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Doppler ultrasonographic measurements of the lateral digital palmar artery in pregnant mares

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Abstract

Cardiovascular changes have been reported in late pregnancy in mares. However, there are no data on changes in peripheral blood flow. Doppler ultrasound represents a sensitive method for assessing the blood flow directed to the hoof. The aims of this study were to evaluate the blood flow parameters of the lateral palmar digital artery (LPDA) in pregnant mares and to assess intra- and interrater agreement between two observers with different levels of experience. The LPDAs of pregnant Italian Standardbred mares were examined. The vessels were located with B-mode ultrasound and analyzed with color and pulsed wave Doppler. The following parameters were recorded by the operators: heart rate (HR), peak systolic velocity (PSV), end-diastolic velocity (EDV), and resistive index (RI). Measurements were performed between 2 and 3 months of gestation (T1), in the last month of pregnancy (T2) and a week after delivery (T3). Seventeen mares aged 3–18 years met the inclusion criteria. Ultrasound examinations of the LPDA were subjectively easy to perform and well tolerated by the mares. Interrater and intrarater agreement were good and moderate, respectively. The HR was higher at T2 than at T1 and T3. The PSV and RI changed significantly during pregnancy, with higher values at T2 and T3, whereas the EDV remained unchanged throughout the examination. Doppler examination showed that peripheral flow changes were present in mares in late pregnancy. However, the persistence of higher values after delivery invites further investigation to assess the correlation between metabolic/endocrine changes related to pregnancy and Doppler parameters.

KEYWORDS
digital blood flow, echo-Doppler, horse, LPDA, pregnancy

1 | INTRODUCTION

In domestic mammals, pregnancy requires a critical and rapid adaptation of the cardiovascular system to manage the increasing nutritional demands of the fetus.[1] Insufficient adaptation can lead to pathological conditions, such as preeclampsia, intrauterine growth restriction, placental detachment, and stillbirth.[2] In human medicine, Doppler ultrasonography is routinely performed during pregnancy to evaluate uterine and fetal blood flow.[3] Indeed, it provides information on the interaction between mother and fetus and may be a valuable screening tool for preventing complications during pregnancy.[4] One such complication is gestational hypertension, which results from adrenergic activation, producing changes in the vascular endothelium and increasing peripheral resistance. In horses, gestational hypertension can lead to premature birth, stillbirth, abortion, or reduced weight in foals at birth.[5] In pregnant mares, metabolic and cardiovascular abnormalities can lead to functional and morphological changes in the vascular network.[6] A study by Nagel et al.[7] found that systolic and diastolic blood pressure of the coccygeal artery decreased significantly in the last 3 months of pregnancy, reaching minimum values during the last 3 days before delivery and increasing between day 1 and day 3 after delivery. Similarly, but with contradictory results, Fowden et al.[8] performed serial evaluations of blood pressure, heart rate, and plasma concentrations of angiotensin-converting enzyme and angiotensinogen in fifteen pregnant ponies in the second half (days 153–336) and the end of pregnancy (days 320–340), showing that none of the recorded data significantly changed over the considered period. Pregnant mares with chronic laminitis present signs of hypertension syndrome and vascular abnormalities in placental vessels, including a reduced vascular lumen and capillary area in the microcotyledons and thickening of the vascular walls.[5] In these patients, evaluation of blood flow parameters, such as the resistive index (RI), also known as the Pourcelot index, of the digital arteries has been hypothesized to be useful for identifying subclinical laminitis, allowing early intervention and hence improving the prognosis.[9] Indeed, the RI represents the resistance of the vascular wall to blood flow during organ perfusion. Consequently, higher RI values may suggest altered capillary flow in the target organ, even if a false normal result is a possible limitation when the systolic and diastolic velocities increase simultaneously.[10] In equine practice, Doppler ultrasound is performed to assess the blood flow of the digital region.[11–17] Indeed, alteration of the blood flow of the lateral palmar digital artery (LPDA) is a finding that is often linked to foot pathologies.[12,15,17,18] Some data related to circulatory and pressure variations during pregnancy in mares have been reported, but to the authors' knowledge, no data are available regarding possible changes in peripheral blood flow parameters in mares in the peripartum period. Therefore, the primary aim of this study was to evaluate whether changes in the peak systolic velocity (PSV), the end-diastolic velocity (EDV), and the RI of the LPDA, measured using Doppler ultrasound, occur during normal and uneventful pregnancy in a group of healthy Italian Standardbred mares. The secondary objective was to evaluate the intra- and interobserver agreement among two observers with different experience levels in performing such measurements. Our hypothesis was that vascular ultrasound indices would change, along with pregnancy-related physiological modifications. Furthermore, a good level of intra- and interobserver agreement in the acquisition of LPDA Doppler ultrasound parameters (PSV and EDV) was expected.

2 | MATERIALS AND METHODS

2.1 | Selection and description of the subjects

This prospective, cohort, observer agreement study was approved by the Ethical Animal Care and Use Committee of the University of Naples "Federico II" (protocol number PG/0113664). All owners signed written informed consent. Clinically healthy female Italian Standardbred horses in the second and third months of pregnancy from three different horse breeding farms in the same geographical area were enrolled in the study. All mares, except one, were multiparous and had no history of previous complications or infertility problems in the last two pregnancies. The exclusion criteria were as follows: (a) any sign of systemic disease at the clinical examination, (b) lameness of any origin at orthopedic evaluation, (c) abortion, (d) stillbirth, (e) birth of premature or maladapted foal, or (f) any clinical or ultrasonographical abnormality of the fetus or pregnancy. Data were collected at three different time points: between the second and third month of pregnancy (T1), in the last month of pregnancy (T2), and one week after delivery (T3). For each subject included in the study, age, month of pregnancy, and heart rate (HR) were recorded in an electronic spreadsheet (Microsoft Excel ver. 16.53 2, Microsoft Corp.) before ultrasonography.

2.2 | Clinical and orthopedic evaluations

Before each clinical and ultrasonographic examination, all mares were allowed to acclimatize for 15 min in a familiar environment in the presence of the examining clinicians. External ambient temperature during the study sessions was 15 ± 4°C, calculated ex-post from an online meteorological repository (https://www.ilmeteo.it/portale/archivio-meteo), and consequently, the examination room has been considered at 17 ± 2°C. In the immediate postpartum period, clinical and orthopedic exams were performed without moving the mare away from the foal to avoid unreasonable stress. All examinations were performed with the mares manually restrained by their grooms and without sedation to avoid pharmacologically induced alteration of the blood flow. Clinical and orthopedic examinations were performed by one of the authors (M.P.P.), a professor in veterinary equine surgery with a Ph.D. in veterinary surgery and more than 20 years of experience in equine practice, before the ultrasound examination to prevent changes in HR and peripheral circulation. Physical examination included the evaluation of rectal temperature, HR, auscultation of the heart, respiratory rate, auscultation of the lungs, and bowel motility. A physical cardiovascular examination was performed and included the evaluation of the pulse at the facial artery, of mucous membranes and capillary refill time, and the auscultation of the heart at the level of the 3rd–5th intercostal space, dorsal to the olecranon. The orthopedic evaluation included inspection, palpation, and evaluation of the hoof's temperature and digital pulse, deep palpation with a hoof tester, and gait evaluation at walk and trot. Once the orthopedic evaluation was performed, pregnancy was confirmed by transrectal ultrasonography at T1 and transrectal/transabdominal ultrasonography at T2. The ultrasound protocol for pregnancy confirmation included pregnancy diagnosis at 14 days with a transrectal probe and then weekly follow-up until day 40 of pregnancy. At T2, parameters assessed by transrectal examination included: (a) cervix, (b) uteroplacental unit of the cervical pole; (c) amniotic membranes, (d) fetal fluids, (e) fetal eyes, (f) biparietal bone diameter, and (g) peripheral pulsations of the fetus (carotid pulsations). By transabdominal examination were evaluated: (a) fetal heart rate, (b) fetal activity, (c) quality, and (d) quantity of the fetal fluids, (e) aortic diameter, thoracic diameter, and (f) thickness of the placenta.

2.3 | Ultrasonographic examination

Ultrasonographic examinations were performed by two operators: D.C. (operator 1), a third-year Ph.D. student in veterinary diagnostic imaging with five years of experience in ultrasonography, and P.C. (operator 2), a veterinary surgeon with a Ph.D. in veterinary equine surgery and ten years of experience in veterinary surgery. The operators performed the examination while seated with the patient standing squarely with the weight equally distributed on all four limbs and manually restrained by their grooms. All exams were performed using a portable ultrasound machine (Esaote MyLab30 VET Gold Cardiovascular, Esaote) equipped with a 6–18 MHz multifrequency, electronic linear transducer (model LA435, Esaote). Transducer-skin contact was achieved using a silicone standoff pad after clipping the fetlock area, scrubbing with soap, rinsing with tap water, moistening the skin with alcohol, and applying acoustic coupling gel. The limb to start the exam and the operator to begin were randomly selected by flipping a coin. During the same session, only one operator at a time was present in the room during image acquisition. After the first operator completed the examination, the second operator performed the ultrasound examination in the same order. After mutual agreement in a preliminary study phase, both operators adopted a standardized scanning approach to limit inter- and intraoperator variability. The ultrasound probe was positioned over the neurovascular bundle on the palmarolateral aspect of the metacarpophalangeal joint at the level of the lateral palmar articular recess of the fetlock (Figure 1).[16] The LPDA of each forelimb was initially identified using transversal scans and then assessed using longitudinal scans since previous studies suggested that transversal scans are more prone to be affected by multiple refraction artifacts that reduce their quality.[19–21] During the examination, each operator was careful not to apply excessive pressure, causing compression of the LPDA walls. The application of color Doppler in duplex mode aided in differentiating the LPDA from the homonymous vein. Pulsed wave Doppler was then applied to the artery in triplex mode using a sample width of 1–2 mm. The wall filter and pulse repetition frequency were set to the smallest values, allowing the flow to be displayed without aliasing. A fixed Doppler angle correction of 70° was applied, aligning the cursor with the long axis of the vessel. The selection criterion for the Doppler spectrum was a minimum of three similar successive waveforms (Figure 2). After the acquisition of the images, using the “resistive index” function of the ultrasound device, the following parameters were obtained: PSV (cm/s), EDV (cm/s), and RI [calculated as RI = (peak systolic velocity − end-diastolic velocity)/peak systolic velocity] by each operator, both present simultaneously at this stage. The HR in beats per minute (bpm) was also retrospectively measured using images obtained at the beginning (HR1) and at the end (HR2) of the exam using the “heart rate” function of the ultrasound device, with measurements taken based on the distance between the systolic peaks of two consecutive waveforms in the spectral trace. These measurements were stored within the ultrasound device, archived in a picture archiving and communication system (dcm4chee-arc-light version 5.11.1, http://www.dcm4che.org), and recorded in an electronic spreadsheet.

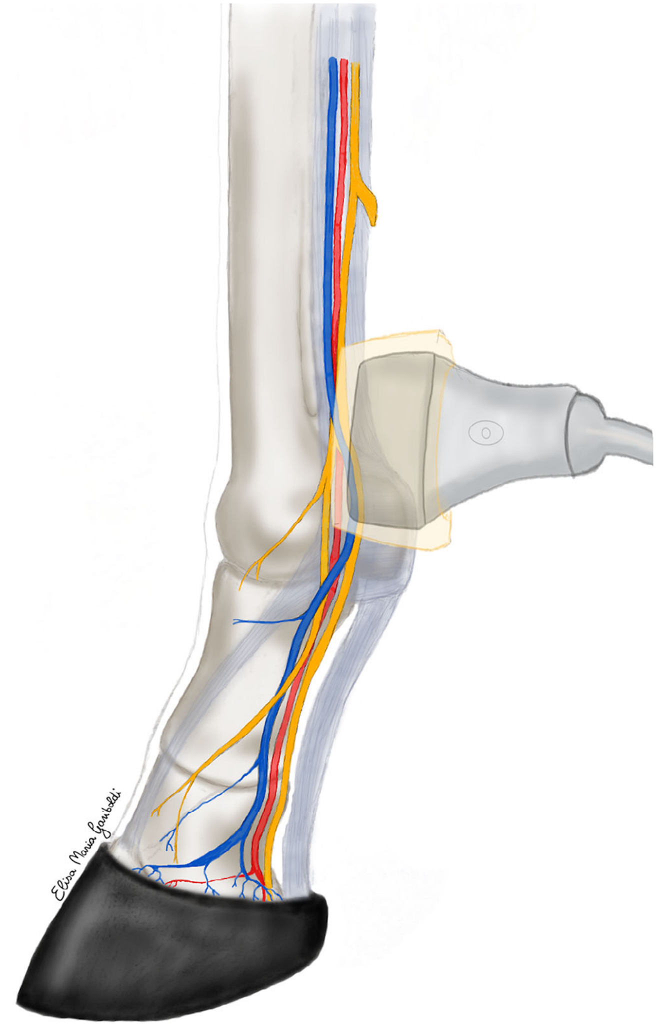

FIGURE 1 Illustration of the equine fetlock showcasing the positioning of the ultrasound probe over the region of interest. The ultrasound probe, equipped with a silicone standoff pad, was placed over the neurovascular bundle on the palmarolateral aspect of the metacarpophalangeal joint at the level of the lateral palmar articular recess of the fetlock. Initial identification of the lateral palmar digital artery (in red) in each forelimb was conducted through transversal scans, followed by comprehensive assessment using longitudinal scans. The illustration also highlights the relevant anatomical structures, including the third metacarpal bone, the lateral proximal sesamoid bone, the lateral suspensory branch of the suspensory ligament, the lateral digital vein (in blue), and the lateral palmar digital nerve with its branches (in yellow). *Source*: Original artwork by Elisa Maria Gariboldi, DVM.

2.4 | Statistical analysis

Statistical analyses were performed by one of the authors (L.A.), a former researcher with a Ph.D. and >10 years of experience and training in statistics, using commercial software (Prism 8 for MacOS, v. 8.2.0, GraphPad Software Inc.; JMP v. 16.0.0 SAS Institute; SPSS 27.01 for MacOS, SPSS Inc.).

Measurements performed by the most experienced operator in imaging (D.C.—operator 1) were recorded in triplicate, and the mean

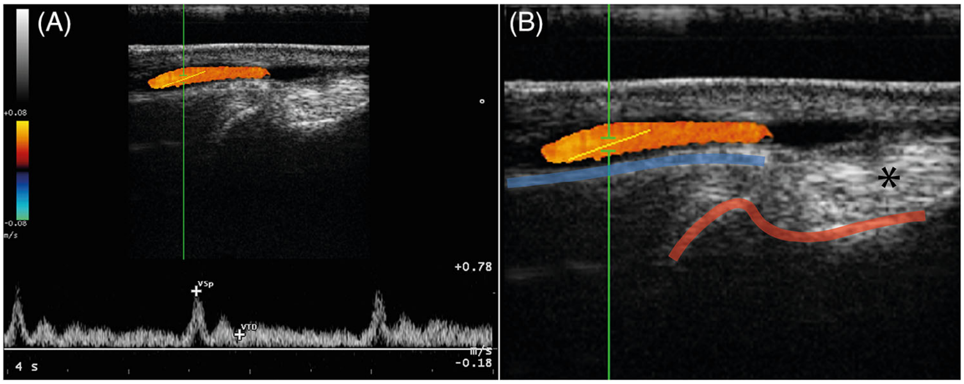

FIGURE 2 Ultrasonographic image (A) and close-up with anatomical landmarks annotation (B) of the ultrasonographic examination of the lateral palmar digital artery (LPDA) using a longitudinal scan. The application of color Doppler in duplex mode aided in differentiating the LPDA (red-yellow blood flow) from the homonymous vein above the distal aspect of the third metacarpal bone (blue highlighter line), the lateral proximal sesamoid bone (red highlighter line) and lateral suspensory branch of the suspensory ligament (asterisk). The pulsed wave Doppler was then applied to the artery in triplex mode using a sample width of 1–2 mm (green parallel lines). After obtaining the waveforms, a fixed Doppler angle correction of 70° was applied aligning a cursor (yellow line) with the long axis of the vessel. Then, the electronic calipers were positioned on the peak systolic velocity (VSp or PSV) and the end-diastolic velocity (VTD or EDV). The ultrasound machine automatically calculated the RI. In the images, the proximal is on the left side of the image. The image was acquired using a multifrequency, linear probe at 12 MHz.

values were used for statistical analysis. Normality was tested with Shapiro–Wilk's *W* test, and data are reported as the mean ± standard deviation (SD) or the median (range) for normally and nonnormally distributed data, respectively. The pooled Student's *t*-test (PSV and RI: at T1 and T3; EDV: at all time points) or the Mann–Whitney *U* test (PSV and RI: at T2) was used to evaluate the effect of side (right vs. left) within groups at any time point. Since any difference between sides (right vs. left) could not be detected, all subsequent analyses were performed by pooling both limbs together. A two-way analysis of variance for repeated measures (RM-ANOVA) with a mixed model for missing values, with a Geisser–Greenhouse correction for sphericity (not assumed), was applied to the pooled right and left measurements to study the effect of time and their interaction on the selected variables. The same statistical approach (i.e., the two-way RM-ANOVA) was applied to evaluate the fluctuation in the HR over the three time points as well as any effect of the procedures performed on the mares (HR1 vs. HR2). The post hoc Fisher's LSD test was used to evaluate differences within the group between each time point.

The effect of age on PSV, EDV, and RI was explored. Since age was not normally distributed, Spearman's rank correlation coefficient (r_s) was applied to explore the association between the age of the mares and the response variables and interpreted as previously reported.[22] Whenever, that is, within any time point, a significant correlation was identified, a least square estimation model for effect leverage was applied, and the leverage diagram was visually evaluated to identify how to eventually categorize age classes. Post hoc, the two age classes were compared with the Student's *t*-test or with Welch's correction after evaluating the homoscedasticity with Levene's test or with the Mann–Whitney's *U* test, according to data distribution.

The intraclass correlation coefficient (ICC) and relative 95% confidence interval (95% CI) were calculated to evaluate intra- and interobserver reliability in measuring PSV and EDV. For the intraobserver reliability test, a two-way mixed effects model with single raters and absolute agreement was applied, using the three measurements performed at each time point for each experimental subject. A two-way mixed effects model with a mean of *k* measurements ($k = 3$ for each operator) and absolute agreement was applied for the interobserver reliability test. An ICC < 0.5 indicated poor reliability, 0.5 < ICC < 0.75 moderate reliability, 0.75 < ICC < 0.9 good reliability, and ICC > 0.90 excellent reliability.[23,24]

3 | RESULTS

Of the 18 mares initially included in the preliminary sample, one was excluded at T2 because of an abortion. Consequently, 17 mares were included in the final sample. The animals were 11.8 ± 4.6 years old (mean ± SD) (range 3–18 years old). All of them were regularly vaccinated and had no history of previous recent significant lameness. Furthermore, they were healthy at the clinical evaluation and showed no signs of ongoing orthopedic disorders. All the mares included in the final sample delivered with no complications, and all newborn foals were healthy at birth. The interobserver reliability test showed good agreement for PSV and moderate agreement for EDV (Table 1). The intraobserver reliability test showed

TABLE 1 Intraclass correlation coefficients and relative 95% confidence intervals and *P*-values for the interobserver agreement among two observers.

Measurement (*n* = 17)	ICC	95% CI	*P*-value
PSV	0.78	0.71–0.84	<.0001
EDV	0.71	0.61–0.78	<.0001

Abbreviations: CI, confidence interval; EDV, end-diastolic velocity; ICC, intraclass correlation coefficient; PSV, peak systolic velocity.

TABLE 2 Intraclass correlation coefficients and relative 95% confidence intervals and *P*-values for the intraobserver agreement among two observers.

	Operator 1 ($n = 17$)			Operator 2 ($n = 17$)		
Measurement	ICC	95% CI	*P*-value	ICC	95% CI	*P*-value
PSV	0.72	0.63–0.79	<.0001	0.67	0.58–0.75	<.0001
EDV	0.59	0.48–0.68	<.0001	0.60	0.49–0.69	<.0001

Abbreviations: CI, confidence interval; EDV, end-diastolic velocity; ICC, intraclass correlation coefficient; PSV, peak systolic velocity.

TABLE 3 Mean ± (standard deviation) or median (range) of the peak systolic velocity (cm/sec), end-diastolic velocity (cm/s), resistive index, and heart rate (bpm) at the beginning and end of the exam at each time point.

Time point ($n = 34$)	PSV (cm/s)	EDV (cm/s)	RI	HR1 (bpm)	HR2 (bpm)
T1	44.2 ± 11.9^{B}	11.7 ± 5.5	0.73 ± 0.12^{B}	45 ± 8^{B}	43 ± 8^{B}
T2	$63.1\ (23.2–41.5)^{A}$	12.3 ± 5.6	0.82 ± 0.09^{A}	$52\ (43–78)^{A}$	$45\ (39–79)^{A}$
T3	61.3 ± 13.1^{A}	11.5 ± 7.1	0.81 ± 0.11^{A}	43 ± 6^{B}	41 ± 6^{B}

Abbreviations: BPM, beats per minute; EDV, end-diastolic velocity; HR1, heart at the beginning of the exam; HR2, heart at the end of the exam; PSV, peak systolic velocity; RI, resistive index; T1, 2–3 months of gestation; T2, last month of pregnancy; T3, 1 week from delivery.
Statistic: A > B for $P < .01$.

moderate agreement for both PSV and EDV for both operators, with slight differences between the two operators (Table 2). Since the RI is derived from the previously mentioned measurements, no ICC was calculated.

The interobserver ICC was good/moderate; therefore, data from the operator with the highest experience in imaging (D.C.—operator 1) were further analyzed. Neither the left nor the right-side measurements for each variable differed within groups at any time point. The pooled right- and left-side recordings of the PSV and EDV and the calculated RI at each time point are summarized in Table 3. For PSV, a significant effect of time ($P < .0001$) was detected (Figure 3A). Measurements were significantly higher at both T2 and T3 than at T1 ($P < .0001$, both), whereas no difference was detected between T2 and T3 measurements. For EDV, no significant effect of time was detected (Figure 3B). For the RI, a significant effect of time ($P < .0001$) was detected (Figure 3C). Indeed, the T2 and T3 RIs were significantly higher than the T1 RI ($P = .002$ and $P = .001$, respectively), with no differences between the T2 and T3 time points. The HR did not differ between the beginning and the end of the exam at any time point. However, both HR1 and HR2 were statistically higher at T2 than at T1 and T3 ($P = .003$; $P = .0006$; $P = .007$; $P = .002$, respectively), with no differences between T1 and T3 (Table 3).

Age did not correlate to PSV, EDV, and RI at T1 and T3. In particular, at T1, $r_s = -.08$, $P = .65$ for PSV, $r_s = -.18$, $P = .30$ for EDV, and $r_s = -.04$, $P = .80$ for RI; at T3, $r_s = .21$, $P = .23$ for PSV, $r_s = -.03$, $P = .88$ for EDV, and $r_s = .04$, $P = .82$ for RI. At T2, EDV did not correlate with age ($r_s = .30$, $P = .08$), whereas PSV and RI showed a significantly low correlation ($r_s = .36$, $P = .03$ and $r_s = .38$, $P = .03$, respectively). Even if the effect leverage evaluation resulted in non-significant ($P = .13$), two age categories were developed (i.e., ≤8 years and >8 years). No significant differences in PSV could be detected between the two age classes (60.6 ± 18.2 vs. 69.3 (46.6–141.5) cm/s; $P = .21$), and no significant differences in RI could be detected between the two age classes (0.80 ± 0.10 vs. 0.83 ± 0.09; $P = .31$).

4 | DISCUSSION

Findings from our study support the hypothesis that the analyzed vascular ultrasound indices, the PSV and RI of the LPDA, change during different stages of pregnancy. Moreover, a moderate and good intra- and interoperator agreement allowed us to assume that the evaluation of the digital flow by Doppler examination is a repeatable and reliable method.

The Doppler examination of the digital arteries was subjectively deemed easy to perform by both operators and well tolerated by the mares, confirming that equine veterinary practitioners can routinely perform this procedure. The HR evaluation has been used as an index of stress levels since, in healthy animals, an increase in HR not associated with physical activity could be related to an emotional reaction or stress.[25] Thus, the HR stability in the mares during the examination can indicate that the procedure was well tolerated and not stressful. The heart rate was significantly higher at the end of the pregnancy, in agreement with the literature.[7,26] Italian Standardbred horses have a docile nature and quiet temperament, which made it easier to collect the data. Reducing restraint to a minimum was adequate for the mares included in this study. Administration of sedative drugs was avoided because it may produce an increase in digital flow, as reported by Leise et al.[27] Moreover, little data have been reported on the use of sedatives in pregnant mares; therefore, it was preferred not to include the use of any drug in the protocol. Pregnant mares exhibit cardiovascular changes comparable to those in pregnant women and rats. The HR increases, and systolic and diastolic blood pressures decrease in mares during the last trimester of pregnancy to adapt to the growing fetus's

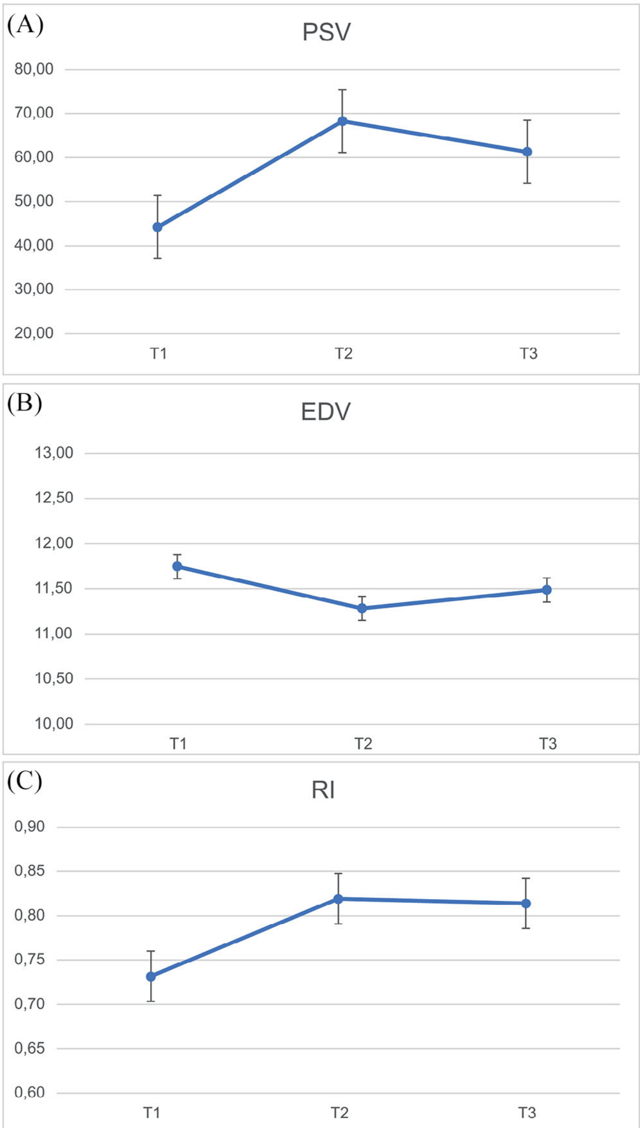

FIGURE 3 Graphic representation of the mean ± standard deviation (between error bars) of the peak systolic velocity (PSV) (A), end-diastolic velocity (EDV) (B), and resistive index (RI) (C) at each time point (T1, T2, T3). The PSV and EDV values on the *y*-axis are expressed as cm/sec.

demands, probably due to an increased vagal tone.[7,26] However, after delivery, the HR returns within the physiological limits, as occurred in the present study.

The Doppler examination allows a quantitative, noninvasive assessment of the blood flow rate and a qualitative evaluation of the vascular tone.[12] Alterations in peripheral flow are associated with several equine pathologies, but scarce data have been reported concerning the physiological, para-physiological, and pathological modifications in the peripheral blood flow due to pregnancy in mares.[12] The placenta is the organ that provides nutritional exchange between the mother and fetus. During gestation, the placenta's vascular network increases to supply the requirements of the growing fetus.[3] Consequently, systemic diseases of the mare, such as metabolic diseases, can compromise the uteroplacental unit, leading to deficits in fetal development and growth. In our study, only uneventful pregnancies were evaluated. At T2, a significantly low correlation between PSV, RI, and mares aged >8 years was observed. Aging exerts a notable impact on the mammalian vascular system, leading to structural and functional changes.[28,29] These alterations encompass increased arterial stiffness, endothelial dysfunction, and modified blood flow regulation.[30] In humans, Plante et al.[28] reported that angiotensin II induces isometric vascular smooth muscle contraction in elderly patients, potentially contributing to heightened vessel rigidity. Such changes may be implicated in the development of conditions like hypertension, coronary artery disease, and heart failure. Notably, age-related arterial wall thickening has also been reported in horses.[31] The observed low correlation between age, PSV, and RI could suggest an age-related decrease in the vessels' adaptive capacity to changes in peripheral circulation, particularly toward the end of pregnancy. Cardiovascular physiological or pathological modification can lead to changes in the equine placenta. In pregnant women, substantial alteration of the cardiovascular system and hydroelectrolytic balance physiologically occurs; the renin–angiotensin system induces homeostasis modifications, with a significant increase in cardiac output and total blood flow, to ensure adequate blood supply to the pregnant uterus.[7,32,33] In horses, from day 210 to the end of normal pregnancy, the total blood flow exponentially increases at the same rate as fetal weight.[34] A decrease in diastolic and systolic pressure and an increase in HR have also been demonstrated in the same period.[7] Similar correlations have been described in sheep, humans, and cattle, where uterine blood flow volume increases by 3, 2.5, and 4.5 times, respectively, during the second half of pregnancy.[35] In horses during the last 3 days before delivery, both systolic and diastolic blood pressure reached minimum values and later increased and remained constant from the first to the third day postpartum.

In this study, the PSV and RI significantly increased in pregnant mares, with higher values at the end of the pregnancy and after delivery than those of the same mares in early pregnancy. This finding may be related to this increase in blood flow volume and the weight gain associated with tumultuous fetal growth in the last 2 months of pregnancy. However, the persistence of high PSV and RI values postpartum suggests that hormone-related mechanisms that occur during pregnancy are also involved. In humans, it has been reported that the PSV and RI of the retrobulbar arteries vary significantly according to the levels of estradiol and testosterone and that parathormone and estrogen affect bone vascularization.[36,37] In cattle, ewes, and sows, uterine perfusion is positively correlated with systemic estradiol concentrations and negatively correlated with systemic progesterone levels.[38] In contrast, in women, a negative correlation has been observed between the uterine blood flow RI and estradiol concentrations during the follicular phase, and a positive correlation between resistance and progesterone levels during the luteal phase has been observed.[38–40] To the authors' knowledge, there are no current studies concerning the effect of physiological hormone levels on circulation parameters in mares. It can be speculated that the increase in estrogen in late pregnancy and during foal heat may be involved in the changes in the PSV and RI observed at

T2 and T3.[41] In our study, the examined vessel, the ultrasound device, the ultrasonographic technique, the weight shift between limbs during examination and the environmental temperature might be considered sources of variability.[13,16,42] To minimize such sources of variability, the same ultrasound device and ultrasound technique were used. During each examination, operators were careful not to compress the LPDA by applying excessive probe pressure, and the silicone standoff pad helped disperse the force over a larger surface area. Despite these precautions, the amount of probe pressure could still contribute to intra- and interoperator variability.

The LPDA is a very superficial vessel, so the operators had to use a Doppler angle correction of 70°. An angle correction greater than 60° can introduce an error of up to 20–30% in calculating PSV and EDV.[43–45] However, in our case, as these were repeated measurements of the same subjects and vessels in a precise location, the same correction angle was maintained between the repeated measurements, reducing this possible source of variability.[43–45] Furthermore, while the angle of insonation influences both PSV and EDV, the RI remains independent of the angle between the ultrasound beam and the insonated vessel, as it is a ratio derived from the aforementioned values. Finally, both operators were right-handed, so the examination of the horses' right LPDA was more difficult for both of them. However, the statistical analysis revealed no difference between measurements of the left and right limbs. A moderate and good intra- and interoperator agreement allows us to assume that the evaluation of the digital flow by Doppler examination has adequate repeatability and reliability. The exam was conducted on weight-bearing horses; Hoffmann et al.[13] highlighted that in a limb that took more weight than the contralateral limb, the velocities in the LPDA decreased. In addition, it has been demonstrated that the increase in pressure on the foot causes an increase in the RI.[14] Although the hour of the day does not significantly affect the PSV, EDV, and RI,[13] a temperature-controlled environment may reduce potential changes in peripheral blood flow. Moreover, the observed values of the PSV, EDV, and RI may depend on several other factors, such as changes in external temperature, weight changes, and feeding. As demonstrated in Murrah Buffalo, an increase in the environmental and body temperature produces a decrease in blood pressure.[46] There are no reported investigations on the influence of temperature on blood flow in mares, but horses might be similarly affected by seasonal changes. The external ambient temperature detected during the experimental sessions was 15 ± 4°C; hence, the examination room has been considered at 17 ± 2°C. Undoubtedly, the lack of precise measurements of the room temperature might be considered a limitation of this study, even if a consistent lack of correlation between wide ambient temperature fluctuations (~ 0–20°C) and PSV, EDV, and RI has been reported.[47] In the present study, body weight was not considered, which might also be considered a limitation of the investigation. Unfortunately, there was no availability of any scale on the farms where the experiment was performed, and indirect methods for body weight evaluation have not been applied since they are considered unreliable in pregnant mares.[48] Another important limitation was the absence of a control group of nonpregnant mares; the study was conducted on Italian Standardbred horse farms, in which most of the animals were pregnant, and there were not enough nonpregnant mares to enroll in the control group. Nonetheless, the placenta reaches full maturity only around day 150 of gestation, and cardiovascular alterations are not described in the first trimester of pregnancy.[49] Consequently, longitudinally following the same group of mares has allowed the identification of significant fluctuations, and T1 values might be considered similar to those of nonpregnant mares.

In human medicine, the detection of cardiovascular changes during normal and complicated pregnancies was found to be an important parameter in the early identification of patients who subsequently developed complications, such as gestational hypertension.[2] The human placenta is hemochorial, that is, the maternal blood comes into direct contact with the fetal chorion, whereas in the horse, the placenta is epitheliochorial, that is, six layers of tissue separate the fetal and maternal blood stores. Therefore, gestational hypertension in these two species may have different consequences on vascular remodeling within the placenta.[2] However, a hypertensive syndrome in horses is often associated with laminitis, which causes the release of proinflammatory factors, including vascular mediators, resulting in an increase in peripheral resistance and lead to abortion, stillbirth, and premature birth.[5] Johnson et al.[6] reported that most owners believe that laminitis is more likely to occur during the third trimester. Further investigations are needed to assess the correlation between pregnancy-related metabolic and endocrine changes and Doppler parameter modification. In conclusion, the technique used was well tolerated by all mares. The evaluation of the digital flow by Doppler examination displayed sufficient repeatability and reliability, with moderate and good intra- and interoperator agreement, respectively. During normal and uneventful pregnancies, changes in the PSV and RI were recorded, and they may be correlated with an increase in fetal weight, metabolic/endocrine changes, and hemodynamic alterations due to pregnancy and placentation. Moreover, considering such fluctuation to be normal, the results of this study might represent a starting point for future investigations concerning modification of the peripheral blood flow in complicated pregnancy.

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Category 1

a. Conception and design: Pasolini, Costanza, Coluccia, Auletta, de Chiara, Greco
b. Acquisition of data: Costanza, Coluccia, Pasolini
c. Analysis and interpretation of data: Montano, de Chiara, Auletta

Category 2

a. Drafting the article: de Chiara, Montano, Costanza, Auletta
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Category 3

a. Final approval of the completed article: de Chiara, Montano, Costanza, Coluccia, Auletta, Greco, Pasolini

Category 4

a. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: de Chiara, Montano, Costanza, Coluccia, Auletta, Greco, Pasolini

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PREVIOUS PRESENTATION OR PUBLICATION DISCLOSURE

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REPORTING CHECKLIST DISCLOSURE

The GRRAS checklist was used by the authors to report studies of reliability and agreement.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

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