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Fetal Distress during the Last Third of Pregnancy in Bitches - Evaluation by Doppler Ultrasound Imaging

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ABSTRACT

***Background*:** Diagnostic ultrasound has revolutionized obstetric practice. Doppler techniques provided *in vivo* studies of the fetus-placental and uterus-placental circulations, in an increasingly precise manner. The assessment of fetal well-being is essential in obstetric practice, however, in veterinary medicine, there is a deficit of ultrasound parameters related to fetal distress. The main of this research was to determine the hemodynamic characteristics and behavior, through Doppler ultrasonography, of maternal-fetal vessels during the final third of gestation in bitches with fetuses under stress, as well as correlating them with each other and with fetal distress.

***Materials, Methods & Results*:** An observational, prospective study was carried out, in which 30 healthy bitches were examined, during the last third of pregnancy, with fetuses under fetal distress. Fetuses were evaluated in 2 stages: (1st): 50-54 days and (2nd): 55-60 days of gestation. All fetuses were calmly assessed, one by one, and fetal heart rate (FHR) was measured 5 times, for at least 3 min, to identify and confirm fetal distress. At least 3 fetuses were evaluated in each bitch and each measurement was performed in triplicate, setting a hemodynamic mean for each value evaluated. The flow pattern in the middle cerebral artery (MCA), internal carotid artery (ICA) and umbilical artery (UA) was evaluated. The analyzed vessel was initially visualized in B mode, followed by evaluation by color and spectral Doppler. The variables observed were: peak systolic velocity (PSV), end diastolic velocity (EDV) and the resistivity (RI) and pulsatility (PI) index, as well as the systole/diastole ratio (S/D). The analysis of RI and PI of the umbilical artery showed a statistically significant difference between the mean values found for the times studied, increasing from time 1 to time 2. For middle cerebral and internal carotid arteries, PI, RI and the relationship systole/diastole (S/D), showed a statistically significant difference between the mean values found for the times studied, decreasing from time 1 to time 2.

***Discussion*:** Fetal heart rate (HR) findings are considered normal above 200 beats per minute (bpm), only if preceded by acceleration and deceleration. This fact confirms the presence of fetal stress, by this parameter, for the fetuses in the present study. Corroborating the picture of fetal distress, another study concluded that HR tends to increase up to 20 days before delivery, a fact that did not occur in the study in question, where animals in the same gestational period were evaluated. Recent research has concluded that an elevated umbilical artery PI at 28 weeks of gestation, in the absence of fetal growth restriction or prematurity, is associated with some adverse cognitive findings in 12-year-olds children. In the present study, a progressive increase in this index was observed throughout the final third of pregnancy. Although the pups were not monitored after birth, this data serves as a warning for veterinarians, and can be useful in the assessment and diagnosis of possible postnatal nervous alterations. Like what was found in the present study, a study reported that from the 4th week to birth, the PSV of the umbilical artery (UA) increased almost linearly, with high significance. For the middle cerebral arteries, the PI showed a statistically significant difference between the mean values found for the times studied, however, decreasing throughout the final third of pregnancy.

Keywords: Imaging diagnosis, obstetrics, reproduction, clinical medicine.

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INTRODUCTION

Diagnostic ultrasound has revolutionized obstetric practice by providing access to information that facilitates clinical performance, quickly and safely [1]. The improvement of equipment and the introduction of Doppler, provided *in vivo* studies of fetal-placental circulations, allowing the assessment of the degree of resistance to blood flow and its relationship with the general health status of the concept [7]. In addition, it made it possible to identify parameters of great diagnostic value, such as the direction and velocity of blood flow in the heart, fetal vessels and placenta [3].

The assessment of fetal well-being is fundamental in obstetric practice, where conditions that characterize fetal distress are frequently observed in the clinical routine. However, for a long time, fetal viability has been evaluated only through the level of fetal movement, as well as the number of heartbeats [11].

In human fetuses, with the evolution of normal pregnancy, there is a decrease in resistance to blood flow in the umbilical circulation and a progressive increase in the end-diastolic component due to the proliferation of the tertiary villous system, thus expanding the areas of exchange in the villi [5,22]. However, in Veterinary Medicine, there is a deficit of ultrasound parameters capable of identifying with greater precision, abnormalities that determine fetal distress.

The objective of this work is to determine the hemodynamic characteristics and behavior, by means of Doppler ultrasonography, of maternal-fetal vessels during the final third of pregnancy in bitches with stressed fetuses, as well as to correlate them with each other and with fetal distress.

MATERIALS AND METHODS

Animals

An observational, prospective study was carried out, in which 30 (thirty) healthy bitches of small to medium breeds (Shihtizu, Poodle, Yorkshire Terrier, Pinscher, French Bulldog and mixed breed) were examined during the last third of pregnancy (40 to 60 days) with fetuses under fetal distress.

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 1A) [14].

On average, 3 fetuses were examined in each pregnant bitch. Fetuses were evaluated in 2 stages: (1st): 50-54 days and (2nd): 55-60 days of gestation, all under fetal distress. All fetuses were calmly assessed, one by one, and fetal heart rate (HR) was measured 5 times, for at least 3 min, to identify and confirm fetal distress [11]. The average of all heartbeats recorded during the examination of each fetus was defined as the HR.

The HR was obtained for each cycle during the automatic acquisition by the software on the machine, measuring the distance of the flow spectra in the analyzed vessels. The mean of all heartbeats recorded during the examination of each fetus was defined as the HR. 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 underwent a general clinical examination to assess their health status. Those who showed signs of systemic changes resulting from parasitic, infectious diseases (fever, vomiting, diarrhea, inappetence), as well as acquired cardiovascular diseases, with the potential to influence fetal development, and change maternal physiological hemodynamic values, such as insufficiencies, were excluded from the work (valves and rhythm disturbances identified on auscultation).

For fetuses, only those that allowed a complete and correct evaluation were included in the experiment, so that all parameters were reliably obtained, due to the position, size quantity, fetuses that presented some type of structural abnormality (cardiac or non-cardiac), electrical, such as arrhythmias or hemodynamics, seen in different ultrasound modalities, were excluded.

Ultrasonographic exam

All animals underwent an abdominal ultrasound examination during the last third of pregnancy to perform the Doppler velocimetric analyses. The bitches were placed in dorsal decubitus, on an adapted sponge bed, with the abdominal surface shaved for scanning after application of conductive gel for ultrasonography. Ultrasonography equipment (M6®, Mindray)[1] was used, coupled to a 12 MHz multifrequency linear transducer. A single, experienced sonographer was

responsible for image acquisition throughout the study. Image quality was maximized by adjusting gain, focus, and depth for each fetus during the exam. 2D sector size has been minimized to improve image quality.

These were submitted to ultrasound examination, where at least 3 fetuses were evaluated in each bitch and each measurement was performed in triplicate, configuring a hemodynamic mean for each value evaluated. The flow pattern in the middle cerebral artery (MCA) [Figure 1D], internal carotid artery (ICA) and umbilical artery (UA) [Figure 1C] was 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 [23]. The variables observed were: peak systolic velocity (PSV), end diastolic velocity (EDV) and resistivity (RI) and pulsatility (PI) index, as well as the systole/diastole (S/D) ratio automatically calculated by means of the following formulas: RI (PSV - EDV) / PSV and PI = (PSV - EDV) / M, where M represents the mean between PSV and EDV (Figure 1) [25].

Statistical analysis

After performing the gestational ultrasound exams, the data were tabulated and then analyzed by ANOVA and by the R Software (R version i3863.5.3)[2] and submitted by the generalized linear model. Data were also analyzed by means of a test of means (Tukey's 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 (RI and PI), of each structure analyzed, using a *P* value equal to 5%.

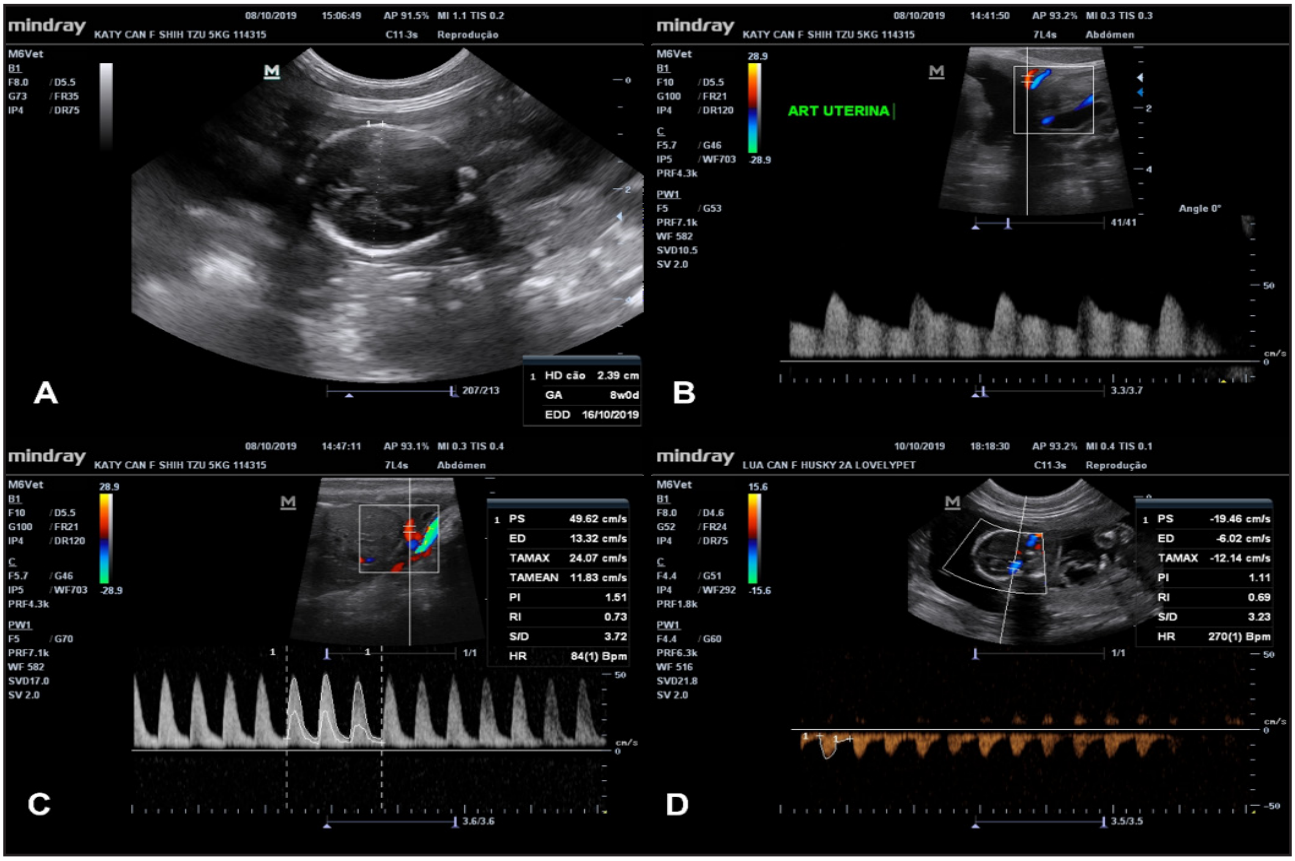

Figure 1. Ultrasound images obtained from canine maternal-fetal structures during the final third of gestation, under fetal distress. A- Measurement of the largest cross-section between the two parietal bones of the skull, from the differentiation of the head and body of the canine fetus, to obtain the Biparietal Diameter (BPD) and estimate the gestational age through the formula: BPD x 15 + 20, obtained automatically by the device software. B- Pulsed wave Doppler for evaluating the flow pattern in the uterine artery. C- Pulsed wave Doppler for evaluating the flow pattern in the umbilical artery and obtaining peak systolic and end-diastolic velocities to calculate resistivity and pulsatility indices. D- Pulsed wave Doppler for evaluating the flow pattern in the middle cerebral artery and obtaining the peak systolic and end-diastolic velocities for calculating the resistivity and pulsatility indices.

RESULTS

Tables 1, 2 and 3 show the measured Doppler velocimetric values for the following maternal-fetal structures: umbilical artery (UA), middle cerebral artery (MCA) and internal carotid artery (ICA), respectively, in the 2 times studied: time 1 (50- 54 days) and time 2 (55-60 days) in animals under fetal distress.

The analysis of the resistivity index (RI) and pulsatility index (PI) from the umbilical artery showed a statistically significant difference between the mean values found for the times studied, increasing from time 1 to time 2 ($P < 0.05$) [Table 1].

For the middle cerebral artery and internal carotid artery (ICA), the PI, RI and the systole/diastole (S/D) ratio showed a statistically significant difference between the mean values found for the times studied, however, decreasing from time 1 to time 2 ($P < 0.05$) [Tables 2 & 3].

Tables 4 and 5 show the mean values obtained for the relationship between PI of CMA and ICA with the PI of UA, respectively, and fetal heart rate (HR), in the 2 periods studied. Both ratios (IPCMA / IPUA and IPICA/ IPUA), as well as HR, showed a significant reduction from time 1 to time 2 ($P < 0.05$).

Figures 2 and 3 show the difference in the mean values obtained for the studied variables, as a function of time, along the final third of pregnancy (40th to 60th day).

In Figure 2, the IP from the UA, in both periods, was greater than 1, with an increase over time. The PI from MCA, in both periods, was less than 1, decreasing until the end of pregnancy. The MCA / UA ratio, in the 2 periods studied, also presented mean values lower than 1, decreasing over the period studied. In Figure 3, the PI from the ICA, as well as the PI from the CMA, in the 2 periods studied, was less than 1, with a progressive decrease over time. The ICA / UA ratio also decreased until the end of pregnancy, with values less than 1.

Table 1. Mean and standard deviations of measured Doppler velocimetric values for the umbilical artery, during times 1 and 2 of canine fetuses.

Umbilical Artery		
Parameters	Gestational time*	
	Time 1 (40-50 days)	Time 2 (51-60 days)
PSV (cm/s)	47.67 ± 1.23[a]	55.16 ± 1.14[b]
EDV (cm/s)	6.71 ± 0.7[a]	6.11 ± 0.8[b]
PI	1.50 ± 0.3[a]	1.60 ± 0.24[b]
RI	0.86 ± 0.04[a]	0.88 ± 0.03[b]

*Thirty healthy bitches. Different letters indicate statistically significant difference between times ($P < 0.05$). PSV: peak systolic velocity; EDV: end diastolic velocity; PI: pulsatility index; RI: resistivity index. *Thirty healthy bitches.

Table 2. Mean and standard deviation of measured Doppler velocimetric values for Middle Cerebral Artery during times 1 and 2 of canine fetuses.

Middle Cerebral Artery		
Parameters	Gestational time*	
	Time 1 (40-50 days)	Time 2 (51-60 days)
PSV (cm/s)	12.00 ±0.56[a]	13.41 ± 1.37[b]
EDV (cm/s)	4.27 ± 0.36[a]	6.09 ± 0.15[b]
PI	0.95 ± 0.2[a]	0.75 ± 0.10[b]
RI	0.64 ± 0.03[a]	0.54 ± 0.02[b]
S/D	2.81 ± 0.12[a]	2.20 ± 0.09[b]

*Thirty healthy bitches. Different letters indicate statistically significant difference between times ($P < 0.05$). PSV: peak systolic velocity; EDV: end diastolic velocity; PI: pulsatility index; RI: resistivity index.

Table 3. Mean and standard deviation of Doppler velocimetric values measured for the Internal Carotid Artery during times 1 and 2 of canine fetuses.

Internal Carotid Artery		
Parameters	Gestational time*	
	Tempo 1 (40-50 days)	Tempo 2 (51-60 days)
PSV (cm/s)	15.35 ± 0.18[a]	19.13 ± 0.26[b]
EDV (cm/s)	5.32 ± 0.04[a]	8.21 ± 0.02[b]
PI	0.97 ± 0.31[a]	0.80 ± 0.08[b]
RI	0.65 ± 0.01[a]	0.57 ± 0.03[b]
S/D	2.88 ± 0.15[a]	2.33 ± 0.14[b]

*Thirty healthy bitches. Different letters indicate statistically significant difference between times ($P < 0.05$). PSV: peak systolic velocity; EDV: end diastolic velocity; PI: pulsatility index; RI: resistivity index.

Table 4. Mean and standard deviation values of the ratio between the middle cerebral artery pulsatility index and the Umbilical Artery Pulsatility Index and fetal heart rate, during times 1 and 2 of canine fetuses from 30 healthy bitches.

	Ratio IP MCA / PI UA	
	Time 1 (40-50 days)	Time 2 (51-60 days)
	0.63 ± 0.05[a]	0.47 ± 0.04[b]
Fetal Heart Rate (FHR)	200.03 ± 8.01[a]	165.11 ± 13.10[b]

Different letters indicate statistically significant difference between times ($P < 0.05$). FHR: fetal heart rate; PI MCA: middle cerebral artery pulsatility index; PI UA: umbilical artery pulsatility index.

Table 5. Mean and standard deviation values of the ratio between the Internal Carotid Artery Pulsatility Index and the Umbilical Artery Pulsatility Index and the heart rate, during times 1 and 2 of canine fetuses from 30 healthy bitches.

	Ratio IP ICA / IP UA	
	Time 1 (40-50 days)	Time 2 (51-60 days)
	0.64 ± 0.07[a]	0.50 ± 0.05[b]
Fetal Heart Rate (FHR)	200.03 ± 8.01[a]	165.11 ± 13.10[b]

Different letters indicate statistically significant difference between times ($P < 0.05$). FHR: fetal heart rate; PI ICC: internal carotid artery pulsatility index; IP UA: umbilical artery pulsatility index.

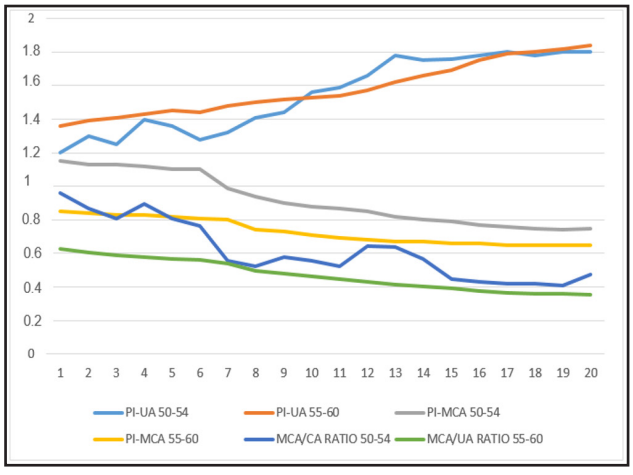

Figure 2. Comparative values of hemodynamic indices of canine fetuses under fetal stress: Middle Cerebral Artery / Umbilical Artery Ratio.

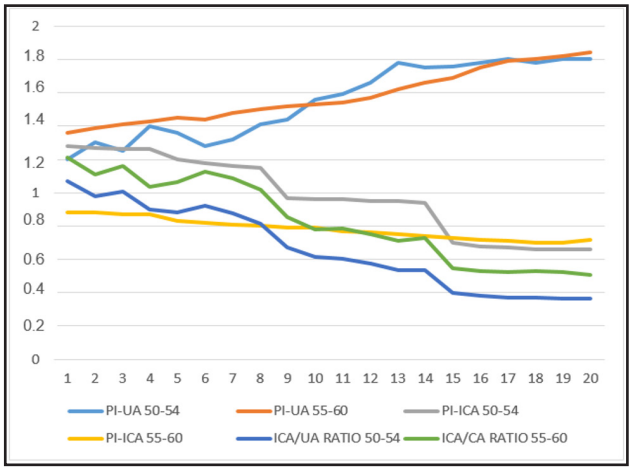

Figure 3. Comparative values of hemodynamic indices of canine fetuses under fetal stress: Common Carotid Artery / Umbilical Artery Ratio.

DISCUSSION

The fetal heart rate (HR) values obtained for the animals in the present study, at times 1 and 2 (200.03 ± 8.01 and 165.11 ± 13.10, respectively), were lower than the normal values found for puppies (up to 220 bpm) [25] and for canine fetuses (220 - 240 bpm), which means, less than 2 to 3 times the maternal heart rate [20]. In dogs it was considered normal, HR above 200 bpm only if preceded by acceleration and deceleration [11]. This fact confirms the presence of fetal distress, by this parameter, for the fetuses in the present study, as this variation was also not observed, where the frequency remained constant throughout the exams, given a detailed, prolonged, and triplicate evaluation of each fetus.

Corroborating the fetal distress picture, other works concluded that the HR tends to increase up to 20 days before birth, a fact that did not occur in the study in question, where animals were evaluated in this mentioned gestational period [24]. In addition, currently, to consider fetal distress, HR must remain below 200 bpm for more than 3 min, which occurred in the study animals, since in the pre-partum phase, intermittent uterine contractions on the fetus generate a temporary reduction of HR significantly but returning to normal or even increasing 1 to 2 min later, under normal conditions [9].

In human obstetric evaluation, the measurement of the umbilical artery Pulsatility index (PI) serves as a marker for fetal well-being in the uterus by assessing the impedance within the fetal-placental circulation and is an indirect measure of the resistance to flow within of the placental vasculature [17,25]. Recent research has concluded that an elevated umbilical artery PI at 28 weeks' gestation, in the absence of fetal growth restriction or prematurity, is associated with some adverse cognitive findings in 12-year-olds children. A possible explanation for this phenomenon is an element of placental insufficiency in the presence of a properly grown fetus, which affects the development of the fetal hippocampus [17]. In the present study, a progressive increase in this index was observed throughout the final third of pregnancy. Although the pups were not monitored after birth, this data serves as a warning for veterinarians, and can be useful in the assessment and diagnosis of possible postnatal nervous alterations.

In healthy dogs, the significant decrease in the umbilical artery PI index during the first third, and the smallest decrease during the remainder of pregnancy, is like the development of the blood flow pattern in human pregnancy [18,22]. During this period, the need to intensify placental circulation leads to an increase in PSV and EDV until the end of pregnancy [12,16]. However, in suffering fetuses, an opposite response was observed, with a significant reduction in EDV, resulting in a progressive increase in the PI until the end of pregnancy, configuring an altered vascular resistance in these vessels, due to fetal stress.

Throughout the evaluations, the umbilical vessels were already visible in B-mode, with the exact position being determined by color Doppler [18], who used two-dimensional ultrasonography in combination with spectral pulsed wave Doppler for to study maternal circulation and the development of normal fetal vascularization in Beagles dogs. Like what was found in the present study, this same author reported that from the 4th week to birth, the PSV of the UA increased almost linearly, with high significance. However, unlike what was found, where the EDV significantly decreased throughout the final third of pregnancy, this index increased linearly from the 6th week to birth in this work with Beagles dogs. This caused the pulsatility and resistivity indices to also increase, differing from what was reported by the author, who showed that they only increased until the 6th week, after which they progressively decreased.

Some researchers recommend monitoring the RI of the umbilical artery, in the pre-partum phases, from 72 h after pre-partum. If this is below 0.71, the pregnant woman is probably 12-6-0 h for delivery, however, if you accompany the female in these last h (24-12-6h) and the IR value reduces and after rising again, it is probably due to fetal distress and the female may be in dystocia [10]. In the present study, only a progressive increase in RI was reported, which characterizes fetal distress in the studied animals. Although the monitoring of the delivery was not carried out, but associating this finding with the HR, it is possible that complications have occurred, as well as the animals have been sent to an emergency cesarean.

In healthy fetuses, when the internal carotid artery was detectable by ultrasound for the first time, no diastolic flow was seen in most fetuses examined. As gestation progresses, slow diastolic flow was measured from the 6th or 7th week [18]. These findings may justify the reduction in the S/D ratio found for the animals in the present study, where it significantly reduced at the end of pregnancy, probably due to a normal increase in the diastolic flow.

In the literature, there are different values of flow measurements in the internal carotid artery, both for canine fetuses and for adult animals of different breeds [13,24]. Such variations occur due to size, body mass, percentage of fat, age and anatomical variations in vessel diameter [4].

Unlike what was found in the present study, preliminary studies of pregnant bitches, the PSV and EDV observed for the fetal internal carotid artery increased significantly from week 6 to week 9 of gestation [8,18]. In this one, the PI varied slightly, but did not show any significant difference between the weeks of gestation, opposing the findings for suffering fetuses, where it significantly reduced throughout the final third of gestation. These findings may suggest the use of observation of this reduction, as a supplementary index for assessing the presence of fetal distress in pregnant dogs, as well as suggesting a possible obstetric intervention, if they persist.

Regarding the internal carotid artery RI, the present study showed a significant reduction over the last third of pregnancy. Studies in pregnant bitches [18,23] demonstrated a similar reduction in normal canine fetuses, from the 6th week of gestation to birth, although this difference was not considered statistically significant. Corroborating these findings, other authors have also described a progressive reduction in this index throughout normal canine pregnancy, implying an adequate perfusion of the placenta and fetal viscera [2,6,15]. Thus, it is suggested to always observe this index together, to confirm fetal distress by Doppler velocity analysis of this vessel.

For the middle cerebral arteries, the PI showed a statistically significant difference between the mean values found for the times studied, however, decreasing throughout the final third of pregnancy. A study carried out with ultrasound evaluation of this vessel in human fetuses in pregnancies with placental insufficiency states that high PSV values predict perinatal mortality and may be useful in the assessment of fetuses with abnormal uterine artery Doppler [19,21].

Research carried out in pregnant bitches, through the longitudinal analysis of the MCA Doppler velocimetry results, demonstrated that the PI was initially abnormal in most of the studied canine fetuses, but observed an increase, with a tendency to normalization before delivery or death fetal, however findings were not observed in our study [19]. Also, by analyzing the PSV, the authors found a well-defined pattern, with a progressive increase with advancing gestational age, and a tendency towards a slight reduction right before delivery or fetal death.

CONCLUSION

The temporal ultrasound evaluation by Doppler velocimetric analysis of the internal carotid, umbilical and middle cerebral arteries of canine fetuses in fetal distress during the last third of pregnancy allowed the determination of their characteristics and behavior during the time studied, as well as the correlation between the same. Although the outcome of pregnancy was not monitored, it can be concluded that the use of these tools proved to be safe for the bitch and the fetuses, as well as being relatively easy and quick to obtain, and the results of this work can be used as a basis for future research, identification of fetal abnormalities, prediction of the day of delivery and clinical guidance regarding obstetric procedures. Still, through this, it is possible a more precise evaluation of indices that indicate fetal distress, in the impossibility of evaluation by conventional methods.

MANUFACTURERS

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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 the paper.

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