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Umbilical Artery Doppler Measurements in Last Week of Pregnancy and Correlation with Vitality of Newborn Lambs

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ABSTRACT

***Background*:** There is a high lamb mortality rate during the first three days of life, resulting in sheep flock economic losses and neonate welfare concerns. A significant number of neonatal deaths could be avoided by preventive management, early diagnosis, and consequently well-timed intervention. The aim of the study was to evaluate the use of umbilical artery Doppler to predict the vitality score (APGAR) of lambs at birth, and its use as a screening tool for detecting high-risk neonatal cases in the last week of gestation, describing changes in the resistivity and pulsatility index in singletons and twins.
***Materials, Methods & Results*:** A total of 27 White Dorper pregnant ewes were studied: 19 multiparous (ewes, 2 to 4-years-old) and 8 primiparous (yearling, up to 1-year-old). From 130 days of pregnancy, gestational ultrasound was performed weekly until delivery, but only data from the last pre-partum scan for each ewe was included. The inclusion criteria for the analysis of the umbilical arteries Doppler were the positioning of the fetus and the quality of the images obtained. Identification of each of the twin fetuses after birth was performed by reference to ultrasound fetometry and its correlation with birth weight. The vitality score at birth (APGAR) was used to classify each group, regardless of the characteristic of eutocic or dystocic delivery, with Group 1 lambs having normal vitality (APGAR ≥ 7) and Group 2 lambs having low vitality (APGAR < 7). A transabdominal Doppler ultrasound of 28 fetal umbilical arteries was performed in the last week before delivery (146 ± 2.4 days of gestation), and the results were compared with the vitality scores of lambs at birth. There were 53.6% of lambs (15/28) with normal vitality scores (Group 1), and 46.4% of lambs (13/28) had low vitality scores (Group 2). The umbilical artery Doppler ultrasound analyzes of the resistance index (RI), the pulsatility index (PI), and the RI and PI oscillations determined by the resistivity index gradient (RIgrad), the resistivity index variations (RIvar%), the pulsatility index gradient (PIgrad) and the pulsatility index variation (PIvar%) were established for each fetus. Pearson's correlation was performed for each umbilical artery Doppler ultrasound variable and vitality score of each lamb, and the T Student's test was performed between Groups 1 and 2. There were no statistically significant differences between groups. Furthermore, the RI values or oscillations did not correlate with the lambs' vitality score, and mildly increased PI values need to be interpreted with caution. Neonatal vitality in lambs was correlated with pulsatility index oscillations: PIgrad ($r = 0.4005$; $P = 0.0346$) and PIvar% (r = 007; $P = 0.0299$).
***Discussion*:** Measurement of umbilical artery RI and PI is possible in ewes with singleton and twin pregnancies in the last week of gestation. High PI of the umbilical artery did not correlate with a low lamb vitality score at birth, as previously described in human fetal medicine. The PI alone cannot be correlated with APGAR in lambs, but the PIgrad and PIvar% analyzes should be performed. In conclusion, the RI and PI values, if analyzed in isolation, show no correlation with the vitality scores of lambs at birth. However, making multiple measurements to derive associations between maximum and minimum values of PI such as PIgrad and PIvar% in the same fetus, could help to screen for high-risk neonates.

Keywords: sheep, ultrasound, neonatal risk, Apgar.

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INTRODUCTION

Mortality in lambs is high (21%) during the 1st 3 days of life, resulting in economic losses and neonate welfare concerns [25]. Vitality score at birth (APGAR) is a neonatal hypoxia examination, allowing the assessment of a neonates' ability to adapt from intrauterine to extrauterine life. It can be used to indicate the presence of fetal abnormalities and to reduce neonatal mortality through early interventions [9,31].

Umbilical artery (UA) Doppler ultrasound has been used to monitor high-risk pregnancies in women, and anomalies in UA blood flow are often considered an early sign of an unhealthy fetus [20,23]. The pulsatility index (PI) and resistance index (RI) of UA Doppler indirectly measure the placental blood flow resistance, providing information for early diagnosis of hypoxia and for predicting catastrophic perinatal outcomes in infants [2,20,33].

Few studies have correlated fetal ultrasound assessments and the neonatal vitality of lambs [32]. This technique could be used to establish fetal viability in sheep, as previously described in pregnant women [2,5,14,21] and pregnant bitches [6,16].

There is little data regarding the accuracy of Doppler examination in peripartum ewes, specifically the analysis of the uterine artery PI and RI. This data may be useful to guide therapeutic strategies, thus improving neonatal survival rates in lambs. The present study aims to determine the feasibility of evaluating UA Doppler in pregnant ewes from 130 days of gestation; to demonstrate the RI and PI in ewes in the last week of gestation; to investigate an association between the RI and PI and lamb vitality at birth; and whether these data will be useful in screening for high-risk births.

MATERIALS AND METHODS

Location and animals

A total of 27 White Dorper pregnant ewes were studied: 19 multiparous (ewes, 2 to 4-years-old) and 8 primiparous (yearling, up to 1-year-old). The WD ram was painted on the chest with a mixture of pigment (Xadrez®)[1] and soybean oil every night before mating, and the lumbosacral region of the ewes was observed for marking by the ram every morning to identify day zero (D0) of gestation. Pregnancy was confirmed by ultrasound between 30 and 45 days after the beginning of the breeding season and the date of mating was confirmed by fetometry in the first 3rd of pregnancy [18]. From 130 days of pregnancy, gestational ultrasound was performed weekly until delivery, but only data from the last pre-partum scan for each ewe was included.

The study was conducted at the Laboratory of Production and Research of Sheep and Goats at the Federal University of Paraná, Pinhais/PR, Brazil. The animals were kept in farm pens, fed with corn silage and nutritionally balanced commercial concentrate, twice daily. Feed quantities were calculated for each group and production stage based on the nutritional needs of the sheep [22]. Mineral salt and drinking water were offered *ad libitum*.

Transabdominal ultrasound examination

Transabdominal ultrasound examinations were performed with the animals restrained in a standing position using a two-dimensional ultrasound device equipped with Doppler (Logic V2®)[2] and with a 2.0-5.0 MHz convex multifrequency transducer (4C-RS Convex Probe®)[3]. The ewe's abdominal wool was cut to optimize ultrasound image acquisition and acoustic gel was applied to the transducer. Image quality was maximized by adjusting the gain, focus, and depth penetration for each fetus during the scan. Sonographic examinations were performed on all ewes by the same sonographer. The examination followed a standard protocol to visualize the entire abdomen by scanning in a counterclockwise direction, starting with the fetus in the right uterine horn (caudal to cranial) followed by the left horn (cranial to caudal).

Thirty days before the start of the experimental period, the animals were conditioned to clipping of the abdominal region and ultrasound exams, to minimize the stress induced by the experimental procedures [28]. The examination was completed in 10 min and under minimal stress conditions for each ewe.

The number of fetuses was determined by B-mode ultrasound examinations and confirmed at delivery. Due to fetal size and position in the last week of gestation, it was not always possible to assess the umbilical cord of both fetuses in twin pregnancies. To identify each twin at birth, fetal measurements were obtained [34], and correlated with birth weight, using the positive correlation between fetal measurements and birth weight in lambs [10].

The resistance index (RI) and the pulsatility index (PI) of the UA were determined by Doppler. A transverse scan of the placenta was performed, and Doppler of the arteries was applied to the floating site of the umbilical cord of each fetus. Data was collected from

at least 3 cardiac cycles for each fetus and assessments were performed on as many fetuses as possible. However, measurements were not made if fetal positioning was suboptimal. In all cases, the images (B-mode or color and pulsed Doppler) were frozen and saved on the equipment's hard disk for later measurements and data analysis. To calculate the mean (mean), the maximum (max), and the minimum (min) of the pulsatility index and the resistance index, measurements were made from at least 2 waves in each cycle obtained from each fetus. For each value of the PI and RI index in each fetus, the gradient (grad) was calculated, by subtracting the minimum from the maximum value. The variation (var%) of PI and RI was calculated by dividing the gradient by the maximum value and multiplying by 100, adapted from umbilical artery Doppler previous study [15] (Figure 1).

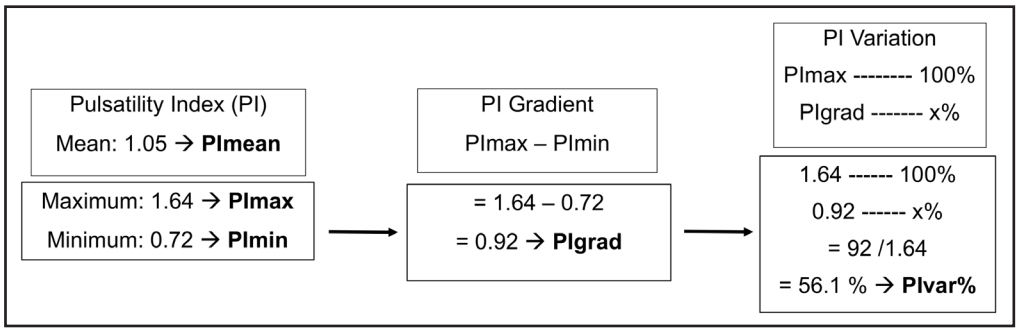

Figure 1. Parameters for analysis of pulsatility index oscillation (PI gradient and PI variation) and mathematical method for such acquisitions, adapted from Giannico [15]. PI: pulsatility index; PImean: average pulsatility index; PImin: minimum pulsatility index; PImax: maximum pulsatility index; PIgrad: pulsatility index gradient; PIvar%: variation of the pulsatility index.

Delivery and neonatal assessment

All ewes were observed during delivery, from the release of amniotic fluid to the passage of the lamb through the vulva and its expulsion. Assistance was provided only if necessary, that is, if the lamb had not been expelled by 60 min after the 1st release of amniotic fluid and/or after repeated and strong abdominal contraction. The vitality of lambs was assessed immediately after expulsion using the APGAR score, as previously described by Vannucchi *et al*. [32], and lambs with a score below 7 were considered to have low vitality [9]. All lambs were weighed 1 h after birth.

Experimental groups

The ultrasound evaluation was performed in 27 ewes in the last week before delivery (gestational duration: 146 ± 2.4 days) and single and twin fetuses were detected. The inclusion criteria for the analysis of the arteries were the positioning of the fetus and the quality of the images obtained. Identification of each of the twin fetuses after birth was performed by reference to ultrasound fetometry and its correlation with birth weight, as previously mentioned. The vitality score at birth was used to classify each group, regardless of the characteristic of eutocic or dystocic delivery, with Group 1 lambs having normal vitality (APGAR ≥ 7) and Group 2 lambs low vitality (APGAR < 7) [31].

Statistical analysis

For statistical analysis, a descriptive analysis of data was performed with simple and relative frequencies of qualitative variables and mean, minimum, maximum, and standard deviation, of quantitative variables. For these variables, the Shapiro-Wilk normality test was also performed. To assess the correlation between 10 proposed predictor variables (RImean, RImin, RImax, RIgrad, RIvar, PImean, PImin, PImax, PIgrad, PIvar) and vitality of lamb after birth, Pearson's correlation coefficients were calculated. To assess mean differences between Groups 1 and 2 for each ultrasound variable T Student test was performed. The significance level was 5%, and all analyzes were performed in the R 4.0.4 environment (R Core Team, 2021).

RESULTS

Twenty-seven ewes were evaluated by gestational ultrasound from 130 days of gestation. There were 16 single pregnancies, and 11 twins, resulting in 38 fetuses measured by ultrasound. Due to the suboptimal positioning of twin fetuses, the RI and PI were measured in 73.7% (28/37) umbilical arteries between 141 ± 3.3 days of gestation. Of the fetuses evaluated by umbilical Doppler 71.4 % were from ewes (20/28) and 28.6% from yearlings (8/28); 57.1% were singletons (16/28), and

42.9% were twins (12/28); 53.6% had eutocic (15/28) and 46.4% had dystocic delivery (13/28). At birth, 53.6% of lambs (15/28) had normal vitality score (APGAR ≥ 7), considered as Group 1, and 46.4% of lambs (13/28) had low vitality score (APGAR< 7), considered as Group 2. Of the lambs in Group 2, 46.2% (6/13) had a severe compromise (APGAR < 3) - 1 abortion, 1 stillbirth, APGAR = 0, and 4 with APGAR 1 to 3 -, and 53.8% (7/13) had moderate compromise APGAR 4 to 6.

The mean, minimum and maximum values of the umbilical artery RI and PI were slightly higher in Group 2 (APGAR < 7), but the means of gradient and variation of RI and PI were slightly lower for the same group compared to Group 1 (APGAR ≥7) (Table 1). The standard deviation of the mean pulsatility index (PImean) and the minimum pulsatility index (PImin) in Group 2 was higher than Group 1. There was no statistically significant difference between the groups for any Doppler variables of the umbilical arteries at the *T* Student test.

When associating the vitality score (APGAR) of the lambs and the Doppler measurements of the UA, a weak Pearson correlation coefficient was found in 2 of the 5 variables of the pulsatility index: PIgrad (r = 0.4005; *P* = 0.0346) and PIvar% (r = 0.4107; *P* = 0.0299). There was no significant correlation between the vitality score at birth (APGAR) and the 5 variables of the resistance index (RI) of the UA.

Table 1. Mean, minimum, maximum, and standard deviation values of the resistance index and pulsatility index of the umbilical arteries and their derivations in the last week of gestation for Group 1 (15 lambs) and Group 2 (13 lambs).

	Normal Vitality Group 1: APGAR ≥ 7					Low Vitality Group 2: APGAR < 7				
Variable	N	mean	minimum	maximum	Standard deviation	N	mean	minimum	maximum	Standard deviation
APGAR	15	8.67	7.00	10.00	1.29	13	3.38	0.00	6.00	2.22
RImean	15	0.65	0.56	0.75	0.06	13	0.67	0.57	0.80	0.08
RImin	15	0.57	0.46	0.69	0.07	13	0.61	0.51	0.75	0.08
RImax	15	0.72	0.59	0.82	0.07	13	0.73	0.59	0.86	0.08
RIgrad	15	0.15	0.06	0.27	0.06	13	0.13	0.04	0.20	0.05
RIvar%	15	20.38	10.17	36.49	6.95	13	17.22	6.78	27.14	6.53
PImean	15	1.14	0.89	1.45	0.18	13	1.19	0.78	1.83	0.31
PImin	15	0.91	0.64	1.17	0.14	13	0.99	0.72	1.62	0.29
PImax	15	1.44	0.95	2.22	0.34	13	1.42	0.81	2.12	0.39
PIgrad	15	0.53	0.07	1.34	0.31	13	0.42	0.06	0.92	0.21
PIvar %	15	34.52	7.37	60.36	13.93	13	30.05	7.41	56.10	11.71

N: Number of samples; APGAR: neonatal vitality score; RImean: average resistance index; RImin: minimum resistance index; RImax: maximum resistance index; RIgrad: resistance index gradient; RIvar%: resistance index variation; PImean: average pulsatility index; PImin: minimum pulsatility index; PImax: maximum pulsatility index; PIgrad: pulsatility index gradient; PIvar%: variation of the pulsatility index.

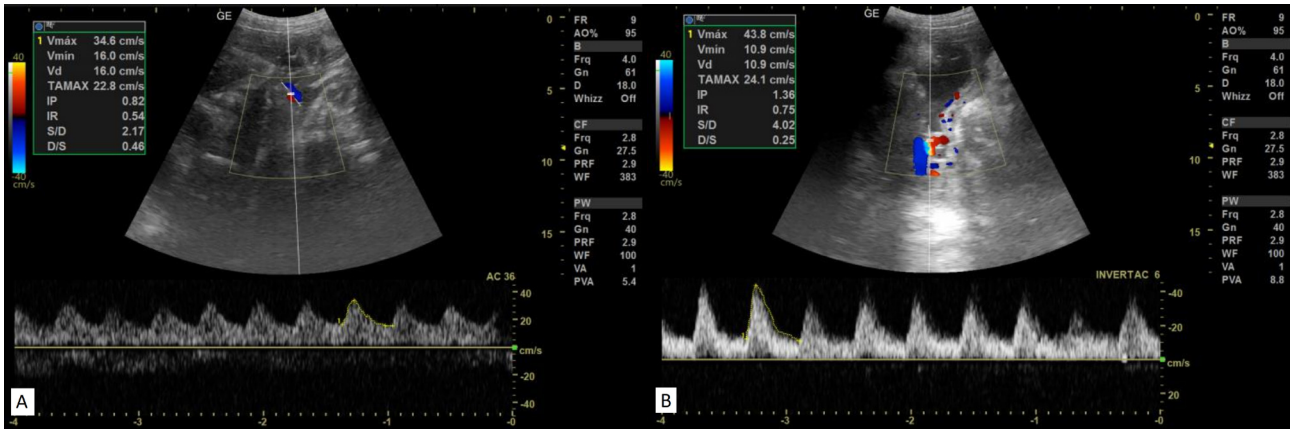

Figure 2. Variation of spectral waves from the umbilical artery of 2 different fetuses, in the last week of gestation in Group 2 (APGAR<7). Waves with a low resistance to blood flow (RI=0.54; PI=0.82) in lamb with APGAR 5 at birth (A) and waves with high resistance to blood flow (RI=0.75; PI=1.36) in lamb with APGAR 2 at birth (B). The images were obtained and processed in a LogicV2® ultrasound system with a convex transducer, image frequency: of 2.8 MHz - scan depth: of 180 mm.

DISCUSSION

The continual improvement in ultrasound technology has allowed access to more specific analyzes of gestational Doppler in a number of species, including ewe. This study shows that the evaluation of the RI and PI is possible at the end of pregnancy in ewes, and that image acquisition takes approximately 10 min, despite the need for post-processing. Doppler is a routine technique in the analysis of uteroplacental and fetal circulation in women [2,21,23,26] and its use has already been established in other species [6,7,16,27,29] and in ewes at other gestational stages [1,3,11,13,19,24,28].

Care was taken during the study to identify which of the twins was being visualized in multiple pregnancies and to ensure correct classification of the lamb as Group 1 and Group 2. This was possible by correlating measurements of specific fetal structures in B-mode ultrasound with birth weight [10,34].

The mean data obtained from UA Doppler in this study were RI (0.65 ± 0.06) and PI (1.14 ± 0.18) for lambs with normal vitality at birth (APGAR ≥7) and RI (0.67 ± 0.08) and PI (1.19 ± 0.31) in lambs with a low vitality score at birth (APGAR <7) [Table 1]. These values are similar to those described by researchers who performed UA Doppler in 12 healthy Chios-cross ewes, 5 single and 7 twin pregnancies, obtaining the indexes at 140 days of gestation RI (0.69 ± 0.02) and PI (1.19 ± 0.05), and at 145 days, RI (0.62 ± 0.02) and PI (0.99 ± 0.07) [4]. Interestingly, the indexes show a slight increase when compared to previous gestational stages, such as at 130 days [30] and 135 days [35].

There was no significant difference in the mean, minimum and maximum values of resistance index (RI) and pulsatility index (PI) of the UA between the 2 groups of lambs, although the mean, minimum and maximum values were slightly higher in Group 2 (APGAR < 7). This suggest that the umbilical blood flow velocity, in the last week of gestation, shows greater resistance in lambs with low vitality at birth. To better express these differences, RI and PI gradient and variation calculations were established (Figure 1). The means of gradient and variation of RI and PI were slightly lower in Group 2 (APGAR < 7) [Table 1], suggesting less oscillations in lambs with low vitality at birth.

The lower mean values and standard deviation of PIgrad and PIvar% in Group 2 demonstrate the differences between the values of PImax, PImin. The percentage differences obtained from the PI of the umbilical artery of each fetus are associated with variations in the fetal placental circulation. The increased PI reflects inadequate vascularization of the placenta. However, PI and RI begin to increase when approximately 60-70% of the placental vascular tree is not functioning [2]. That is, only 6 of the newborns in this study had a severe impairment at birth (APGAR < 3), and 2 died (APGAR = 0), and therefore no significant differences were identified.

Pearson's correlation coefficient showed a weak association between the PI index (PIgrad and PIvar%) and the APGAR score used to analyze lamb vitality. The positive correlation shows that the umbilical artery blood flow velocity is more variable in lambs with normal vitality, than in lambs with low vitality at birth. However, since this approach has not been previously studied, comparisons were not possible. We propose that future studies are required to establish the effectiveness of this type of mathematical analysis.

It was also not possible to associate a high umbilical artery PI (> 1.2 humans) with a low APGAR score at birth in babies [8]. This concurs with our findings, as shown in Figure 2. The mean and a specific cut-off value of the PI were not associated with the lamb's vitality score at birth. However, although the mean PI value alone cannot be correlated with the APGAR score in lambs, analysis of PIgrad and PIvar% should be performed to obtain more data. Despite this, we were unable to identify significant differences that would inform improvements in the management of ewes at the end of pregnancy.

Contrary to our hypothesis, there was no association between the umbilical artery RI and the newborn lamb's vitality (APGAR). In a study in pregnant ewes and with different blood load challenges to fetuses, the researchers observed that fetal hypotension, induced by 10 to 15% blood removal (hypovolemia), increased the pulsatility index of the umbilical artery by 42%, but was not associated with increased total placental vascular resistance (RI) [17]. The same authors also found that a 10 to 15% increase in fetal blood volume (hypervolemia), resulted in a 29% increase in blood pressure, but did not affect the umbilical artery PI or the placental vascular RI. They concluded that the increase in velocity indices

in umbilical artery blood vessels are not necessarily related to placental vascular disease, but may be a sign of fetal hypotension [17].

Previous studies have suggested a correlation of Doppler PI and RI values, with fetal vitality in the newborn child [21]. The progressive decrease in the diastolic flow of the umbilical arteries increases the Doppler indices of the umbilical artery studied (RI and PI). Diastolic flow progressively reduces in the umbilical arteries due to deformations of the vascularization of the placental villi, and placental insufficiency promotes a chronic deficiency of nutrition, oxygenation, growth retardation, movement, circulatory compensatory mechanisms in response to hypoxia [23]. Together, researches stated that a single brief episode of fetal asphyxia at the end of gestation in ewes results in significant disruption of gray and white matter in the developing brain [12] and causes a significant behavioral retardation in newborn lambs and influences their vitality score (APGAR) [31].

The limitations of this study correspond to the number of fetuses analyzed, and the inability to analyze all the Doppler indices in both twins due to the positioning of the fetuses at the time of the examination.

In summary, Doppler analysis can be performed in ewes at the end of gestation, even with twin fetuses. The RI values do not correlate with the vitality score of the lambs at birth. The PI value alone should be interpreted with caution. The use of the PIgrad, PIvar% value of the umbilical artery needs more than one measurement for each fetus at the same examination. Further studies are required to validate this proposed model for early diagnosis of a low vitality score in lambs.

CONCLUSIONS

The values of resistance index (RI) and pulsatility index (PI) of the umbilical artery of single or twin fetuses, if analyzed in isolation, do not show association with the vitality score of lambs at birth. The proposal of making several measurements in the same fetus and analyzes of maximum and minimum values of PI to calculate PIgrad and PIvar% could help in the detection of high-risk neonates.

MANUFACTURERS

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Ethical approval. All study procedures were previously approved by the Ethics Committee on the Use of Animals of the Campus of Agricultural Sciences of the Federal University of Paraná, Brazil (051/2020).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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