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Fetal echocardiography in B and M modes for to standardize morphological and functional cardiovascular variables of canine fetuses in the final third of gestation: fetal echocardiographic measurements

Ecocardiografia fetal nas modalidades B e M para padronização de variáveis cardiovasculares morfológicas e funcionais de fetos caninos no terço final da gestação: medidas ecocardiográficas fetais

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ABSTRACT: Fetal echocardiography allows early diagnosis of cardiovascular changes. However, few studies have described physiological values that allow a quantitative evaluation for this test in dogs. The objective was to describe the presumed physiological values of echocardiographic variables obtained in B and M modes, and cardiac function, by canine fetal echocardiography in the last trimester of pregnancy. Twenty fetuses were submitted to fetal echocardiography. The variables that showed a statistically significant correlation coefficient in relation to fetal age were the diameter of the aorta and the thickness of the interventricular septum in systole and in diastole. The morphological evaluation allowed the observation and determination that the heart occupied an average of one third of the thorax, most of it located in the left hemithorax and with the apex turned to the left. By identifying the spine, the anterior wall of the chest was observed opposite to it, represented by the sternum. The right ventricle was found just below, separated from the right atrium by the tricuspid valve. Both ventricles showed similar size, thickness and contractility. The measurement of ejection fractions and shortening allowed the determination of the left ventricular systolic function. This study determined physiological values for the main echocardiographic variables obtained in B and M modes for the investigated group.

KEYWORDS: Cardiology; diagnostic imaging; internal medicine; morphology; small animals.

RESUMO: A ecocardiografia fetal permite o diagnóstico precoce de alterações cardiovasculares. Entretanto, poucos estudos descreveram valores fisiológicos que permitam uma avaliação quantitativa deste teste em cães. O objetivo foi descrever os valores fisiológicos presumidos das variáveis ecocardiográficas obtidas nos modos B e M, e da função cardíaca, pela ecocardiografia fetal canina no último trimestre de gestação. Vinte fetos foram submetidos à ecocardiografia fetal. As variáveis que apresentaram coeficiente de correlação estatisticamente significativo em relação à idade fetal foram o diâmetro da aorta e a espessura do septo interventricular na sístole e na diástole. A avaliação morfológica permitiu observar e determinar que o coração ocupava em média um terço do tórax, a maior parte localizado no hemitórax esquerdo e com o ápice voltado para a esquerda. Ao identificar a coluna, observou-se a parede anterior do tórax oposta a ela, representada pelo esterno. O ventrículo direito foi encontrado logo abaixo, separado do átrio direito pela valva tricúspide. Ambos os ventrículos apresentaram tamanho, espessura e contratilidade semelhantes. A medida das frações de ejeção e de encurtamento permitiu a determinação da função sistólica do ventrículo esquerdo. Este estudo determinou valores fisiológicos das principais variáveis ecocardiográficas obtidas nas modalidades B e M para o grupo investigado.

PALAVRAS-CHAVE: Cardiologia; diagnóstico por imagem; medicina interna; morfologia; pequenos animais.

INTRODUCTION

Fetal cardiology allows, through exams, the diagnosis of alterations and early detection of anatomical and functional particularities of the fetal heart and great vessels. This practice has implications for pre and postnatal management and patient prognosis (Schneider *et al.* 2005; Wang et al., 2020[a]; Wjtowicz *et al*, 2017).

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Among the tests used, fetal echocardiography stands out, which is sensitive for obtaining morphological data in real time. This makes it possible to establish bases to evaluate the heart and detect congenital diseases (Donofrio *et al*. 2014; Fontes Pedra *et al*. 2019). However, unlike that observed by echocardiography in adult animals, which is widely described and with well-standardized protocols, for fetuses, very few studies have reported physiological values for this method (Boon, 2011).

In Human Medicine, numerous studies have been described, and there are guidelines that deal exclusively with fetal cardiology and fetal echocardiography (Anuwutnavin *et al*. 2014; FONTE PEDRA et al., 2019; Wang *et al*., 2020[a]; Wang *et al*. 2020[b]). In Veterinary Medicine, a single study applied techniques from Human Medicine to develop regression equations that describe the relationship between fetal heart size and biparietal diameter in fetuses, by echocardiography (Giannico, 2017).

Thus, additional studies that show presumed physiological parameters are extremely important, as well as quantitative data that can be used as reference values for this exam, allowing the identification of possible congenital alterations and abnormalities by two-dimensional fetal echocardiography in dogs.

The objective of this study was to describe the physiological values of the main echocardiographic variables obtained in B and M modes, and cardiac function, by fetal echocardiography of bitches in the last trimester of pregnancy.

MATERIAL AND METHODS

The protocols used in this work were approved by the Ethics Committee on Animal Use - CEUA/XXX, registered under No. 649/2020.

An observational, prospective study was carried out, which included 20 (twenty) canine fetuses, from healthy bitches, which weighed between 6 and 15 kg and carried up to 4 fetuses, during the last trimester of pregnancy (47 to 60 days). At least 03 fetuses from each bitch were always analyzed. All pregnant bitches included had homogeneous fetuses regarding the measurements used in the study and their measurement values used did not differ. Thus, 1 fetus from each bitch was chosen to compose the study group, corresponding to the comparison of 20 fetuses from different bitches.

Gestational age was estimated by measuring the biparietal diameter (BPD), which was measured in the largest cross-section from the head and body differentiation. After obtaining the BPD measurement, the formula "BPD x 15 + 20" was used to estimate the gestational age, automatically calculated by the software of the ultrasound equipment (Figure 1) (Luvoni; Beccaglia, 2006).

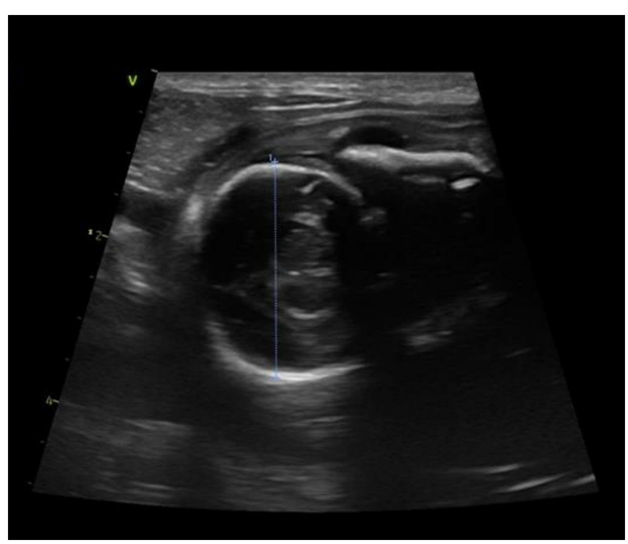
Exclusion criteria were applied to bitches and fetuses. The bitches underwent a general and specific clinical examination of the cardiovascular system (Feitosa, 2014) and complementary examinations (hematology and electrocardiogram) to assess their general health status. Those which showed signs of systemic changes resulting from acquired parasitic, infectious and cardiovascular diseases, with the potential to influence fetal development and alter maternal physiological hemodynamic values, such as valvular insufficiency and rhythm disturbances identified on the electrocardiogram, were excluded from the study.

Fetuses that presented some type of structural abnormality (cardiac or non-cardiac), electrical, such as arrhythmias or hemodynamics, visualized in the different ultrasound modalities, were excluded. Those that did not allow a complete and correct echocardiographic evaluation due to the positioning, size and number of puppies in the litter were also excluded.

All females underwent an abdominal ultrasound examination during the last trimester of pregnancy for fetal echocardiography. The bitches were positioned in dorsal decubitus, on an adapted sponge trough, and the abdominal surface was shaved for scanning after applying conductive gel for ultrasound. *Vivid IQ* ultrasound equipment (*GE Healthcare*) was used, coupled to a 6S-RS multifrequency (2.7-8 MHz) cardio pediatric sector transducer.

The largest possible number of fetuses was evaluated on each occasion, only on those that presented optimal conditions for the examination. The echocardiographic studies were recorded according to the "Guidelines and Standards for Performing Fetal Echocardiography", from the "Brazilian Guidelines for Fetal Cardiology," from the Brazilian Society of Cardiology (Fontes Pedra *et al*. 2019). A single experienced examiner was responsible for image acquisition throughout the study.

The morphological evaluation of the fetal heart was based on transversal and longitudinal sections of the fetus, starting from the fetal abdomen, sweeping it from the region caudal to the diaphragm to the cranial mediastinum, passing



Source: author's collection (55 days of gestation).

Figure 1. Measurement of the largest cross-section between the two parietal bones of the skull, based on the differentiation of the head and body of the canine fetus, to obtain the biparietal diameter (BPD) and estimate the gestational age using the formula: "BPD x 15 + 20", obtained automatically by the device software.

through several levels (four-chamber view, short axis view and cardiac long axis, cava long axis view, ductal view and aortic arch view), up to a complete scan of all cardiac structures, as proposed by the Brazilian Fetal Cardiology Guideline (Fontes Pedra *et al*. 2019). From there, subtle rotations were made until images similar to those obtained in canine echocardiography could be recognized. Image quality was maximized by adjusting gain, focus, and depth for each fetus during the scan. The size of the 2D sector had been minimized to improve image quality.

Images were obtained in B (Two-Dimensional – 2D) and M (Movement) modes to obtain the morphological and functional variables, following previously established protocols for humans and small animals (Boon, 2011; Fontes Pedra *et al*. 2019). Three representative cardiac cycles were analyzed, and the mean was calculated for each variable.

Heart rate (HR) was obtained for each cycle during automatic acquisition by the software on the machine, measuring the distance between two diastolic peaks, in M-mode of the left ventricle, in longitudinal section at the level of the papillary muscles. All fetuses were carefully evaluated, and fetal heart rate (FHR) was measured five times for at least 5 min (Gil *et al.* 2014). The average of all heartbeats recorded during the examination of each fetus was defined as the HR.

The cardiac chambers were recorded during their greatest diameter, at the end of diastole. The measurements included: in the transversal and/or longitudinal view (four-chamber view) of the left ventricle, at the level of the papillary muscles when images were acquired in systole and diastole, in M-mode, to calculate the thickness of the interventricular septum (IVS), the free wall (LVFW), and the internal diameter of the left ventricle (IDLV). These measurements were used to calculate the ejection and shortening fractions, automatically measured by the device, which were used for the functional assessment of the heart.

The diameter of the aortic root was measured in the transverse plane of the fetal heart, at the level of the annulus valve, in B-mode. In the same plane, the diameter of the left atrium was measured. These values were used to acquire the left atrium/aorta (LA/AO) ratio (Figure 2).

The Microsoft Office Excel software (Microsoft 356®) was used to analyze the data and perform descriptive statistics to obtain the mean, standard deviation, confidence interval and coefficient of variation for the variables analyzed. Pearson's correlation coefficient was used to assess the statistical correlation between the different variables and fetal age and with each other. The variance reference ranges were determined by calculating a 95% tolerance range, designed to cover 99% of all future events.

RESULTS

Since, in most cases, fetal sexing was not possible, the study was based on a total sample of 20 fetuses.

Table 1 shows the presumed physiological values obtained for the two-dimensional echocardiographic variables in B and M modes of the studied group. The tabulation of data referring to 55 days of gestation appears to be better representative of the final echocardiographic dimensions of the canine fetal heart, while linear regression made it possible to highlight the fluctuation of these values within the period of analysis (47 to 60 days of gestation). Thus, Figures 3, 4 and 5 represent the scatter diagrams for evaluating the linear regression between these variables and fetal age.

The variables that showed statistically significant correlation coefficients in relation to fetal age were: AO (r = 0.56), IVSs (r = -0.63) and IVSd (r = 0.59). The other variables that showed correlations with each other are described below: the diameter of the aortic root showed a positive correlation with the IDLVd (r = 0.56) and with the IDLVs (r = 0.50). The LVFWd showed a negative correlation with the IDLVd (r = -0.52). Heart rate was positively correlated with the LA/AO ratio (r= 0.62) and negatively correlated with the IDLVs (r = -0.54). Maternal age was positively correlated with IVSd (r = 0.64).

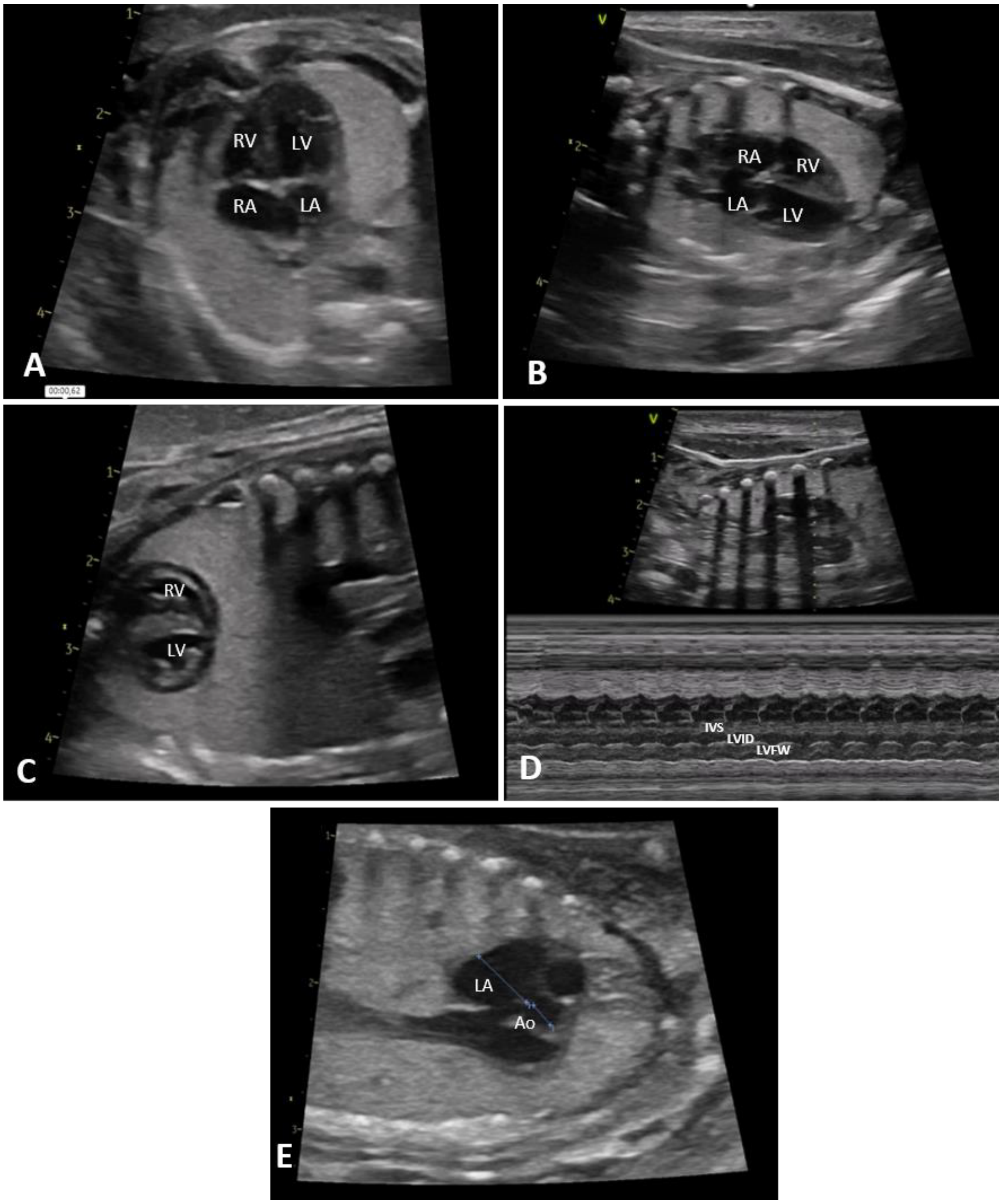
The morphological evaluation made it possible, through the visualization of the four-chamber projection, to observe and determine the heart occupying, on average, one third of the thorax, most of it located in the left hemithorax and with the apex facing to the left. By cross-section, the four heart chambers were shown allowing the differentiation of right and left structures.

By identifying the spine, the anterior wall of the chest was observed opposite it, marked by the sternum. Immediately below it, the right ventricle (RV) was found, separated from the right atrium by the presence of the tricuspid valve with its two leaflets (septal and parietal), located in the right atrioventricular ostium. This, in cross-section, showed a half-moon shape, molding itself to the LV surface but did not extend to the cardiac apex.

The left ventricle was identified by its conical shape, and by the presence of its apex forming the apex of the heart. Both ventricles presented approximately equal size, thickness and contractility, although the RV was slightly larger than the LV.

The atrial and ventricular septa were in the center of the heart with the left and right atrioventricular valves. The LA was identified by its proximity to the vertebral column and the descending aorta and its position was superior to the LV, separated from it by the mitral valve, composed of two coapted valves, which appear as thin lines of increased echogenicity following the cardiac cycle. Similarly, the right atrioventricular valve was shown to have an equal opening orifice, separating the RV and the right atrium (RA), approximately the same size as the LA.

Fetal heart rate (FHR) values averaged 225.14 bpm and accelerated and decelerated throughout the examination. The measurement of EF and SF allowed the determination of left ventricular systolic function.



LV: left ventricle; *RV*: right ventricle; *LA*: left atrium; *RA*: right atrium. Source: author's collection.

Figure 2. Echocardiographic images in B and M modes obtained from canine fetuses during the final trimester of pregnancy (55 days of gestation). (A) Apical view of the four chambers. (B) Longitudinal view of the four chambers. (C) Short axis view at the level of the papillary muscles, showing RV molding to the LV surface. (D) Passage of the M-mode cursor, used to obtain measurements of the interventricular septum wall (IVS), left ventricular free wall (LVFW) and left ventricular internal diameter (LVID), through the longitudinal view of the four chambers. (E) Two-dimensional short-axis view at the base of the heart used to measure the LA/AO ratio.

The examination proved to be safe, both for pregnant women bitches? and fetuses, and was possible, relatively easy and quick to perform.

DISCUSSION

The echocardiographic measurements in this study were obtained following previously established protocols to guarantee safe measurements and diagnostic images, similar to studies carried out with canine fetuses and adult animals (Boon, 2011; Carvalho *et al*. 2016; Giannico *et al*. 2015). In small animal veterinary medicine, echocardiographic parameters are well discussed and have standardized values for different breeds of dogs (Boon, 2011). Some studies were conducted in reference to fetal heart diseases diagnosed by ultrasound in the prenatal period in dogs (Veiga *et al*. 2013), However, this is one of the few studies that show physiological values of echocardiographic variables for a specific group of canine fetuses.

FHR values were similar to those found for puppies (up to 220 bpm) (Tilley, 1992) and within the physiological range standardized for fetuses (220-240 bpm), that is, two to three times the maternal heart rate (Nyland; Mattoon, 2002). A recent study considered that FHR greater than 200

Table 1. Presumed physiological values for two-dimensional echocardiographic variables obtained in B and M modes of canine fetuses in the last trimester of pregnancy, last third of gestation (55 days of gestation) – Teresina/PI/Brazil – 2022.

Variables	Average and SD	CI	CV (%)	Min-Max	*r*
AO (cm)	0.21 (0.03)	± 0.013	14.28	0.18 – 0.3	0.56[a]
LA (cm)	0.40 (0,04)	± 0.02	10	0.3 – 0.5	-
LA/AO ratio	1.89 (0.36)	± 0.161	19.04	1.25 – 2.55	-
IVSd (cm)	0.14 (0.04)	± 0.017	28.57	0.1 – 0.2	0.59[a]
IVSs (cm)	0.15 (003)	± 0.017	20	0.1 – 0.2	- 0.63[a]
IDLVd (cm)	0.45 (0.08)	± 0.037	17.77	0.3 – 0.6	-
IDLVs (cm)	0.21 (0.04)	± 0.017	19.04	0.13 – 0.29	-
LVFWd (cm)	0.11 (0.02)	± 0.012	18.18	0.1 – 0.2	-
LVFWs (cm)	0.14 (0.04)	± 0.018	28.57	0.1 – 0.22	-
EF (%)	81 (1.2)	± 2.29	9.7	72 – 88.09	-
SF (%)	52 (5.23)	± 1.79	10	42.42 – 59.52	-
FHR (BPM)	225.14 (4.1)	± 6.24	1.82	200 – 250	-
Fetus age (days)	53 (3.37)	± 1.47	6.35	47 – 59	-
Maternal age (year)	2.71 (1.17)	± 0.51	43.17	1 – 5	-

[a] Variables significantly correlated with fetal age. *AO*, diameter of the aortic root; *LA*, diameter of the left atrium; *LA/AO* ratio, left atrium/aorta ratio; *IVSd*, interventricular septum in diastole; *IVSs*, interventricular septum in systole; *IDLVd*, internal diameter of the left ventricle in diastole; *IDLVs*, internal diameter of the left ventricle in systole; *LVFWd*, left ventricular free wall in diastole; *LVFWs*, left ventricular free wall in systole; *EF*, ejection fraction; *FS*, shortening fraction; *FHR*, fetal heart rate; bpm, beats per minute; *CI*, confidence interval; *CV*, coefficient of variation; *SD*, standard deviation; *Min-Max*, minimum and maximum; *r*, Pearson's correlation coefficient; *BPM*, beats per minute.

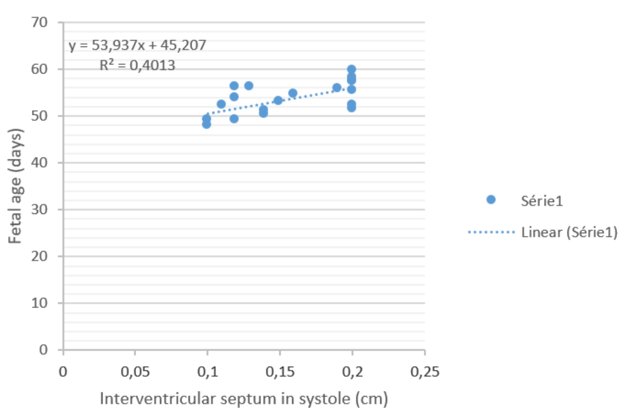


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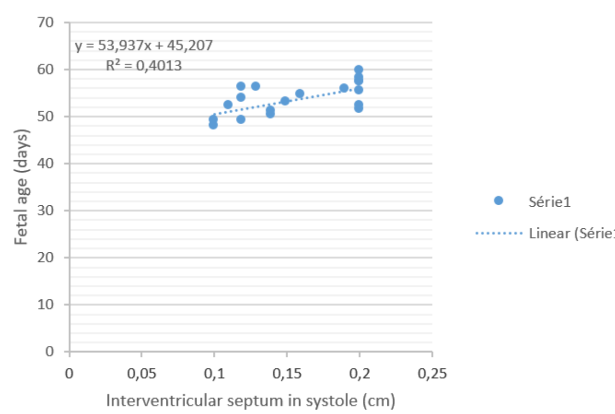
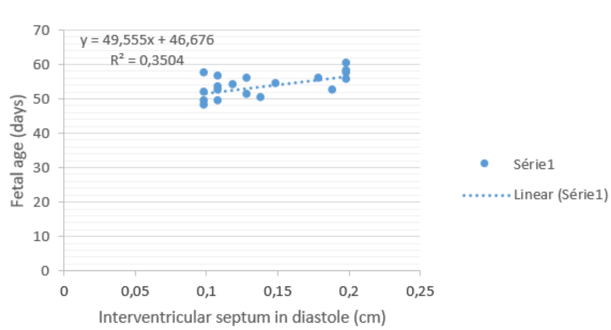
Figure 3. Scatter diagram to evaluate the linear regression between the interventricular septum in systole and fetal age in the last third of gestation (47 to 60 days of gestation)– Teresina/PI/Brazil – 2022.



Source: author's collection.

Figure 5. Scatter diagram to evaluate the linear regression between aortic diameter and fetal age, last third of gestation (47 to 60 days of gestation) – Teresina/PI/Brazil – 2022.



Source: author's collection.

Figure 4. Scatter diagram to evaluate the linear regression between the interventricular septum in diastole and fetal age, last third of gestation (47 to 60 days off gestation) – Teresina/PI/Brazil – 2022.

bpm is physiological, if preceded by acceleration and deceleration. This fact confirms the presumed physiological values found, as this variation was also observed during the exams through a detailed and prolonged evaluation of each fetus (Gil *at al.* 2014).

Corroborating these findings, a study concluded that FHR tends to increase up to 20 days before delivery, the same period of the study in question (Verstegen *et al.* 1993). In addition, currently, in order to be considered fetal distress, the FHR must remain below 200 bpm for more than three minutes, which did not occur in the study animals, since in the prepartum phase, intermittent uterine contractions on the fetus generate a significant temporary decrease in FHR, that returns to physiological heart rate or even increases one to two minutes later (GIANNICO *et al.* 2015). This fact justifies the negative correlation found for FHR and fetal age.

The average found for the diameter of the aortic root and left atrium showed similar values to those found for fetuses of brachycephalic (0.31 and 0.43, respectively) and non - brachycephalic breeds (0.29 and 0.40, respectively) (Giannico *et al.* 2015). Such similarity is probably related to the similar weight of bitches from both experiments (Giannico *et al.* 2015), which results in fetuses with approximate size. In addition, all echocardiographic measurements were performed in the same period, from the 40th day of pregnancy, period in which the differentiation of the cardiac chambers is perceived, thus avoiding large morphological variations (Yeager *et al.* 1990). This is justified by the known positive correlation between weight and cardiac dimensions, causing a proportional increase in the diameter of the aorta and left atrium, as the size of the animal increases (Hanton *et al.* 1998). Corroborating this, the diameter of the aorta was positively correlated with fetal age, that is, the older the fetus, the greater the diameter of the aorta.

The left atrium/aorta ratio (LA/AO) showed slightly higher values when compared to healthy adult animals (up to 1.6) (Keene *et al.* 2019). In adult dogs, increased LA is usually related to volume and pressure overload in several heart diseases, acquired or congenital. In this group of animals, the size of the AE is of interest for clinical assessment of disease severity (Hansson *et al.* 2002). However, it is known that in fetuses there is an overload of physiological pressure due to anatomical and functional changes that occur during fetal life, such as the foramen ovale and the ductus arteriosus. Most of the blood arriving at the heart is directed by the *crista dividens* through the foramen ovale into the left atrium, which mixes with a relatively small amount of blood returning from the lungs through the pulmonary veins (Elderstone; Rudolph; Heyman, 1980; Moore; Persaud; Torchia, *et al.* 2019).

A study involving 420 human fetuses evaluated by fetal echocardiography showed that the enlargement of the right atrium (RA), in most cases (202 fetuses), was due to physiological reasons, while the others were often related to congenital abnormalities that were ongoing, with increased right heart pressure, such as pulmonary atresia (Yeager; Concannon, 1990).

The Brazilian Guideline for Human Fetal Cardiology guides the identification of the LA through its proximity to the spine and identification by the characteristic movement of the foramen ovale valve, which regulates blood flow from the right atrium to the left atrium (Fontes Pedra *et al.* 2019). This valve, although also present in canine fetuses (Hyttel; Snowatzn; Vejlsted, 2012), is difficult to visualize on echocardiographic examination. This is probably due to the extremely small size of the structures when compared to human fetuses. Despite the advanced gestational age, and the use of a good ultrasound device, associated with correct image adjustment, some structures are still difficult or impossible to visualize, which is one of the main limitations of the exam. The same probably happened with the moderator band (septomaginal trabecula), which, despite being present in the canine species, was not visualized in fetal echocardiography.

The mitral and tricuspid valves showed an aspect similar to that seen for human fetuses. However, in human fetuses, the septal leaflet of the tricuspid valve is inserted closer to the cardiac apex, resulting in a minimal difference in the level of implantation. This fact was not seen for dogs, and no reports were found in the veterinary literature of any description of this difference. It is believed, however, that due to the similarity of the cardiovascular anatomy existing between dogs and humans (Camacho *et al.*, 2016), such a difference may also exist, however, because it is minimal, it is impossible to detect. Even in human fetuses this implantation difference is reported to be difficult to observe, emphasizing the observed difficulty (Fontes Pedra *et al.* 2019).

Similarly to that observed for human fetuses, the right and left ventricles showed similar morphology, with the RV being slightly larger than the LV. This fact is justified due to the greater thickness of the right ventricular wall in fetuses and neonates, resulting from the greater work during intrauterine life, which causes physiological hypertrophy (Swanson *et al.* 2015).

The results obtained for left ventricular free wall thickness, interventricular septum and left ventricular internal diameter, both in systole and diastole, when compared with adult dogs of different breeds and sizes (Chetboul *et al.*, 2016; Lombard *et al.* 1984) confirm the known correlation between the increase in body weight and increase in cardiac dimensions for the parameters evaluated (Hanton *et al*, 1998). In this study, the thickness of the interventricular septum in diastole was positively correlated with fetal age, that is, the older and more developed the fetus, the greater the diameter of this structure. The females in the present study, despite being different breeds, varied relatively little in weight. Even though the sex of the fetuses was not identified in most cases, this fact is probably not an obstacle, because previous studies suggest that this variable does not influence echocardiographic measurements.

The assessment of fetal cardiac function in humans has enabled cardiac analyzes in intrauterine life, in order to diagnose congenital heart diseases and analyze systemic disorders of the fetus and/or mother that damage the fetal heart (Pineda *et al.*, 2000). In Veterinary Medicine, a single study showed the assessment of cardiac function (systolic, diastolic and global) in canine fetuses, possibly allowing the intrauterine diagnosis of congenital heart disease and its correlation with the moment of delivery (Giannico *et al.* 2015).

EF and FS analysis allowed the establishment of left ventricular systolic function for this group of animals. For fetuses, a single study has shown mean values of fractional shortening during the final trimester of pregnancy ranging from 25.3

to 29.40, values lower than those obtained in the present study (GIANNCO et al., 2017). Physiological SF values in adult dogs range from 33.7 to 45.9. However, although it is taken as an estimate of systolic function, it is an index largely influenced by preload and afterload, so that conditions that increase preload or decrease afterload result in an increase in shortening fraction and vice versa (Boon, 2011; Chetboul; Bussadori; Madron, 2016). In fetuses, these conditions are present under physiological conditions, justifying the increase in FS compared to adult dogs.

The mean EF value obtained in the present study was higher than that found for adult dogs of different breeds, when calculated by the same method used in the present study (*Teichholz*) (70.9 ± 6.0) and by different methods (*Simpson*) (66.5 ± 6.4) (Chetboul; Bussadori; Madron, 2016). However, this variation can be considered physiological, as there is a wide variation for this index in dogs, due to numerous factors, such as elevated heart rate, which has the potential to significantly alter this index beyond the maximum physiological values (Crippa *et al.* 1992).

CONCLUSION

Fetal echocardiographic evaluation of dogs during the final trimester of pregnancy allowed the establishment of values presumed physiological for the main variables observed, obtained in B and M modes, for the investigated group. The analysis of the data obtained reflects an indication of the general healthy condition of the heart and fetal cardiac function.

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