Presumed Normal Hemodynamic Values of the Arteries in the Final Third Period of Gestation in Bitches

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ABSTRACT

Background: Doppler ultrasonography enables the investigation of vascular blood flow indexes in gestational assessment, being able to detect vascular resistances that can affect fetal and maternal circulation, such as cases of placental insufficiency, associated with fetal cerebral oxygenation deficit and fetal distress. The study aims to assume hemodynamically normal values in the final third of gestation in bitches, of the umbilical, uteroplacental, middle cerebral and internal carotid arteries, correlating the obtained Doppler velocimetric indexes, for the assessment of the feto-placental circulation, and prediction of fetal viability indexes, fetal centralization and probable date of delivery.

Materials, Methods & Results: Thirty healthy bitches were examined in the final third of gestation (40-60 days). These were evaluated by Doppler ultrasonography at 2 times (T): T1: between 40-50 days; T2: between 51-60 of gestation. At each time point, the peak systolic velocities (PSV) and end-diastolic velocities (EDV) used to obtain the pulsatility (IP) and resistivity (IR) indexes of the umbilical, uteroplacental, middle cerebral and internal carotid arteries of fetuses were evaluated. Also, the systole/diastole (S/D) ratio of these vessels was evaluated. The data obtained were subjected to analysis of variance and Tukey test, using a P value equal to 5%. A significant difference was observed between velocities and Doppler velocimetric indexes between the 2 phases of the gestational final third in all studied vessels. There was an increase in the values of PSV and EDV and a decrease in the indexes, as the probable date of delivery approached. The analysis of the umbilical cord IR showed an increase from P1 to P2 (P < 0.05), while the IP decreased at the same time. For all studied variables there was a statistically significant difference (P < 0.05). In the uteroplacental artery, similarly to the umbilical artery, the PSV and EDV values showed an increase between P1 and P2, while the S/D ratio decreased up to 60 days of gestation (P2). The IR and IP of these vessels decreased during the study interval in pregnant bitches. The studied variables showed a statistically significant difference when analyzed comparatively between P1 and P2 (P < 0.05). As for the middle cerebral artery and internal carotid artery, the PSV and EDV values increased until the end of pregnancy. Likewise, the Doppler velocimetric values and the S/D ratio decreased until the end of pregnancy (P2).

Discussion: The hemodynamic values obtained for the umbilical artery and uterine artery were significantly different between 40 and 50 days of gestation (P1) and 50 and 60 days of gestation (P2), with a decrease in vascular resistance and an increase in velocities. It can be related to a greater association of maternal-fetal blood perfusion, due to the increased demand for the development of the fetus. The Middle Cerebral Artery (MCA) also showed changes between P1 and P2, with an increase in PSV and EDV in the final third of pregnancy, and the S/D ratio was reduced, differing significantly between P1 and P2. The systolic (PSV) and diastolic (EDV) flow velocities of the internal carotid artery increased progressively, while the IR and IP and the S/D ratio decreased, between the evaluated periods (P1 and P2), providing greater flow in canine fetuses, maintaining normal heart rate, indicating positive fetal viability.

Keywords: Doppler, gestational ultrasound, bitches, fetal circulation, umbilical artery, internal carotid, fetal middle cerebral artery.
INTRODUCTION

Two-dimensional Doppler ultrasound in pregnant women is able to aid in the pregnancy diagnosis, evaluating the development of the fetus and maternal-fetal circulation throughout this period [20,21]. Fetal growth depends on vascular development in the maternal and fetal compartments, so that the fetus can obtain an adequate nutritional supply, it is important to assess maternal-fetal vascularization through the study of vessels such as the uteroplacental artery and umbilical cord [42,43].

The study of fetal cerebral vascularization allows the assessment of cerebral blood perfusion, correlating with the development of the nervous system and fetal well-being. The study of the middle cerebral artery and fetal internal carotid artery makes it possible to assess cerebral blood flows, being able to predict cases of cerebral oxygenation insufficiency, and signs of earlier fetal distress [34,37,45].

The objective of this study is to evaluate, during the final third of pregnancy in bitches, by means of Doppler velocimetric ultrasonographic analysis, the blood flow of the umbilical and uteroplacental arteries, in order to characterize the maternal-fetal blood flow. In addition, the objective is to evaluate the flow of the internal carotid and middle cerebral arteries, to determine the presumed normal hemodynamic values, and to correlate them with each other and with fetal heart rate, for indirect prediction of fetal cerebral irrigation, fetal viability and the probable date of delivery.

MATERIALS AND METHODS

Local

The study was developed at the Center for Specialized Veterinary Diagnostic Imaging - NUDIVE/UFPI, at the Federal University of Piauí, Portella, Teresina, PI, Brazil.

Animals

An observational, prospective study was carried out, in which 30 (thirty) healthy bitches, of small to medium-sized breeds, with ages ranging from 1 to 7 years of age and weighing between 5 and 35 kg, were examined. The bitches were submitted to a complete physical examination and laboratory tests, where they were considered without evidence of hematological and biochemical alterations, as well as presenting an absence of concomitant affections (endocrine, infectious or reproductive).

Gestational age was estimated by measuring the biparietal diameter (BPD), which was measured at the largest cross-section from the differentiation of head and body. After obtaining the BPD measurement, the formula DBP X 15 + 20 was used to estimate the gestational age, automatically calculated by the device software (Figure 1 A) [34].

On average, 3 fetuses were examined in each bitch. Fetuses were evaluated in 2 periods (P): (P1): 40-50 and (P2): 51-60 days of gestation, for bitches with normal gestation and without signs of fetal stress. Bitches whose gestation reached 60 days and who showed signs of labor were selected, and were later submitted to elective cesarean surgery, at the option of the tutor. The fetuses were evaluated, one by one, and the fetal heart rate (FHR) was measured 5 times, for at least 3 min, to exclude the possibility of fetal stress [27]. The mean of all heartbeats recorded during the examination of each fetus was defined as the FHR.

The FHR was obtained for each cycle during the automatic acquisition by the software on the device, measuring the distance of the flow spectra in the analyzed vessels. The average of all heartbeats recorded in 3 consecutive cycles during the examination of each fetus defined the FHR. 2D sector size has been minimized to improve image quality. The highest pulse repetition frequency (PRF) was used to avoid aliasing signals in normal flows.

Exclusion criteria

Exclusion criteria were applied to bitches and fetuses. The bitches were submitted to a general clinical examination to assess their health status. Dogs that showed signs of systemic changes resulting from parasitic, infectious diseases (fever, vomiting, diarrhea, inappetence), with serum changes observed in the blood count and biochemical tests, acquired or congenital cardiovascular diseases, with the potential to influence fetal development, as well as changes in maternal physiological hemodynamic values (valvular insufficiency and disturbances in cardiac rhythm or maternal cardiac conduction) identified in auscultation and electrocardiographic examination, respectively, were excluded from the work.

For fetuses, only those that allowed the assessment of all parameters listed for the study were included in the experiment, where data acquisition could be assumed to allow reliable clinical measurements. Fetuses that presented some type of structural (cardiac or non-cardiac) or electrical abnormality, congenital malformations or exhibiting signs of fetal distress identified on ultrasound examination were excluded.
Ultrasound exam

All females underwent abdominal ultrasound examination during the last third of pregnancy to analyze their Doppler velocimetric indices. The bitches were placed in dorsal decubitus, on an adapted sponge gutter, with the abdominal surface shaved for scanning after application of conductive gel for ultrasonography. Ultrasonography equipment (M6® - Mindray) was used, coupled to a 12 MHz multifrequency linear transducer. The acquisition of images and videos of the exams were performed by the same experienced ultrasonographer throughout the study. Image quality was maximized by adjusting gain, focus, and depth for each fetus during the exam.

At least 3 fetuses were evaluated in each bitch and each measurement was performed in triplicate, throughout the cardiac cycle, configuring a hemodynamic mean for each value evaluated. The flow pattern of the umbilical artery (UA), uteroplacental artery (UPA), middle cerebral artery (MCA) [Figure 1 D] and internal carotid artery (ICA) [Figure 1 C] were evaluated.

The analyzed vessel was initially visualized in B mode, followed by evaluation by color and spectral Doppler. For spectral exams, the configuration of velocity ranges and the size of the spectral window (gate) were adjusted during each exam, to obtain a sequence of spectral Doppler graphics with symmetric and distinct systolic and diastolic cardiac cycles, without aliasing. Gain, filters, and pulse repetition frequency (PRF) settings were uniform across all exams.

The Doppler spectrum obtained was later analyzed, using 3 pulses of the cardiac cycle, the central one being chosen as the real representative of the blood flow velocity [46]. The variables observed were: peak systolic velocity (PSV), end diastolic velocity (EDV) and resistivity (IR) and pulsatility (IP) indexes, as well as...
the systole/diastole (S/D) ratio, automatically calculated by means of the following formulas: IR = (PSV - EDV) / PSV and IP = (PSV - EDV) / M, where M represents the mean between PSV and EDV (Figure 1) [26].

Statistical analysis

After performing the exams, the data were tabulated and then analyzed by ANOVA and by the R Software² (Version R i3863.5.3), being submitted by the generalized linear model. Data were also analyzed by a test of means (Tukey test) to assess the presence of significant difference between the 2 evaluation times, along the final third of pregnancy, in velocity values (PSV and EDV) and indexes (IR and IP) of each analyzed structure, using a P value equal to 5%.

RESULTS

Tables 1, 2 and 3 evidence the measured Doppler velocimetric values for the following maternal-fetal structures: umbilical cord (UC), middle cerebral artery (MCA) and internal carotid artery (ICA), respectively, in the 2 periods studied: P1 (40-50 days) and P2 (51-60 days), with all bitches able to continue until 60 days of gestation, monitored by heart rate (HR) variability, always above 180 bpm.

PSV and EDV showed a progressive increase over the study period. Consequently, the S/D ratio decreased between the periods studied. The analysis of IR and IP of the umbilical cord showed an increase in IR from P1 to P2 (P < 0.05), while there was a decrease in IP between P1 and P2. For all variables studied, there was a statistically significant difference (P < 0.05), as shown in Table 1.

In the uteroplacental artery, similarly to the umbilical artery, the PSV and EDV values showed an increase between P1 and P2, while the S/D ratio decreased up to 60 days of gestation (P2). IR and IP decreased during the study interval. The studied variables showed a statistically significant difference when analyzed comparatively between P1 and P2 (P < 0.05) [Table 2].

For the middle cerebral artery and internal carotid artery, the PSV and EDV values increased until the end of pregnancy. Similarly, to the previously described Doppler velocimetric values, the S/D ratio decreased until the end of pregnancy (P2). The comparative analysis between periods P1 and P2 showed a statistically significant difference for all measured variables (P < 0.05).

The analysis of the cerebroplacental relationship (MCA/UA) showed decreasing values between periods P1 (0.92 ± 0.03) and P2 (0.87 ± 0.06), this difference being statistically significant (P < 0.05). Another index proposed in this study, the relationship between the internal carotid artery and the umbilical artery (IntCarArt/UA) presented values close to those verified for the MCA/UA ratio, reducing from P1 (0.91 ± 0.05) to P2 (0.85 ± 0.04), likewise presenting a statistically significant difference (P < 0.05). Heart rates between periods P1 (250.09 ± 12.14) and P2 (228.11 ± 18.20) showed normal values, with no events associated with fetal stress.

<table>
<thead>
<tr>
<th>Table 1. Umbilical artery Doppler velocimetric values at 40-50 and 51-60 days of gestational age.</th>
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<td>Parameter</td>
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<td>PSV (cm/s)</td>
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<td>IP</td>
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<td>IR</td>
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<td>S/D</td>
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Different letters indicate statistically significant difference between times (P < 0.05). PSV: Peak Systolic Velocity; EDV: End Diastolic Velocity; IP: Pulsatility Index; IR: Resistivity Index.

<table>
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<th>Table 2. Doppler velocimetric values of the uteroplacental artery at 40-50 and 51-60 days of gestational age.</th>
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<td>Parameter</td>
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<td>PSV (cm/s)</td>
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<td>IP</td>
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<td>IR</td>
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Different letters indicate statistically significant difference between times (P < 0.05). PSV: Peak Systolic Velocity; EDV: End Diastolic Velocity; IP: Pulsatility Index; IR: Resistivity Index.
Table 3. Middle cerebral artery Doppler velocimetric values at 40-50 and 51-60 days of gestational age.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>40-50 days</th>
<th>51-60 days</th>
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<tbody>
<tr>
<td>PSV (cm/s)</td>
<td>15.51 ± 1.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.77 ± 1.37&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>EDV (cm/s)</td>
<td>4.95 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.44 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IP</td>
<td>1.28 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IR</td>
<td>0.68 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.63 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>S/D</td>
<td>3.13 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.75 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Different letters indicate statistically significant difference between times (<i>P</i> &lt; 0.05). PSV- Peak Systolic Velocity; EDV- End Diastolic Velocity; IP- Pulsatility Index; IR- Resistivity Index.

Table 4. Doppler velocimetric values of the common carotid artery at 40-50 and 51-60 days of gestational age.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>40-50 days</th>
<th>51-60 days</th>
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<tr>
<td>PSV (cm/s)</td>
<td>16.42 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.21 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>EDV (cm/s)</td>
<td>3.31 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.15 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IP</td>
<td>1.27 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>IR</td>
<td>0.80 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S/D</td>
<td>4.96 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.31 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
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Different letters indicate statistically significant difference between times (<i>P</i> &lt; 0.05). PSV- Peak Systolic Velocity; EDV- End Diastolic Velocity; IP- Pulsatility Index; IR- Resistivity Index.

DISCUSSION

The identification of problems related to the course of pregnancy in bitches remains challenging in Veterinary Medicine. Countless researchers have been dedicated to the acquisition of data that can guide the prediction of childbirth, or even the risks regarding the decision of vaginal or cesarean delivery [6,25,27,28,44]. Human medicine has long observed that newborns born between 39 and 40 weeks of gestation have better results, given the fact that periods shorter than these make the fetus more prone to complications resulting from immaturity and, culminating in an increase in neonatal or postpartum mortality [38]. Therefore, our studies were carried out from bitches in the final third of gestation between 40 and 60 days (P1 = 40-50 days; P2 = 51-60 days).

Faced with a human fetal medicine with a greater repertoire of information, perhaps our greatest challenge is to find an “intersection” between the stages of pregnancy that are so different between humans and small animals, so that we can better implement our obstetric behaviors, reducing the risks for the fetus at birth. All bitches were evaluated and considered able to follow up to 60 days of gestation, especially monitored by HR variability. In this study, HR was always above 180 bpm, especially between 12 and 72 h before delivery, as previously described for bitches [3,25,26].

Not infrequently, pregnancies that develop “apparently normal” have an unexpected outcome in pregnant bitches, evolving with stillborn fetuses or that die postpartum, a situation often observed in human pregnancies [18]. There is still a need to understand the magnitude of these problems, observing key factors, such as gestational age, for which existing information in Veterinary Medicine, compared to human obstetrics, is still imprecise [24,29]. In this context, it is important to emphasize that, for this study, bitches that went into labor at 60 days of gestation were selected (the borderline date for the time of gestation in bitches), and were subsequently submitted to cesarean surgery, all with viable fetuses. In human obstetrics the increased risk of stillbirths resulting from prolonged pregnancies beyond 41 weeks is known [5].

Fetal hemodynamic indexes are valuable tools in defining the fine line between presumably normal flow values and those that may denote abnor-
malities during pregnancy. Umbilical artery, middle cerebral artery and uteroplacental artery were chosen to be analyzed in this clinical trial because they are the most important vessels in the assessment of fetal well-being and in the non-invasive monitoring of the “fetoplacental unit” in humans [31] and animals [2,27,39]. Auxiliary evaluation was also performed through the fetal carotid arteries. The hemodynamic values obtained for the umbilical artery were significantly different between 40 and 50 days (P1), when compared to the rest of the gestation [51 to 60 days] (P2), which was also documented by other authors for this vessel [7-9]. The umbilical artery of the fetuses studied was characterized by its low-resistance vascular bed, with continuous forward flow throughout the cardiac cycle and diastolic phase increasing between the 2 periods evaluated (P1 = 6.60 ± 1.51; P2 = 14.13 ± 1.20), so that the S/D ratio decreases with advancing pregnancy (P1 = 4.79 ± 0.25; P2 = 3.20 ± 0.11), as described in the canine specie [2,9,17,39]. We adopted the vascular interrogation of the umbilical artery in its floating portion, as recommended by the 2013 guideline of the International Society of Ultrasonography in Obstetrics and Gynecology, to perform obstetric Doppler ultrasonography [10], as there is a significant difference between the flow values measured in the cord insertion portion in the fetus, its middle and distal portion [17,23,39]. Similar to previous studies [31,33], in our assessments, the IR and IP of the umbilical artery varied during pregnancy. Recently, the doppler flow analysis of the umbilical cord of bitches showed that the IR of the umbilical artery decreased during the periods of 12-6 and 6-1 h before delivery, and that the lowest values were less than or equal to 0.7 in bitches with normal delivery, when compared to bitches that developed fetal stress, where the IR was shown to be increased, especially 6-1 h before the cesarean [25]. The umbilical artery IP of the fetuses studied showed a dignified decrease between times P1 and P2 in this study. Corroborating our results, other researchers described that IP increases consistently from the 5th to 6th week of gestation (35 to 42 days) decreasing from then until birth [39,40]. However, there are reports that this decrease can also occur more clearly between the 6th and 7th weeks of gestation (42 and 49 days) [17]. These last values are more similar to our data, possibly due to a small variation in the point of the vascular interrogation between authors. The increase in IP, as well as the absence of end-diastolic flow or reverse diastolic flow in umbilical arteries have been used for the detection and surveillance of high morbidity and mortality subgroups in fetuses with growth disorders [12].

Uterine artery flow characteristics (IR, IP, parabolic profile, monophasic, low resistance, no notches) were also similarly described in dogs [17,39]. The diastolic flow increased significantly in late pregnancy (P1 = 17.96 ± 3.51; P2 = 24.06 ± 4.51), with well-defined systolic and diastolic phases [8,25]. Such correlations are important to identify abnormalities during pregnancy. In non-pregnant women, the wave profile of the uterine artery is of high resistance, with low diastolic flow and the presence of an “early notch” (nouching), which disappears at the end of pregnancy. In humans, the persistence of this characteristic is associated with adverse outcomes such as: fetal growth restriction, pre-eclampsia, increased risk of preterm birth [22,32].

During pregnancy, the small uteroplacental vessels are usually sensitive to color Doppler examination [39,40]. Although in the final third of pregnancy the uterine artery can be identified close to the zonal placenta, in this study, for the most part, they were seen between two gestational sacs [15]. IR, IP and S/D ratio show progressive reductions in the final third of pregnancy. It is believed that this reduction is related to placental development and histological alterations of the spiral arteries, which are invaded by trophoblastic cells that consume their musculoskeletal coverage, making these arteries larger and establishing low-resistance connections [1,41]. This remodeling is essential for successful placentation, changing the vascular bed of the uterine artery to a continuous low-resistance diastolic flow.

The middle cerebral artery (MCA) had its vascular flows widely discussed during pregnancy in humans [4,11,13,30]. The MCA of the studied fetuses showed an increase in PSV and EDV in the final third of pregnancy, and the S/D ratio was reduced, differing significantly between P1 and P2. Only 1 study [21] provides information on the MCA flow values in Veterinary Medicine, and the results are similar to those found for the fetuses in this research. Literature is still scarce regarding the study

of the hemodynamic profile of MCA throughout pregnancy in female dogs. Both the IR and the IP decreased substantially in the 2 studied moments. In human fetuses, the analysis of MCA flows has been performed in at least 2 situations: study of fetal anemia and for the calculation of the MCA/UA ratio [31]. For the fetuses evaluated, the MCA flows were of high resistance, anterograde and with a continuous diastolic phase that increases until the end of pregnancy [16] and in pregnancies with a normal course in humans [19]. The MCA/UA ratio, known as the cerebroplacental ratio (CPR) is obtained from the simple ratio between the IP of the middle cerebral artery and that of the umbilical artery. In fact, alterations in CPR in human medicine have been shown to be a predictor of pregnancy complications, characterizing redistribution of fetal circulation as a result of the compensatory adaptation of nutrients and oxygen, resulting from placental insufficiency [14]. This study demonstrated for the first time the behavior of CPR in canine fetuses, proposing presumably normal values of this index for the species.

The PSV and the EDV of the internal carotid artery progressively increased, while the IR, IP and the S/D ratio decreased, between P1 and P2. The first author to describe the hemodynamics of the common carotid artery and its behavior during pregnancy, contributing new information on cerebral flow parameters in canine fetuses was in 1998 [39,40]. This author evidenced the almost constant behavior of this index throughout pregnancy and, unlike our findings, they observed an increase in IP at the end of pregnancy in bitches. For him, the diastolic flow was often undetectable in many fetuses between 6 and 7 weeks of gestation, unlike our findings, where the diastolic phase was well evidenced. We believe that this difference was mainly due to the technological improvement of the ultrasound equipment currently used, the type of filter used to analyze the diastolic flow signal, or even as a result associated with the Doppler insonation angle. In addition, our analyzes were performed on the internal carotid artery, a branch of the common carotid artery most closely related to cerebral circulation.

Doppler ultrasound of cerebral vessels has been used in human obstetrics to analyze perinatal hypoxia and acidosis [35,36]. In this context, the relationship between the IP of the internal carotid artery and the umbilical artery (IntCarArt/UA) analyzed in this study showed similar values (P1 = 0.91 ± 0.05; P2 = 0.85 ± 0.04) to those obtained for the relationship between the IP of the MCA/UA in the final third of pregnancy and with a significant difference between P1 and P2. It is noteworthy that the internal carotid artery is a vessel whose Doppler interrogation is less difficult depending on the operator performing the evaluation. In humans, a decrease in the IntCarArt/UA ratio has been reported throughout pregnancy in normally developing fetuses [46]. These authors reported that in fetuses with growth restriction, high values of the pulsatility index in the umbilical artery occur associated with reduced values of the pulsatility index in the internal carotid artery, suggesting the presence of a brain-sparing effect. As we reported for the MCA/UA ratio, to our knowledge, this is the first time that the fetal viability assessment index using the IntCarArt/UA ratio is reported in Veterinary Medicine.

CONCLUSION

The vascular flow velocities of the uteroplacental artery, umbilical artery, middle cerebral artery and internal carotid artery showed values similar to those previously described in the literature for bitches in the normal course of pregnancy, supporting the other results obtained in this research. The cerebroplacental ratio (CPR) and the IntCarArt/UA ratio were proposed in this study for the first time in Veterinary Obstetrics and showed results similar to those found in the human literature. We believe that these data can be useful in identifying fetal centralization, as it occurs in human obstetrics, contributing to the characterization of fetal viability, providing valuable data for a more effective scheduling of cesarean delivery, when necessary, increasing the survival of newborns.

MANUFACTURERS
1Mindray do Brasil. São Paulo, SP, Brazil.
2R Software (Version R i386.3.5.3). São Luís, MA, Brazil.

Ethical approval. The protocols used in this work were approved by the Animal Experimentation Ethics Committee - CEEA/UFPI, under No. 657/2021.

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


