittent sampling during the last month of gestation (Kim-Egloff et al. 2016; Waldvogel and Bleul 2014; Demir, Kaçar, and Polat 2022; Hussein 2013). These studies mostly focus on investigating the effects of different medical agents on perfusion or assessing pathological conditions (Kim-Egloff et al. 2016; Waldvogel and Bleul 2014; Hussein 2013). A literature search did not reveal any information on how daily ultrasonographic parameters physiologically change during the last 10 days of pregnancy, particularly regarding the process of placental maturation.

4.1 | Feasibility of Measurements

Ultrasonographic imaging is a condition that requires precision. It is one of the reasons that makes it difficult to apply intestinal spasmolytics during transrectal sonographic examinations in cows. Intestinal movements make it difficult to keep the transducer fixed on the uterine artery and placentomes for a long time. Therefore, the transducer was temporarily removed from the measurement side and measurements were made again after waiting for a while. This eliminates problems caused by peristalsis (Waldvogel and Bleul 2014; Thijssen et al. 2011). In addition, some of the other problems encountered during image acquisition are the angle of the transducer, the presence of faeces in the rectum, the movement of the foetus, the mobility of the mother and repeated measurements of the same area. Necessary precautions should be taken to minimize such problems, and the examination can be repeated after a certain period of time. To minimize errors,

TABLE 1 | Pearson correlation coefficients between blood flow volume (BFVo), blood flow velocity (BFVe), pulse rate (PR), pulsatility index (PI), uterine artery diameter (DM), resistance index (RI) and placentome perfusion for pregnant and non-pregnant horns.

Parameters	BFVo		RI		DM	
	PH	NPH	PH	NPH	PH	NPH
Placentome perfusion	0.202	0.265*	−0.362**	−0.323**	0.343**	0.540**
BFVe PH	0.818**	0.785**	−0.091	−0.075	0.391**	0.543**
BFVe NPH	0.746**	0.758**	−0.121	−0.058	0.362**	0.505**
PR PH	−0.049	−0.143	−0.006	−0.197	−0.411**	−0.302**
PR NPH	−0.046	−0.107	−0.087	−0.135	−0.254*	−0.229*
BFVo PH	—	0.696**	−0.067	−0.019	0.328**	0.540**
BFVo NPH	0.696**	—	−0.125	−0.063	0.412**	0.482**

Abbreviations: NPH, non-pregnant horn; PH, pregnant horn.
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

TABLE 2 | Pearson correlation coefficients between variables determined with B-Mod.

Parameters	Myometrium thickness	Myometrium-MGV	Placentome-MGV	Placentome perfusion
Placentome area	0.622**	−0.430**	−0.498**	0.351**
Myometrium thickness	—	−0.378**	−0.546**	0.366**
Myometrium-MGV	−0.378**	—	0.233*	0.228*

Abbreviation: MGV, mean grey value.
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

the risk of error can be reduced by ensuring that the image of the area to be examined is homogeneous and by taking multiple images at the specified sampling point and averaging them.

4.2 | Echotexture

Due to the high margin of error in evaluations conducted by eye on echotexture, there has been a need for computer-aided systems. Although tissue densities or characteristics determined by USG can be observed, they are not easily measurable by the human eye, and everyone's perception of greyscale, leading to excessive variability in image interpretation. Therefore, there is a tendency to achieve more quantitative results by evaluating ultrasonographic images using computer-aided programs (Scully et al. 2015).

The placenta is the organ that transfers some hormones and nutrients from the mother to the foetus during pregnancy. Researchers have been investigating the echotextural properties of the placenta for a long time (Demiṙ, Kaçar, and Polat 2022; Cengiz et al. 2014; King 1973). A correlation has been reported between placental MGV and maternal age in humans, including the first trimester of pregnancy (Pala et al. 2015). Measurements of placental MGV in the second and third trimesters in women have shown an increase in the level of calcification in matured and ageing placentas (de Paula et al. 2009). The increase observed in MGV obtained in this study may be associated with increased calcification. Additionally, the distance between foetal and maternal blood vessels shortens in order to meet the increasing demands of the foetus in the last months of pregnancy (Gross, Williams, and Russek-Cohen 1991). Accordingly, the increased connective tissue within the crypt can be considered the cause of the increase in placentome MGV.

The myometrium of cattle consists of smooth muscles, thus possessing the ability for spontaneous contractions (Kaufmann et al. 2008). This property, also known as myogenic activity, is regulated by hormonal and neural stimuli (Popescu et al. 2007). The relationship between various hormones during the periparturient period and the regulation and expression of receptors involved in myometrial contractility have not been fully investigated in cows towards the end of pregnancy (Górriz-Martín et al. 2017). Most in vitro studies conducted so far have focused on the myometrial contractility of cyclic cows and cows in the first 7 months of pregnancy (Kaufmann et al. 2008; Hirsbrunner et al. 2002). In one study, weekly ultrasonographic examinations from the second month of pregnancy until parturition in cows showed no change in myometrial thickness throughout pregnancy (Zoller et al. 2019). In mares, the thickness of the uterus and placenta did not change between Months 4 and 8 of pregnancy but significantly increased between Months 10 and 12. However, in mares with delayed delivery, the thickness of the uterus and placenta significantly decreased (Renaudin, Troedsson, and Gillis 1999). When the foetus enters the birth canal, a sensory stimulus initiates a neural pathway that terminates in the paraventricular

nucleus of the posterior pituitary, causing a release of abundant oxytocin, which in turn stimulates myometrial contraction. These myometrial contractions result in pressure changes in the foetal villi, leading to alterations in hyperaemic and ischaemic conditions and ultimately, physical separation of the feto-maternal unit (McNaughton and Murray 2009). Our study suggests that the progression of myometrial thickness remains stable until the fourth day prepartum and decreases in the last 2 days, indicating the initiation of myometrial activity.

Various changes occur in the cell population of placenta during pregnancy. Such changes are necessary for the maturation of the placenta and the subsequent expulsion of the foetal part of the placenta after delivery. Placental maturation involves complex mechanisms encompassing morphological, functional and endocrinological processes (Woicke et al. 1986). Around the ninth month of pregnancy, the endometrial epithelium begins to thin due to a decrease in the number of trophoblast giant cells, which directly expose the foetal trophoblast epithelium to the endometrium (Boos, Janssen, and Mulling 2003). Additionally, the decrease in foetal blood flow at the time of delivery leads to rapid contraction of cotyledon villi (Leiser et al. 1998). In a study conducted in cows, the size of the placenta decreased after the 250th day of pregnancy (Laven and Peters 2001). Similarly, another study in cows found both increases and decreases in placental size during the last month of pregnancy (Singh et al. 2017). In this study, a decrease in placental area during the last 10 days prepartum was identified, which can be associated with the mechanism described above.

4.3 | Placentome Perfusion

Differences in uterine artery blood flow variables directly affect placental perfusion values. This suggests that placental perfusion can be predicted using uterine artery blood flow. Many foetal chorionic epithelial cells undergo apoptosis when the foetus goes out into the external environment. In cow placentomes, this is seen as a stage of placental maturation (Boos, Janssen, and Mulling 2003). At least some of this chorionic epithelial cell apoptosis is a result of decreased blood flow to the uterus (Kamemori et al. 2011). There are few studies examining the perfusion of placentomes physiologically in the late pregnancy in cows (Demiṙ, Kaçar, and Polat 2022). In one study, the perfusion of placentomes in both pregnant and non-pregnant horns in cows did not change significantly in the last month prepartum (Kim-Egloff et al. 2016). In this study, the decrease in placentome perfusion 3 days before delivery could be associated with both placental maturation and reduction in placentome area. Additionally, the positive correlation between placentome perfusion and placentome area supports the mechanism described above.

4.4 | Spectral

In the last trimester, the increase in the size of the foetus and the uterus is accompanied by an increased demand for nutrients and oxygen. It is assumed that this demand is met by the increased perfusion of the uterus and foetus (Satterfield et al. 2010). In cows, there has been a linear increase in uterine blood flow during the second half of pregnancy (Herzog et al. 2011). In a different study, from Days 30 to 270 of pregnancy, BFVe increased 3-fold, uterine artery DM increased 20-fold and BFVo increased 17-fold (Panarace et al. 2006). Furthermore, in cows, as pregnancy progresses, RI decreases, whereas BFVe, BFVo and DM increases (Bollwein, Baumgartner, and Stolla 2002). In this study, the RI of the ipsilateral uterine artery was lower compared to the contralateral uterine artery, but DM, BFVe and BFVo were higher. These results were consistent with other studies (Bollwein, Baumgartner, and Stolla 2002; Panarace et al. 2006).

Throughout pregnancy, there is generally an upward trend in the physiological changes of both uterine BFVe and its associated BFVo (Bollwein, Baumgartner, and Stolla 2002; Herzog et al. 2011). In this study, a positive correlation was found between BFVe and BFVo in both pregnant and non-pregnant uterine horns. Regarding BFVo, a statistically significant decrease was identified in the late pregnancy period (from prepartum 4.5 to 0.5 weeks) (Kim-Egloff et al. 2016). Another study reported that uterine artery BFVe and BFVo did not significantly change throughout the study period during the last month of pregnancy (Waldvogel and Bleul 2014). Additionally, in cows, BFVo increased during the last month of pregnancy, whereas BFVe and BFVo numerically decreased in the last 2 days (Demiṙ, Kaçar, and Polat 2022). Although the numerical decrease in BFVe and BFVo data 2 days before parturition was not statistically significant ($p < 0.05$), it was considered a noteworthy finding. These findings suggest a decrease in blood flow in the last 2 days, indicating a decrease in perfusion. We can consider this change as an effect of placental maturation. Perhaps in future studies using a larger number of cows, this difference could be better elucidated.

When studies conducted in late pregnancy are examined, it is observed that the spectral parameters RI and PR have not shown significant changes (Kim-Egloff et al. 2016; Waldvogel and Bleul 2014; Demiṙ, Kaçar, and Polat 2022; Hartmann et al. 2013). The increase in PR in our study is similar to other studies (Kim-Egloff et al. 2016; Waldvogel and Bleul 2014). The increase in PR in pregnant women is associated with an increase in plasma volume and is most pronounced in the second half of pregnancy (Guyton and Hall 2005). The decrease in RI in women accompanies the increase in plasma volume, allowing more blood to be carried to the placenta. This is achieved by structural expansion of the uterine vascular bed and reduction of vascular tone (Thornburg et al. 2000). This increase is also likely to be associated with the increased size and efficiency of the heart in bovine and human foetuses (Mary and James 1999). In a study conducted in cows, RI decreased throughout the 36th week of pregnancy (Panarace et al. 2006). The difference in RI in the late pregnancy was not statistically significant (Waldvogel and Bleul 2014; Demiṙ, Kaçar, and Polat 2022). Similarly, in this study, there was no significant difference in RI throughout the study period, but there was a noticeable increase numerically in the last 2 days before delivery. This situation indicates compatibility with decreased perfusion in the last 2 days.

4.5 | Uterine Artery DM

In our study, uterine artery RI did not change significantly and numerically decreased from prepartum day 2 to parturition.

Additionally, although blood flow velocities during the last 2 days prepartum BFVe, BFVo and uterine artery DM moved in a similar direction, the same was not true for RI. Studies in cows have determined that there is no statistically significant change in uterine artery DM between the 37th and 39th weeks of pregnancy (Herzog et al. 2011; Körte 2007), which is consistent with our findings.

5 | Conclusion

The use of B-mode and Doppler USG in the evaluation of placental maturation in cows is quite limited, and there are important gaps in this regard. In this study, physiological changes in perfusion, echotexture and certain measurements (thickness/area) occurring in the last days of pregnancy were determined, and significant differences in these parameters were observed 2 days before delivery. Furthermore, it is considered that ultrasonographic examinations can be effectively used to evaluate inflammatory changes in the uterine wall and placental abnormalities in late pregnancy.

Author Contributions

Murat Can Demir: writing – review and editing, validation, methodology, project administration, investigation, formal analysis, data curation. **Merve Sena Demir**: investigation, writing. **Burak Büyükbaki**: investigation. **Mushap Kuru**: formal analysis, validation, editing, software. **Semra Kaya**: methodology, investigation, formal analysis. **Cihan Kaçar**: writing – review and editing, investigation.

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Ethics Statement

The study was conducted in accordance with ethical principles following approval from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK-2023/146).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

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