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CARDIOVASCULAR IMAGINOLOGICAL EVALUATION IN CANINE FETUSES: ECOCARDIOGRAPHY AND DOPPLER

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ABSTRACT

The monitoring of gestational development in both medicine and veterinary medicine is performed through two-dimensional ultrasonography and Doppler techniques, which offer anatomical and functional information of the fetus. Methods of gestational assessment have improved in human obstetrics, resulting in the early diagnosis of abnormal pregnancy or fetal distress, allowing intervention as needed. In veterinary medicine, studies that analyze the efficacy of this modality are limited. Few studies have characterized maternal and fetal blood flow through Doppler ultrasonography and their progressive changes during canine pregnancy. This is the first study to show the differences in these flows in pregnant bitches on the day of delivery. Fetal echocardiography is the detailed ultrasonographic evaluation of the heart used to identify and characterize fetal cardiac abnormalities and has been performed for several years in human medicine. Although the major focus in fetal echocardiography is the identification of cardiac defects and fetal arrhythmias, the analysis of fetal heart function is also gaining importance. This research is a pioneer in veterinary medicine, and, to the authors knowledge, no data on fetal echocardiography has been published prior to this study.

This doctoral thesis is divided into four chapters. The first two assess the hemodynamic characteristics of the umbilical artery in the week of delivery in bitches. Chapter 1 is published in the journal “Animal Reproduction Science” and describes changes in umbilical artery blood flow within the last 120 hours of canine gestation. It reveals how changes in the umbilical artery resistive index (RI) in the prepartum period provide information on the time of delivery and helps in the diagnosis of possible fetal dystocia and distress. Chapter 2 is published in “Theriogenology” and performs a quantitative analysis of RI along with fetal heart rate oscillation, showing that the correlation between them provides a more accurate prediction of the time of delivery in bitches.

Chapter 3 and 4 have the objective, through fetal echocardiography, to qualitatively and quantitatively monitor fetal cardiac development in dogs, allowing accurate evaluation of the growth of heart structures and cardiac function. Chapter 3, which is published in “Veterinary Research Communications”, reveals regression models for correlating canine fetal heart development with fetal body size, characterizing normal development or suggesting cardiac abnormalities. Chapter 4 describes values of canine fetal heart function, demonstrating systolic, diastolic and global function parameters and their correlations with the moment of delivery.

Key-words: dogs, gestation, fetal ultrasonography, resistive index, heart rate, fetal echocardiography

RESUMO

O monitoramento do desenvolvimento gestacional tanto na medicina como na medicina veterinária é realizado por meio da ultrassonografia bidimensional e Doppler, que permite informações anatômicas e funcionais do feto. Os métodos de avaliação gestacional têm aumentado na obstetrícia humana, resultando no diagnóstico precoce da gravidez anormal ou sofrimento fetal, que permite intervenção assim que necessária. Na medicina veterinária os estudos que analisam a eficácia dessa modalidade são limitados. Poucos são estes que caracterizaram por meio da ultrassonografia Doppler o fluxo sanguíneo materno e fetal e suas mudanças progressivas durante a gestação canina. Este presente estudo é o primeiro a demonstrar a alteração destes fluxos em cadelas gestantes no dia do parto. A ecocardiografia fetal é a avaliação ultrassonográfica detalhada do coração utilizada para identificar e caracterizar anomalias cardíacas fetais e vêm sendo realizada há vários anos pela medicina. Embora o maior foco da ecocardiografia fetal seja a identificação de defeitos cardíacos e arritmias fetais, outra área que tem crescido é a análise da função cardíaca fetal. Este presente trabalho é pioneiro na medicina veterinária, não havendo estudos descritos sobre ecocardiografia fetal anteriores à nossa publicação.

Esta tese de doutorado está dividida em quatro capítulos. Os dois primeiros avaliam as características hemodinâmicas da artéria umbilical na semana do parto em cadelas. O Capítulo 1 está publicado na revista *Animal Reproduction Science* e descreve as alterações no fluxo sanguíneo da artéria umbilical nas últimas 120 horas da gestação canina. Ele revela como as alterações do índice de resistividade (IR) da artéria umbilical no período pré-parto fornecem informações sobre o momento do parto e auxiliam no diagnóstico de uma possível distocia e sofrimento fetal. O Capítulo 2 está publicado na *Theriogenology* e faz uma análise quantitativa do IR juntamente com a oscilação da frequência cardíaca fetal, mostrando que a correlação entre tais dados fornece uma previsão mais precisa do momento do parto nas cadelas.

Os Capítulos 3 e 4 tem o objetivo de, por meio da ecocardiografia fetal, acompanhar de forma qualitativa e quantitativa o desenvolvimento cardíaco fetal em cães, permitindo exata avaliação do crescimento das estruturas do coração e função cardíaca. O Capítulo 3, que está publicado na *Veterinary Research Communications*, revela modelos de regressão para correlacionar o desenvolvimento do coração fetal canino com o tamanho do corpo fetal, caracterizando o desenvolvimento normal ou sugeririndo anomalias cardíacas. O Capítulo 4 descreve valores de função cardíaca fetal canina, demonstrando os parâmetros sistólicos, diastólicos e de função global e suas correlações com o momento do parto.

Palavras-chave: cães, gestação, ultrassonografia fetal, índice de resistividade, frequência cardíaca, ecocardiografia fetal

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LIST OF ABBREVIATIONS AND ACRONYMS

AC	- Abdominal cross-sectional area
ANOVA	- Analysis of variance
Ao area	- Aorta area
Ao Vmax	- Aortic flow
Ao	- Aorta
AUC	- Area under the curve
BPD	- Biparietal diameter
bpm	- Beats per minute
CCO	- Combined cardiac output
CI	- Confidence intervals
cm	- Centimeter
cm/s	- Centimeter per second
cm^2	- Square centimeter
CV	- Coefficient of variation
DICOM	- Digital imaging and communications in medicine
EDV	- End diastolic velocity
FC	- Frequência cardíaca
FL	- Femoral length
FS%	- Fraction shortening
h	- Hour
HR	- Heart rate
IQR	- Interquartile range
IR	- Índice de resistividade
IVCT	- Isovolumic contraction time
IVRT	- Isovolumic relaxation time
LA	- Left atrium
LCO	- Left cardiac output
LR (-)	- Negative likelihood ratio
LR (+)	- Positive likelihood ratio
LV	- Left ventricle
LVd	- Left ventricular cavity during diastole
LVET	- Left ventricular ejection time

LIST OF ABBREVIATIONS AND ACRONYMS (continuation)

LVs	- Left ventricular cavity during systole
m/s	- Meter per second
MHz	- Megahertz
Min-Max	- Minimum and maximum
mL	- Milliliter
mL/min	- Milliliter per minute
mm	- Millimeter
MPI	- Myocardial performance index
ms	- Millisecond
n	- Number
NPV	- Negative predictive value
P area	- Pulmonary area
P Vmax	- Pulmonary flow
Pa	- Pulmonary artery
PPV	- Positive predictive value
PSV	- Peak systolic velocity
R^2	- Regression coefficient
RA	- Right atrium
RCO	- Right cardiac output
RI	- Resistive index
RMSE	- Root mean square error
ROC	- Receiver operating characteristic
RV	- Right ventricle
SD	- Standard deviation
SE	- Standard error
SV	- Stroke volume
TVI	- Time-velocity integral

LIST OF SYMBOLS

+	- Addition sign
≈	- Approximately equal
±	- Both addition and subtraction operation
÷	- Division sign
=	- Equal
·	- Multiplication sign
( )	- Parentheses: calculate expression inside first
%	- Percent
π	- Pi constant: 3.141592654...
<	- Strict inequality: less than
>	- Strict inequality: more than
-	- Subtraction sign

SUMMARY

1 CHAPTER 1

THE USE OF DOPPLER EVALUATION OF THE CANINE UMBILICAL ARTERY IN PREDICTION OF DELIVERY TIME AND FETAL DISTRESS

ABSTRACT

The aim of this study was to describe changes in umbilical artery blood flow in the latter stages of canine pregnancy. Seventeen pregnant bitches were examined sonographically to evaluate umbilical artery blood flow at the following antepartum times: 120-96h, 96-72h, 72-48h, 48-24h, 24-12h, 12-6h and 6-1h. The peak systolic velocity and end diastolic velocity were measured to calculate the resistive index (RI). Bitches were classified into 2 groups according to delivery method: normal delivery (Group 1, n = 11) and Caesarean section, due to fetal distress, (Group 2, n = 6). During the study, the RI of the umbilical artery in bitches in Group 1 significantly declined in the time periods 72-48h, 24-12h, 12-6h and 6-1h before delivery when compared to the reference RI (120-96h antepartum period), with values below 0.7 in the 12-6h and 6-1h periods. In Group 2, the RI decreased significantly in the antepartum periods 96-72h, 72-48h, 48-24h with respect to the period 120-96h, and increased in the periods from 24-12h, 12-6 and 6-1h (being significantly higher in this last period) until the time of Caesarean section. Therefore monitoring of changes in umbilical artery RI in the pre-partum period may provide information about time of delivery in bitches and also assist in the diagnosis of possible dystocia and fetal distress.

Key-words: canine, fetal ultrasonography, Doppler ultrasonography, pregnancy, gestational ultrasound, resistive index

AVALIAÇÃO DOPPLER DA ARTÉRIA UMBILICAL DE CÃES PARA PREVER O MOMENTO DO PARTO E O SOFRIMENTO FETAL

RESUMO

O objetivo deste estudo foi descrever alterações no fluxo sanguíneo da artéria umbilical nos últimos momentos da gestação canina. Dezessete cadelas gestantes foram examinadas ultrassonograficamente para avaliação do fluxo sanguíneo da artéria umbilical nos seguintes momentos pré-parto: 120-96h, 96-72h, 72-48h, 48-24h, 24-12h, 12-6h e 6-1h. A velocidade sistólica máxima e a velocidade diastólica final foram mensuradas para calcular o índice de resistividade (IR). As cadelas foram classificadas em dois grupos de acordo com o tipo de parto: parto normal (Grupo 1, n = 11) e cesariana, secundariamente ao sofrimento fetal (Grupo 2, n = 6). Durante o estudo, o IR da artéria umbilical nas cadelas do Grupo 1 diminuiu significativamente nos períodos de 72-48h, 24-12h, 12-6h e 6-1h pré-parto quando comparado ao IR considerado referência (120-96h pré-parto), com valores abaixo de 0,7 nos períodos 12-6h e 6-1h. No Grupo 2, o IR diminuiu significativamente nos períodos pré-parto 96-72h, 72-48h, 48-24h em relação ao período 120-96h, e aumentou nos períodos 24-12h, 12-6 e 6-1h (sendo significativamente maior neste último período) até o momento da cesariana. Assim, o monitoramento das alterações do IR da artéria umbilical no período pré-parto pode fornecer informações sobre o momento do parto nas cadelas além de auxiliar no diagnóstico de distocia e sofrimento fetal.

Palavras-chave: cães, ultrassonografia fetal, ultrassonografia Doppler, gestação, ultrassonografia gestacional, índice de resistividade

1.1 INTRODUCTION

Conventional two-dimensional ultrasonography is a non-invasive, safe and efficient technique for monitoring fetal development and viability in veterinary medicine (ENGLAND; EDWARD, 1990; YEAGER; CONCANNON, 1990). However, two-dimensional ultrasonography provides little information about blood flow and therefore has been used in conjunction with Doppler ultrasound for clinical assessment in obstetrics and gynecology in a number of animal species (NAUTRUP, 1998; DI SALVO et al., 2006; DOMINGUES et al., 2007; BLANCO et al., 2008). Doppler ultrasound can be used to evaluate anatomical and functional vascular information such as blood flow velocity, direction and type (NICOLAIDES et al., 2000).

Pulsed wave Doppler allows analysis of blood flow within a single vessel. The Doppler waveform represents changes in the velocity of the blood flow during the cardiac cycle and a deflection in the late systolic or early diastolic flow is characteristic of high-resistance arterial blood flow waveforms (BLANCO et al., 2008). The main flow parameters measured using this technique are the peak systolic velocity (PSV) and end diastolic velocity (EDV). The first parameter, PSV, is formed by the opening of the semilunar valves and the forward ejection of blood. This forward blood flow starts to decelerate when cardiac contraction provides insufficient forward force to overcome the elastic properties of the downstream vascular bed and the viscosity of blood. The EDV varies in vascular beds predominantly due to differences in downstream vascular resistance and input pressure (GOSLING; KING, 1975).

Another commonly measured parameter is the resistive index (RI), also known as the Pourcelot ratio, which is calculated from blood flow velocities. This index, expressed by (PSV – EDV) ÷ PSV, indicates the downstream resistance in arteries (ranging from 0 to 1, where 0 is no resistance and 1 maximum resistance) (POURCELOT, 1974). These parameters have also been used to assess fetal blood flow during pregnancy in a number of species (REED et al., 1996; BOLLWEIN et al., 2000; BOLLWEIN et al., 2002a; BOLLWEIN et al., 2002b; BOLLWEIN et al., 2003; BOLLWEIN et al., 2004; REYNOLDS et al., 2006).

Fetal blood flow in the dog was first described in 1998 by Nautrup and further research has shown that Doppler ultrasonography can provide significant information on canine pregnancy (MIRANDA; DOMINGUES, 2010; BLANCO et al., 2011;

BATISTA et al., 2013; FELICIANO et al., 2013). Normal Doppler measurements of umbilical artery blood flow in canine fetuses have been reported (DI SALVO et al., 2006). In this species, the RI of the umbilical artery progressively decreases throughout normal gestation to ensure adequate perfusion of the placenta and fetal viscera (NAUTRUP, 1998; DI SALVO et al., 2006; MIRANDA; DOMINGUES, 2010). However, there is no published information on the RI of the canine umbilical artery in the last days of gestation. The aim of this study was to describe and evaluate changes in umbilical artery blood flow at the end of canine pregnancy with a view to developing a method for predicting the parturition day and/or diagnosing possible dystocia and fetal distress.

1.2 MATERIALS AND METHODS

Seventeen, clinically healthy (primiparous or pluriparous), pregnant bitches of different breeds ranging from 1 to 7 years of age were included in this study. The data of the dams are shown in Table 1.1. All procedures were conducted in accordance with the Animal Use Committee guidelines. Two-dimensional and Doppler ultrasonographic evaluations were carried out using ultrasonographic equipment (MyLab 30 – Esaote, Genova, Italy) with a 7.5 to 12 MHz linear multifrequency transducer (LA523 reference – Esaote, Genova, Italy). Sonographic examinations were performed in all bitches by an echocardiographer – image acquisition was checked by one sonographer who was a member of the Brazilian College of Veterinary Radiology. The bitches were positioned in dorsal recumbency using a sponge trough, acoustic gel was applied to the transducer and abdominal hair was clipped to optimize ultrasonographic image acquisition.

The pregnant bitches were examined sonographically twice a week throughout pregnancy to determine the end of fetal organogenesis (YEAGER; CONCANNON, 1990). After detection of fetal intestinal peristalsis (YEAGER; CONCANNON, 1990), sonographic examination was carried out every day until delivery to evaluate blood flow in the umbilical artery. Doppler measurements were made in up to six fetuses in each pregnant bitches. When fewer than six fetuses were present, measurements were repeated in each fetus to obtain a total of six measurements in each pregnant bitch for every time period. A cross-sectional scan of the placenta zonaria was performed and the umbilical arteries in the mid-cord site of the free-floating umbilical cord were examined.

Color Doppler was used to the position of the arteries. The sample volume of pulse-wave Doppler was placed in the center of the color-coded blood flow to obtain the waveforms (DI SALVO et al., 2006). Three uniform consecutive waveforms were included and the average of the three was used. PSV and EDV were measured in m/s. Resistive index [(PSV − EDV) ÷ PSV] was automatically calculated by the ultrasound software. The measurements were always obtained when the fetal heart rate was greater than 200 bpm.

When fetal distress was present flow measurements were acquired at the highest possible heart rate. The bitches were divided into two groups after data collection: Group 1, bitches with normal delivery; and Group 2, bitches that underwent Caesarean section due to fetal distress. Antepartum time was determined after data collection and analysis, by counting backwards from the date of delivery (where time of birth was zero hour), and divided into the following periods: 120-96h, 96-72h, 72-48h, 48-24h, 24-12h, 12-6h and 6-1h before parturition.

Fetal distress was assessed in all time periods by reference to fetal heart rate (HR) (VERSTEGEN et al., 1993). The fetal HR was recorded using M-mode echocardiography (VERSTEGEN et al., 1993) and the degree of variation in heart rate (oscillation) was assessed. Fetal distress was defined as a constant fetal HR between 150 and 180 bpm (SMITH, 2012; GIL et al., 2014). Bitches presented with fetuses in distress were sent for Caesarean section within one hour of the ultrasound examination.

Data analysis was performed using R for Windows (version i386 3.1.0, R Foundation for Statistical Computing, Vienna, Austria, 2014). For analysis of RI, two models were adjusted using the method of generalized linear models proposed by Nelder and Wedderburn (1972), in which the distribution of the response variable; matrix model and link function is taken into account. To adjustment of the linear model covariates "period before delivery" and "bitch" were considered. The selected model for the curve adjustment was based on normal data distribution. The Kruskal-Wallis nonparametric test was used to compare the RI of the umbilical artery in each period, between bitches with normal delivery and bitches which were referred for Caesarean section (Groups 1 and 2), and thus to determine which periods were similar. A P-value less than $\alpha = 5\%$ was considered significant, and the significance of each parameter was analyzed relative to the value set.

1.3 RESULTS

The 17 bitches included in the study were categorized into two groups: Group 1 comprised 11 bitches delivered by normal parturition (bitches 1–11) and Group 2 included six bitches referred for a Caesarean section with fetuses in distress (bitches 12–17) (Table 1.1). The velocities (PSV and EDV) and RI values of umbilical artery of each group during the last five days of gestation are reported in Table 1.2 and 1.3.

TABLE 1.1 – Characteristics of the bitches in Group 1 and 2, their type of delivery and numbers of fetuses.

Group	*Bitch*	*Breed*	*Age*	*Primiparous or pluriparous*	*Parturition*	*Number of fetus*
1	1	Chihuahua	2	Primiparous	Normal delivery	3
	2	Pekingese	2	Pluriparous	Normal delivery	5
	3	Maltese	2	Primiparous	Normal delivery	3
	4	Pinscher	3	Primiparous	Normal delivery	6
	5	Pug	2	Pluriparous	Normal delivery	7
	6	Schnauzer	5	Primiparous	Normal delivery	2
	7	English Cocker Spaniel	3	Primiparous	Normal delivery	6
	8	American Pit Bull Terrier	2	Primiparous	Normal delivery	6
	9	Boxer	7	Primiparous	Normal delivery	13
	10	Siberian Husky	1	Primiparous	Normal delivery	7
	11	X-breed	3	No clinical history	Normal delivery	11
2	12	Yorkshire Terrier	7	Primiparous	Caesarean – fetal distress	4
	13	Pug	1	Primiparous	Caesarean – fetal distress	3
	14	Pug	3	Pluriparous	Caesarean – fetal distress	6
	15	Schnauzer	4	Pluriparous	Caesarean – fetal distress	4
	16	Schnauzer	6	Pluriparous	Caesarean – fetal distress	4
	17	American Staffordshire Terrier	3	Primiparous	Caesarean – fetal distress	3

TABLE 1.2 – Values of peak systolic velocity (PSV), end diastolic velocity (EDV) and resistive index (RI) of umbilical artery in 11 bitches with normal delivery (Group 1) assessed in hours before parturition.

Period	*120-96h*	*96-72h*	*72-48h*	*48-24h*	*24-12h*	*12-6h*	*6–1h*
PSV							
Mean ± SD (m/s)	0.32 ± 0.10	0.40 ± 0.13	0.33 ± 0.08	0.43 ± 0.14	0.36 ± 0.15	0.27 ± 0.06	0.34 ± 0.09
Range (m/s)	0.21-0.62	0.20-0.61	0.19-0.55	0.21-0.74	0.18-0.74	0.16-0.38	0.20-0.50
EDV							
Mean ± SD (m/s)	0.08 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.04	0.10 ± 0.04	0.09 ± 0.02	0.12 ± 0.03
Range (m/s)	0.04-0.14	0.06-0.15	0.05-0.15	0.06-0.18	0.05-0.19	0.06-0.13	0.07-0.17
RI							
Mean ± SD	0.75 ± 0.04	0.76 ± 0.04	0.74 ± 0.03	0.75 ± 0.04	0.72 ± 0.04	0.67 ± 0.03	0.65 ± 0.04
Range	0.70-0.85	0.70-0.84	0.69-0.83	0.71-0.86	0.67-0.82	0.61-0.70	0.56-0.70

SD, standard deviation

TABLE 1.3 – Values of peak systolic velocity (PSV), end diastolic velocity (EDV) and resistive index (RI) of umbilical artery in six bitches which subsequently underwent caesarean section due to fetal distress (Group 2) in hours before parturition.

Period	*120-96h*	*96-72h*	*72-48h*	*48-24h*	*24-12h*	*12-6h*	*6–1h*
PSV							
Mean ± SD (m/s)	0.34 ± 0.10	0.33 ± 0.11	0.34 ± 0.19	0.29 ± 0.08	0.36 ± 0.19	0.30 ± 0.09	0.35 ± 0.17
Range (m/s)	0.18-0.49	0.19-0.52	0.18-0.97	0.18-0.56	0.20-0.86	0.17-0.46	0.16-0.74
EDV							
Mean ± SD (m/s)	0.07 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	0.09 ± 0.02	0.10 ± 0.04	0.08 ± 0.02	0.09 ± 0.03
Range (m/s)	0.04-0.10	0.05-0.11	0.06-0.19	0.06-0.15	0.05-0.19	0.05-0.12	0.04-0.15
RI							
Mean ± SD	0.79 ± 0.04	0.76 ± 0.03	0.72 ± 0.06	0.70 ± 0.05	0.71 ± 0.06	0.71 ± 0.06	0.74 ± 0.03
Range	0.70-0.85	0.69-0.80	0.59-0.80	0.59-0.78	0.60-0.80	0.61-0.80	0.71-0.81

SD, standard deviation

Figure 1.1 shows the boxplot of the distribution of the RI of the umbilical artery before the delivery period in the bitches in Group 1. The RI of the umbilical artery declined during the periods 12-6h and 6-1h before delivery, and the lowest values were less than or equal to 0.7 (Figure 1.2).

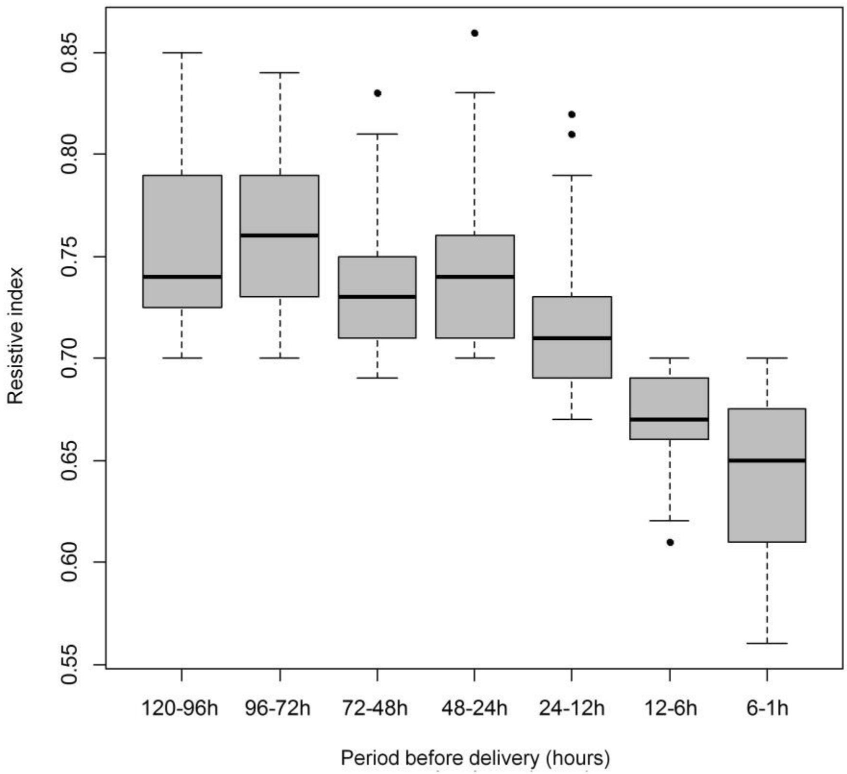

FIGURE 1.1 – Boxplot of resistance index during the antepartum period in bitches which subsequently experienced a normal delivery (Group 1).

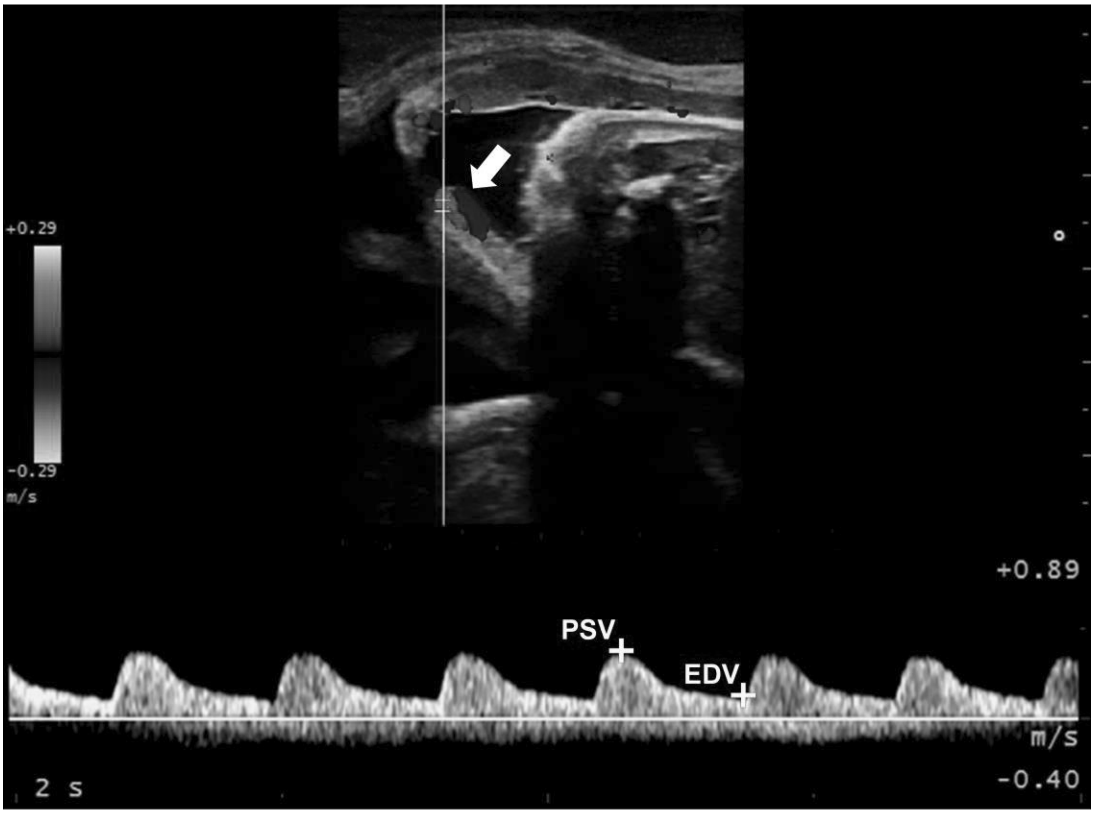

FIGURE 1.2 – Doppler ultrasonography of a fetal umbilical artery in a bitch from Group 1 at 6-1h before normal parturition. The umbilical cord is visualized by color Doppler (arrow), with the umbilical artery in red and umbilical vein in blue. The pulse-wave Doppler sample volume was placed in the center of the umbilical artery to obtain the waveforms (bottom). Peak systolic velocity (PSV), 0.37 m/s; end diastolic velocity (EDV), 0.12 m/s; resistive index, 0.67; heart rate, 212 bpm.

Table 1.4 shows the adjusted generalized linear model for the RI of the umbilical artery in Group 1, the estimates of the period 120-96h pre-partum were used as a reference for the comparison with other estimates and tested the null hypothesis of its regression parameter with a significance level of 5%. The RI in the 72-48h, 24-12h, 12-6h and 6-1h periods were significantly lower compared to the reference RI (120-96h antepartum period) (Table 1.4). Table 1.4 also shows the standard errors. The t-value is the quotient of the parameter estimate and its standard error.

In all Group 2 bitches (bitches 12–17) at least one fetus showed signs of fetal distress 6-1h preoperatively, and the bitch showed no signs of labor. Therefore, Caesarean section was performed. Figure 1.3 shows a boxplot of the distribution of the umbilical artery RI by period before delivery in the bitches in Group 2. The lowest values of RI occurred within 48-24h of delivery and the highest in the period 6-1h before delivery.

TABLE 1.4 – Estimates of the parameters of normal adjusted model to the data of resistance index of the umbilical artery in bitches which experienced normal delivery (Group 1).

Parameter	*B*	*Estimate*	*SE*	*t*	*P-value*
Intercept	β 0	0.7471	0.0078	95.8500	< 2E-16 ***
Period					
96-72h	β 1	0.0043	0.0071	0.6120	5.413E-01
72-48h	β 2	-0.0177	0.0069	-2.5840	1.031E-02 *
48-24h	β 3	-0.0115	0.0069	-1.6610	9.795E-02
24-12h	β 4	-0.0442	0.0066	-6.7190	1,110E-10 ***
12-6h	β 5	-0.0849	0.0079	-10.7010	<2E-16 ***
6-1h	β 6	-0.1097	0.0087	-12.6600	<2E-16 ***

SE; Standard error
*** 0.001; ** 0.01; * 0.05

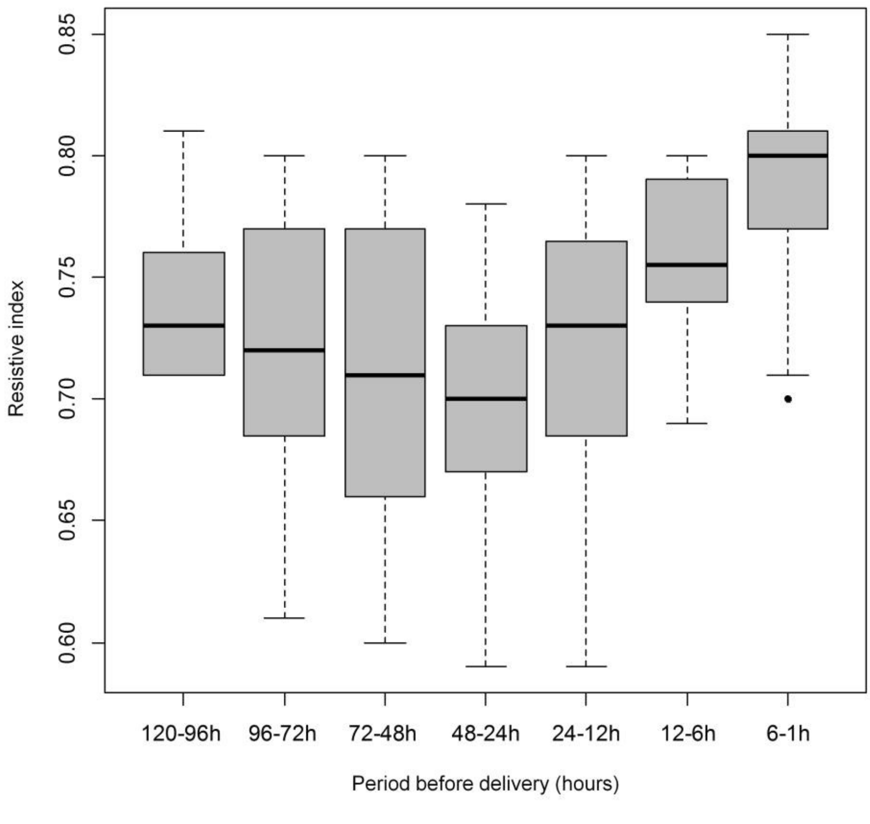

FIGURE 1.3 – Boxplot of resistance index during the antepartum period in bitches with fetal distress that subsequently underwent cesarean section (Group 2).

Figure 1.4 shows the change in the RI of the umbilical artery in each bitch in Group 2, demonstrating that in all animals there was a marked decline in the RI of the umbilical artery at one time period, but in the following periods RI elevation occurred before parturition. This reduction in RI occurred during different time periods in individual bitches (ranging from the 72-48h period to 12-6h before parturition) before fetuses became distressed.

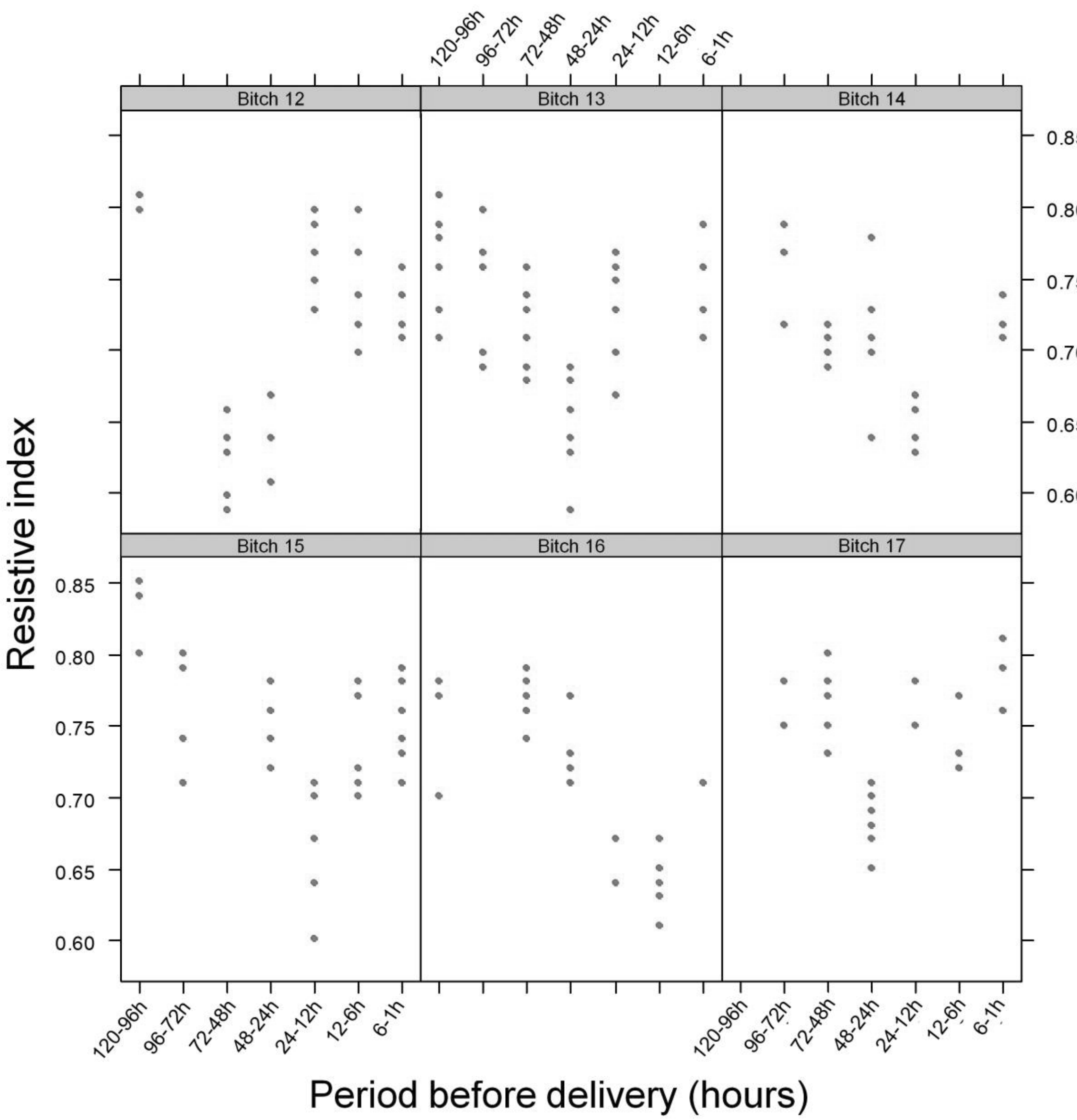

FIGURE 1.4 – Dispersion graph of average resistive index of umbilical artery in each antepartum period (hours) in six bitches from Group 2.

Table 1.5 shows the adjusted generalized linear model for the RI of the umbilical artery in Group 2. Estimates from the 120-96h pre-partum period were used as reference values for the comparison with other estimates. The estimates for the periods 96-72h, 72-48h, 48-24h and 6-1h showed significant differences in relation to the reference (120-96h pre-partum) period. It can be seen that the estimates in the pre-partum periods 96-72h, 72-48h, 48-24h the RI decreased significantly with respect to the 120-96h period. During the 6-1h period, RI increased significantly with respect to the 120-96h period. The 24-12h and 12-6h periods were not statistically different to the 120-96h period. This table also shows standard errors. The t-value is the ratio of the parameter estimate and its standard error.

TABLE 1.5 – Estimates of the parameters of normal model to the data of the resistance index of the umbilical artery in dogs in which underwent caesarian section due to fetal distress (Group 2).

Parameter	*B*	*Estimate*	*SE*	*t*	*P-value*
Intercept	β 0	0.7368	0.0110	67.1340	< 2E-16 ***
Period					
96-72h	β 1	-0.0311	0.0129	-2.4040	1.720E-02*
72-48h	β 2	-0.0318	0.0116	-2.7360	6.820E-03**
48-24h	β 3	-0.0433	0.0115	-3.7570	2.300E-04***
24-12h	β 4	-0.0198	0.0122	-1.6220	1.065E-01
12-6h	β 5	0.0091	0.0132	0.6860	4.936E-01
6-1h	β 6	0.0510	0.0128	3.9920	9.430E-05***

SE; Standard error
*** 0.001; ** 0.01; * 0.05

The comparison between the periods of Group 1 and 2, using the Kruskal-Wallis test, showed no significant differences in the RI of the umbilical artery between some periods, shown in Table 1.6. In the remaining periods there are differences between compared pre-partum periods. All puppies were born healthy; there was no mortality including those that were previously in distress.

TABLE 1.6 – Pre-partum periods were considered similar using the Kruskal-Wallis test.

Compared periods		*Kruskal-Wallis value*	*P-value*
Group 1	*Group 2*		
96-72h	24-12h	0.102	7.94E-01
72-48h	12-6h	0.652	4.193E-01
6-1h	48-24h	0.861	3.534E-01

1.4 DISCUSSION

A number of papers have shown the value of two-dimensional ultrasound analysis of umbilical artery blood flow for the assessment of fetal development and viability (NAUTRUP, 1998; ACHARYA et al., 2004; DI SALVO et al., 2006; BLANCO et al., 2011). These studies were conducted at different gestational stages but, to date, no research has demonstrated the changes in RI in the terminal phase of gestation in the days to hours before delivery.

In order to conduct a Doppler examination it is essential to understand the hemodynamics of the artery, the capabilities and limitations of Doppler ultrasound, and the parameters to be measured. In the authors' opinion, the umbilical cord is best evaluated in a transverse section in the placenta zonaria. The location of the Doppler sampling site in the umbilical cord affects the Doppler waveform and the indices are significantly higher at the fetal end of the cord than at the placental end (MAULIK, 1989; MAULIK et al., 1990). In addition to making measurements in the correct location measurements must be taken when the fetal heart rate is elevated, to obtain the most reliable RI value. There is an inverse relation between fetal heart rate and length of cardiac cycle and therefore fetal heart rate influences the configuration of the arterial Doppler waveform. When the heart rate drops, the diastolic phase of the cardiac cycle is prolonged and the end-diastolic frequency shift declines (MAULIK, 1989; MAULIK et al., 1990). Unfortunately, it was not possible to measure flow at heart rates above 200 bpm in all fetuses since those fetuses in distress had heart rates that were consistently lower than this. Thus, in this study, the flow was measured at the highest possible heart rate to minimize these potential changes in waveform.

Analysis of results from the bitches in Group 1 showed that the RI of the umbilical artery decreases within the final 24 hours before delivery (Table 1.4). This reduction becomes more evident as parturition approaches. In the 12-6h period RI remained below 0.7 (range 0.7 to 0.61), with the largest reduction occurring in the 6-1h period when the RI dropped to 0.56. These findings may assist in monitoring labor and predicting the time of delivery which may assist the breeder and veterinarian to provide optimal care.

Di Salvo et al. (2006) monitored the blood flow of the umbilical artery in canine fetuses weekly until the eighth week of pregnancy, reporting a RI of 0.75, similar to that obtained in this investigation in 120-96h period. The explanations proposed in the literature for the reduction in the RI of the umbilical artery as pregnancy advances are related to reduced vascular resistance. With the maturation of placento/fetal circulation and cardiovascular development of the fetus, peripheral vascular resistance reduces (GUZMAN et al., 1990; DILMEN et al., 1994; CHARNOCK-JONES; BURTON, 2000; COAN et al., 2004; KAUFMANN et al., 2004; DI SALVO et al., 2006; UM; ADAMSON, 2006; BLANCO et al., 2014). There is still some doubt about the influence of RI in the final phase (hours before delivery). It is not known whether the reduction in RI is purely a feature of the maturation of the maternal/fetal circulation or whether other maternal factors and the onset of labor may cause this decrease in RI.

Not all fetuses show reductions in umbilical artery RI values to 0.7 or less before 12 hours of the time of delivery (Figure 1.1). In this study this average RI values were not useful, as low RI is only a predictor of birth within 12 hours if all fetuses have an RI below 0.7. However the detection of RI below 0.7 in any fetus should alert the sonographer to the fact that RI may begin to decrease in other fetuses and that time of delivery may be approaching. Our data suggest that an RI value of less than 0.7 in all fetuses, indicates that the delivery will occur within 12 hours, as shown in Table 1.2. The variations in RI within the same uterus may correlate with fetal maturity, as fetuses of different ages may be present in the same litter (TSUTSUI, 1989; REYNAUD et al., 2005; TSUTSUI et al., 2006).

In the present study, six of 17 bitches underwent Caesarean section (Group 2) due to fetal distress in at least one fetus. Regardless of the cause of dystocia, changes in the umbilical artery RI may alert the clinician to the potential indication for Cesarean section. In all cases, the decision to refer the dog for a cesarean section was made taking into account information and opinions from the sonographer, clinical veterinarian and bitch owner. Regardless of the fetal distress in Group 2, all puppies were born healthy, indicating appropriate timing of the surgical interventions.

The lowest values of the RI in the umbilical artery occurred during the 48-24h antepartum period in bitches in Group 2 (Figure 1.3), which was statistically similar to the period 6-1h bitches in Group 1 (normal delivery). In other words, this would be the time that the bitch should have gone into labor, and when labor did not occur, there was a subsequent increase RI values above 0.7, until such time as the fetuses became distressed and were referred for cesarean delivery (Figure 1.3 and 1.4). Statistical analysis reveals that, following the significant decrease of RI, during periods 24-12h and 12-6h there is a return to values statistically similar to those observed in the period 120-96h in the same group (Table 1.5), as well as being statistically similar to values before whelping (96-72h and 72-48h) in Group 1. Furthermore, in the 6-1h period in bitches of Group 2, fetal distress resulted in a significant increase in umbilical artery RI, with values larger than those observed during the period 120-96h. Explanations for the increase in the RI of the umbilical artery in bitches of Group 2 include the changes in placental blood flow, fetal oxygenation and vascular reactivity.

For some researchers the changes of umbilical flow measured by Doppler may be the first sign of subtle reductions in perfusion of the fetal villosities (RIGANO et al., 2001). The abnormal ramifications or progressive occlusion of the vessels of the fetal villosities vascularity results in high flow resistance, which in turn causes changes in the Doppler waveform of the umbilical artery (RIGANO et al. 2001). Changes in blood flow in the umbilical artery become apparent when there is 30% reduction in vascular supply to villosities (MORROW et al., 1989). The risk of hypoxia and fetal acidosis is proportional to the severity of the umbilical artery Doppler abnormality (BILARDO et al., 1990; WEINER, 1990). Such variations in the RI of the umbilical artery were also observed in pathological canine pregnancies (bitches in which pregnancy interruption occurred between days 52 and 60, and those that had perinatal death greater than 60%) (BLANCO et al., 2011).

These findings also agree with studies in man, where sonographic examination of the umbilical artery has been shown to be useful for identification of fetuses that are at risk (ACHARYA et al., 2004; GHOSH; GUDMUNDSSON, 2009; MAULIK et al., 2011; LI et al., 2014). Moreover, fetal hypoxia results in release of several vasoactive substances that act in concert to regulate fetoplacental blood flow (WALTERS; BOURA, 1991; MACARA et al., 1993). Amongst those with pronounced vasoconstrictor properties (increasing vascular resistance) are the cathecolamines, arginine vasopressin, and angiotensin II, and release of these is particularly marked during hypoxia (HANSON, 1997). Also, an increase in umbilical artery flow resistance is commonly associated with a variety of placental lesions (GILES et al., 1985; ARABIN et al., 1992), changes that were not studied here but require histomorphological investigation. The change in RI can also be due to a reduction in fetal heart rate secondary to distress. It is known that the decrease in heart rate prolongs the diastolic phase and decreases EDV, modifying the configuration of the

Doppler waveform and the RI (MAULIK, 1989; MAULIK et al., 1990). Unfortunately, it is not possible to adjust for change in heart rate and thus, the interpretation of the increase of RI in these circumstances should be made with caution. Knowing the interactions between these possible causes, it is likely that changes in the umbilical artery blood flow have a multifactorial etiology.

Table 1.3 shows the changes of the RI in the umbilical artery in all bitches in Group 2, but it is worth remembering that decrease of RI occurred in a different pre-partum period in each bitch. This results in a larger standard deviation and a greater range of values, clearly shown in Figure 1.4. Figure 1.4 shows the changes of RI for each bitch during the periods before the fetal distress (6-1h) and subsequent Caesarean section. It is important to realize that RI begins to increase (with reference to the period 6-1h) at 48 to 6 hours before referral for Caesarean section. In other words, fetal distress may not manifest for up to 2 days after the return of RI values to above 0.7, or may begin within six hours of the increase in RI.

The limitations of this research relate to the low number of bitches in which fetal distress was seen and which were subsequently referred for Caesarean section. Despite the low number of cases, useful information was obtained. The influences of the type of dystocia, physical characteristics of the bitch and litter size need further investigation. Failure to obtain data for all periods in some bitches is a factor that must be considered, because some owners found it hard to present their bitches to the veterinary hospital at the times required.

Changes in umbilical artery blood flow may help predict the time of parturition as well as indicate potential future fetal distress. This data is most useful when analyzed in context with the previously published data on variations (oscillations) in fetal heart rate (GIL et al., 2014). Further studies are needed to determine the association between these two parameters and how to best use the information. Additionally, changes in the umbilical artery RI may indicate that the bitch may not deliver normally and that the fetuses are in early fetal distress and should be monitored carefully to ensure best outcomes.

Serial ultrasound assessments should be performed to better evaluate and interpret the changes in blood flow of the umbilical artery. The authors believe that fetuses should also be monitored by evaluating the RI of the umbilical artery and variations in heart rate every 12 hours from 72 hours before expected delivery date.

This will allow more accurate detection of early signs of fetal distress (since changes to do not occur simultaneously in all fetuses).

In conclusion, analysis of RI, through monitoring of blood flow in the umbilical artery, is useful to predict imminent delivery in bitches and the use of this index may assist in detection of early fetal distress.

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2 CHAPTER 2

ASSESSMENT OF UMBILICAL ARTERY FLOW AND FETAL HEART RATE TO PREDICT DELIVERY TIME IN BITCHES

ABSTRACT

The aim of this study was to quantitatively investigate the oscillation of the fetal heart rate (HR) in advance of normal delivery and whether this index could be used to indicate impending delivery. In addition, fetal HR oscillation and umbilical artery resistive index (RI) were correlated to determine if the combination of these parameters provided a more accurate prediction of the time of delivery. Sonographic evaluation was performed in 11 pregnant bitches to evaluate the fetal HR and umbilical artery RI at the following antepartum times: 120-96h, 72-48h, 24-12h and 12-1h. Statistical analysis indicated a correlation between the oscillation of fetal HR and the umbilical artery RI. As delivery approached a significant reduction in the umbilical artery RI was documented and greater oscillations between maximum and minimum heart rates occurred. We conclude that the quantitative analysis of fetal HR oscillations may be used to predict the time of delivery in bitches. The combination of fetal HR and umbilical artery RI together may provide more accurate predictions of time of delivery.

Key-words: dog, fetal ultrasonography, Doppler ultrasonography, resistive index, heart rate

AVALIAÇÃO DO FLUXO DE ARTÉRIA UMBILICAL E DA FREQUÊNCIA CARDÍACA FETAL PARA PREVER O MOMENTO DO PARTO EM CADELAS

RESUMO

O objetivo deste estudo foi investigar quantitativamente a oscilação da frequência cardíaca (FC) fetal antes do parto normal e se este índice pode ser utilizado para indicar a iminência do parto. Além disso, a oscilação da FC fetal e o índice de resistividade (IR) da artéria umbilical foram correlacionados para determinar se a combinação desses parâmetros pode fornecer uma previsão mais precisa do momento do parto. A avaliação ultrassonográfica foi realizada em 11 cadelas gestantes para avaliar a FC fetal e o IR da artéria umbilical nos seguintes momentos pré-parto: 120-96h, 72-48h, 24-12h e 12-1h. A análise estatística indicou uma correlação entre a oscilação da FC fetal e o IR da artéria umbilical. À medida que o parto se aproxima, é observada uma redução significativa no IR da artéria umbilical com maiores oscilações entre as frequências cardíacas máxima e mínima. Concluímos que a análise quantitativa das oscilações da FC fetal pode ser utilizada para prever o momento do parto em cadelas. A combinação da FC fetal com o IR da artéria umbilical oferece previsões mais precisas sobre este momento.

Palavras-chave: cão, ultrassonografia fetal, ultrassonografia Doppler, índice de resistividade, frequência cardíaca

2.1 INTRODUCTION

Following pregnancy diagnosis, a successful outcome requires that the neonates can survive in the extrauterine environment (INDREBØ et al., 2007; LAMM; NJAA, 2012; TØNNESSEN et al., 2012). For veterinarians, owners and breeders, the major concern when planning cesarean delivery is to maximize neonatal survival forwarding the bitch for surgery with mature fetuses (MANKTELOW; BASKERVILLE,1972). Ability to predict an appropriate time of delivery to maximize viability, before the onset of intrauterine fetal distress, would improve neonatal survival.

Ultrasonography can be used to estimate gestational age in dogs. A variety of methods have been described involving morphological evaluations, for example crown-rump length at the beginning of pregnancy and biparietal diameter, or the evaluation of fetal organogenesis by sequential examinations (ENGLAND; ALLEN 1990; KUTZLER et al., 2003; BECCAGLIA; LUVONI, 2006, LUVONI; BECCAGLIA, 2006, LAMM; MAKLOSKI, 2012). However, these ultrasonographic methods are not sufficiently accurate so as to predict the exact date of parturition, and can result in premature preparation for normal delivery or planning for caesarean delivery [BECCAGLIA; LUVONI, 2006; LUVONI; BECCAGLIA, 2006).

In a recent publication we introduced new sonographic parameters which provide more accurate estimation of the expected delivery date in dogs. These parameters include fetal heart rate (HR) acceleration and deceleration during the antepartum period (GIL et al., 2014) and changes in umbilical artery blood flow, more specifically the resistive index (RI), at the end of canine pregnancy (GIANNICO et al., 2015).

Our hypothesis is that a combination of fetal HR oscillations and umbilical artery RI could provide more accurate predictions of delivery date. Therefore, the purpose of this study was fourfold: 1. To provide quantitative definition of the oscillations in fetal HR in bitches with normal delivery; 2. To identify percentage (relative to maximum) of fetal HR variation at different times close to the delivery time; 3. To document any correlation between the fetal HR oscillations and the RI of umbilical artery flow; 4. To determine if the combination of fetal HR variation and the RI of umbilical artery flow would provide a more accurate prediction of delivery date than either parameter alone.

2.2 MATERIALS AND METHODS

The study was conducted on 11 clinically healthy pregnant bitches which went on to have a normal delivery. Several breeds were represented including Boxer (1), Chihuahua (1), English Cocker Spaniel (1), Maltese (1), Pekingese (1), Pinscher (1), Pit Bull Terrier (1), Miniature Schnauzer (1), Siberian Husky (1), X-breed (1) and Yorkshire Terrier (1). The bitches ranged from 1 to 4 years of age and delivered between 3 and 11 fetuses each. All procedures were conducted in accordance with the institutional Animal Use Committee guidelines. Inclusion criteria were bitches without concomitant disease and with a positive pregnancy diagnosis that were available for serial examinations.

Two-dimensional and Doppler ultrasonographic evaluations were carried out using a MyLab 30 machine (Esaote, Genova, Italy) with a 7.5 to 12 MHz linear multifrequency transducer (LA523 reference – Esaote, Genova, Italy). Abdominal hair was clipped to optimize the ultrasonographic image, the bitches were restrained in dorsal recumbency using a sponge trough, and acoustic gel was applied to the transducer. Image quality was maximized by adjusting the gain, focus and depth penetration for each fetus during examination.

The bitches were followed by ultrasonographic examination from the 30th day after the first mating or insemination to pregnancy diagnosis. Ultrasonography examinations were performed every four days until the eighth week of gestation, and on each examination fetal organogenesis and age was evaluated (YEAGER; CONCANNON, 1990). After the examination at the 8th week assessments were performed daily until delivery to assess umbilical artery blood flow and fetal HR.

The examination protocol included scanning the whole abdomen by circling clockwise, starting with the fetus in the left uterine horn (cranial to caudal) followed by the right horn (caudal to cranial). Measurements were made in as many fetuses as possible, and at least three fetuses from each bitch were examined at each time point.

For the Doppler parameters, a cross-sectional scan of the placenta zonaria was performed and the umbilical arteries in the mid-cord site of the free-floating umbilical cord were examined (DI SALVO et al., 2006; GIANNICO et al., 2015). Color Doppler was useful to locate the position of the umbilical arteries and the pulsed-wave Doppler sample gate was placed at their center to obtain the waveforms (DI SALVO et al., 2006). Peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured in m/s to calculate the RI, using the following equation: (PSV − EDV) ÷ PSV (Figure 2.1A). The fetal HR, in beats per minute, was recorded for three minutes using M-mode (Figure 2.1B).

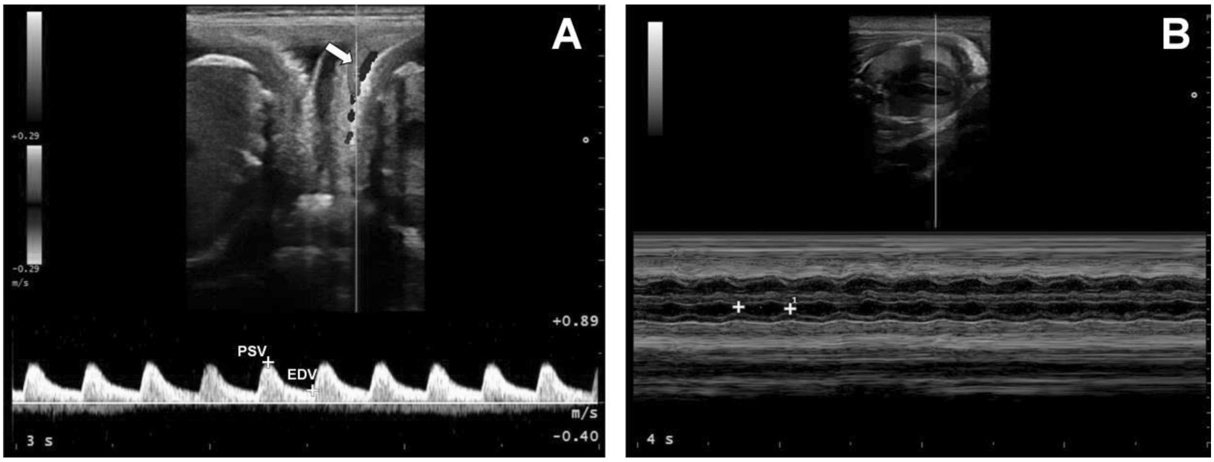


FIGURE 2.1 – Images of gestational ultrasonography in a bitch. A) Evaluation of a fetal umbilical artery. The umbilical cord is visualized by color Doppler (arrow), umbilical artery is red and umbilical vein is blue. The pulsed-wave Doppler sample volume was placed in the center of the umbilical artery to obtain the waveforms. Peak systolic velocity (PSV), 0.42 m/s; end diastolic velocity (EDV), 0.12 m/s; resistive index, 0.71. B) Representative fetal heart M-mode ultrasonography to measure the heart rate. Heart rate, 166 bpm.

For better analysis of oscillations of fetal HR, two parameters were created: the gradient between the highest and lowest HR (HR Gradient), calculated as the number of beats between the fastest and slowest recorded heart rate; and the percentage variation of HR in relation to the maximum heart rate recorded (HR Variation) (Figure 2.2).

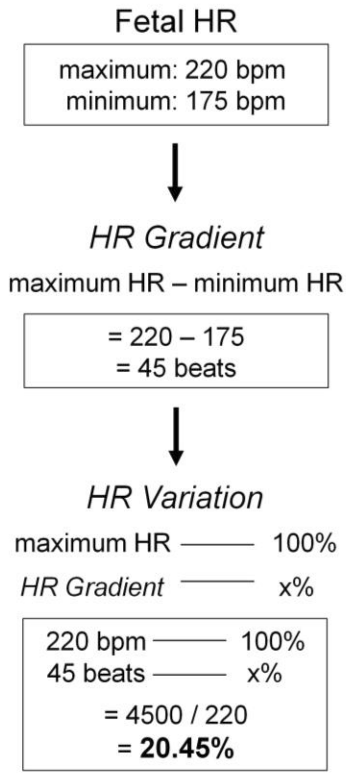


FIGURE 2.2 – Parameters for analysis of the oscillation of the fetal heart rate (HR Gradient and HR Variation) and mathematical method for such acquisitions. HR, heart rate. Bpm, beats per minute.

Antepartum time was determined after data collection and analysis, by counting backwards from the date of delivery, where time of birth was zero hours. For the purpose of analysis, data was grouped into the following periods: 120-96h, 72-48h, 24-12h and 12-1h before parturition.

Data analyses were performed using GraphPad Prism software (Version 5.0 - San Diego, CA, USA) using default settings. All data was tested for normality with the Shapiro-Wilk test. For normally distributed data, ANOVA was used to check for differences between time periods (prepartum periods), while data that were not normally distributed were analyzed with the Kruskal-Wallis nonparametric test. Linear and non-linear regression models were tested to establish the correlation between the oscillation of fetal HR and the RI of the umbilical artery flow. Both HR Gradient and HR Variation were correlated with the RI average and regression equations were constructed to correlate the RI and the HR Gradient, as well as the RI and HR Variation. A P-value of 5% was considered significant for all analyses. For the analysis of fetal HR oscillation (HR Variation), the 95% confidence interval for each pre-partum period was calculated. The sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio were calculated for the RI and

HR Variation, either isolated or in combination, to predict delivery within the following 12 hours.

2.3 RESULTS

Measurements from at least three fetuses from 11 pregnant bitches were analyzed at each time period of the study. All bitches included in this investigation had three or more fetuses. Thus, six replicates of the proposed parameters were collected for each pregnant bitch.

The umbilical artery RI values, fetal HR, HR Gradient and HR Variation in different periods, as well as the results of Kruskal-Wallis and ANOVA are reported in Table 2.1. Noteworthy in this table are the minimum and maximum values of the umbilical artery RI. For the 12-1h pre-partum period the maximum value does not exceed 0.7. As delivery approaches the minimum fetal HR decreases, resulting in a greater range between the maximum and minimum values. This is best demonstrated using the HR Gradient and HR Variation. Table 2.1 shows that these two parameters progressively increase as delivery approaches.

TABLE 2.1 – Data of resistive index (RI) of umbilical artery, fetal heart rate (HR) in beats per minute, HR Gradient in beats and HR Variation in percentage, and the P-value of the respective test applied, in 11 bitches with normal delivery assessed in hours before parturition.

Parameter	*Data*	*120-96h*	*72-48h*	*24-12h*	*12-1h*	*P-value*
RI	Minimum	0.70	0.69	0.67	0.56	<0.0001 *(Kruskal-Wallis)*
	25% Percentile	0.72	0.71	0.69	0.63	
	Median	0.74	0.73	0.71	0.67	
	75% Percentile	0.79	0.75	0.73	0.69	
	Maximum	0.85	0.83	0.82	0.70	
	Coefficient of variation	5.71%	4.64%	5.15%	5.34%	
	N	43	46	54	53	
Fetal HR (bpm)	Minimum	172	162	160	133	<0.0001 *(Kruskal-Wallis)*
	25% Percentile	210	210	184	169	
	Median	222	225	208	199	
	75% Percentile	230	236	225	216	
	Maximum	255	248	249	252	
	Coefficient of variation	6.89%	9.66%	11.58%	14.87%	
	N	48	48	54	60	
HR Gradient (beats)	Minimum	17	18	29	45	0.0097 *(ANOVA)*
	25% Percentile	27	21	41	51	
	Mean	36	50	68	66	
	75% Percentile	52	72	70	84	
	Maximum	54	76	88	106	
	Coefficient of variation	34.91%	50.78%	31.43%	27.68%	
	N	8	8	9	10	
HR Variation (%)	Minimum	7.20	7.26	13.55	19.57	0.0029 *(ANOVA)*
	25% Percentile	11.57	8.72	18.26	23.42	
	Mean	15.08	20.88	27.31	29.12	
	75% Percentile	20.95	29.81	29.30	34.50	
	Maximum	23.56	31.36	35.48	44.35	
	Coefficient of variation	34.91%	50.94%	27.77%	24.56%	
	N	8	8	9	10	

The values of N for the parameters of RI and fetal HR refer to total repetitions analyzed, and values of N for *HR Gradient* and *HR Variation* parameters are related to the mean of repetitions analyzed.

A variation of 5.7% was observed in the RI variation coefficient during the 120-96h period, which became relatively stable until the 12-1h period (Table 2.1). The coefficient of variation for fetal HR progressively increased, starting at 6.9% in 120-96h period, and reaching 14.9% in 12-1h period (Table 2.1). Figure 2.3 shows the distribution of each parameter, with a reduction in the medians of umbilical artery RI (Figure 2.3A), and an increase in the medians of both HR Gradient and HR Variation (Figure 2.3C and 2.3D) as delivery approached. This table also demonstrates that the use of the median fetal HR does not clearly illustrate the changes in HR (Figure 2.3B). Figure 2.4 shows the distribution of RI and fetal HR together in all fetuses analyzed in different bitches during the prepartum period, demonstrating a decrease in RI associated with increased fetal HR oscillation as delivery approaches.

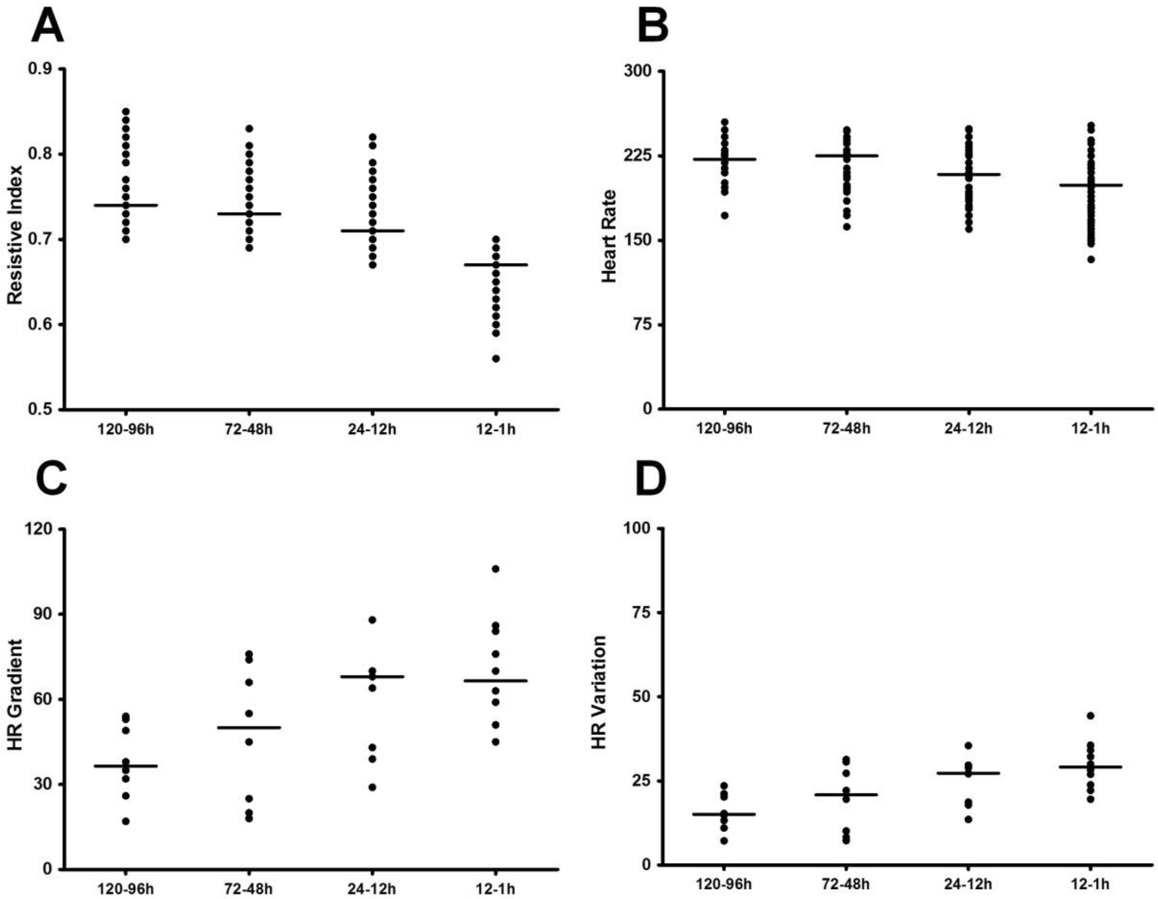


FIGURE 2.3 – Graphic of the distribution of each parameter during the antepartum period in the 11 bitches with normal delivery. A) Resistive index, showing the decrease in the median as delivery approaches. B) Fetal heart rate (in beats per minute) demonstrating that the use of the median is not useful for evaluating HR oscillation. C) HR Gradient (in beats), demonstrating the increase in the median as delivery approaches. D) HR Variation (in percentage), showing the increase in the median as delivery approaches. HR, heart rate.

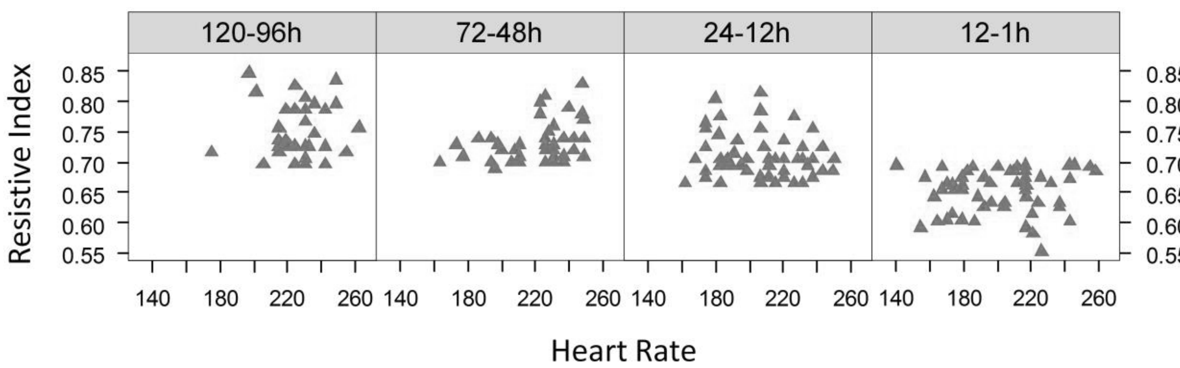


FIGURE 2.4 – Dispersion of resistive index of umbilical artery and fetal heart rate in each antepartum period in 11 bitches with normal delivery.

The 95% confidence interval of HR Variation in each of the prepartum periods is shown in Figure 2.5. This figure shows the overlap of confidence intervals on these prepartum periods. Note that in some fetuses HR Variation values greater than 24.48% may occur in the 12 hours prior to delivery, although this same percentage might indicate that the fetus is still 72-48 hours or 24-12 hours antepartum. The same finding applies to other values of HR Variation (ex. 20.44%, 27.92%). However, values higher than 30.67% in the HR Variation occurred only in the immediate prepartum period (12-1h).

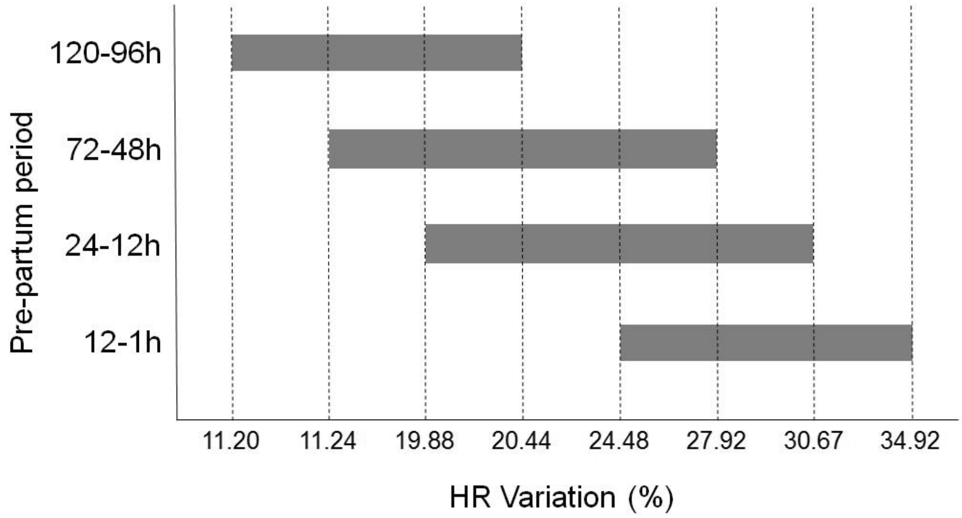


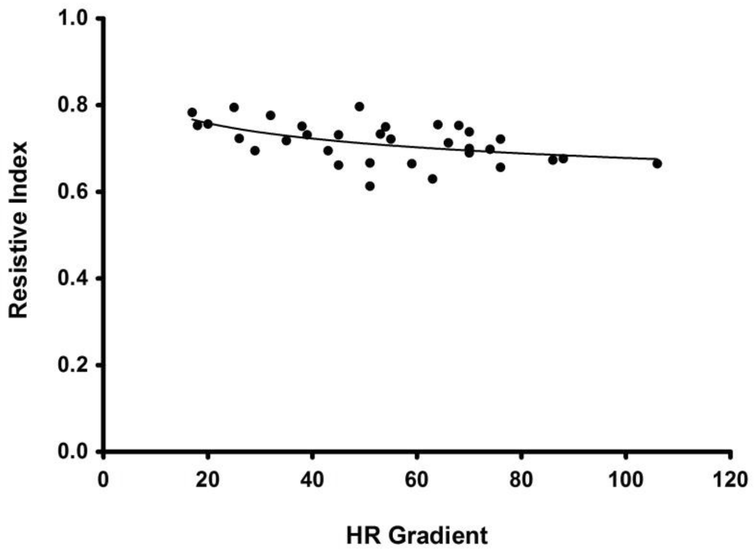
FIGURE 2.5 – Confidence interval of 95% for HR Variation. Notice the overlap of these confidence intervals in the pre-partum periods. HR, heart rate.

Sensitivity, specificity, positive predictive value, negative predictive value, and the likelihood ratio for delivery occurring within the next 12 hours were calculated using the parameters RI less than 0.7 and HR Variation of 24.48-27.92%, 27.92-30.67% and >30.6%, either alone or in combination (Table 2.2). It is clear that a RI below 0.7 has a lower sensitivity and specificity than any value of HR Variation concerning the occurrence of delivery within the next 12 hours. However, when the prediction of delivery was based on the association of HR Variation (either 27.92-30.67% or >30.67%) with a RI lower than 0.7, we documented a greater sensibility and specificity as compared to HV Variation utilized in an isolated manonener. Therefore, the association of these two parameters attained a better likelihood ratio that the expected delivery truly occurred within 12 hours following sonographic evaluation.

TABLE 2.2 – Comparison of sensibility, specificity, positive predictive value, negative predictive value, and likelihood ratio of resistive index (RI) <0.7, heart rate variation (HR Variation) 24.48 - 27.92%, 27.92 - 30.67% and >30.6%, or a combination of both in documenting delivery within the next 12 hours. 95% confidence intervals are shown for sensibility, specificity, positive predictive value, negative predictive value.

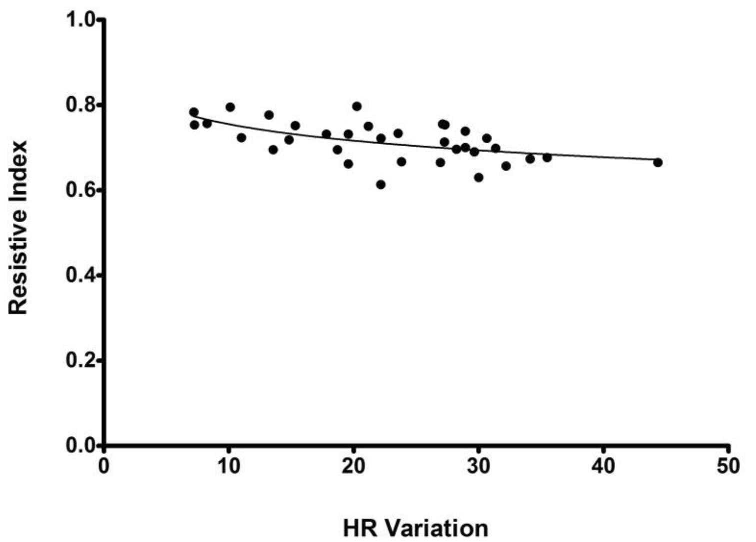
	Sensibility	*Specificity*	*Positive predictive value*	*Negative predictive value*	*Likelihood ratio*	*P (Fisher's exact test)*
RI < 0.7	0.0800 (0.0098 to 0.2603)	0.1111 (0.0028 to 0.4825)	0.2000 (0.02521 to 0.5561)	0.0417 (0.0010 to 0.2112)	0.0900	<0.0001
HR Variation 24.48 - 27.92%	0.1200 (0.0255 to 0.3122)	0.8889 (0.5175 to 0.9972)	0.7500 (0.1941 to 0.9937)	0.2667 (0.1228 to 0.4589)	1.080	1.0000
HR Variation 27.92 - 30.67%	0.1200 (0.0255 to 0.3122)	0.7778 (0.3999 to 0.9719)	0.6000 (0.1466 to 0.9473)	0.2414 (0.1030 to 0.4354)	0.5400	0.5908
HR Variation > 30.67%	0.8800 (0.6878 to 0.9745)	0.5000 (0.1871 to 0.8129)	0.8148 (0.6192 to 0.9370)	0.6250 (0.2449 to 0.9148)	1.760	0.0274
Combined RI < 0.7 and *HR Variation* 24.48 - 27.92%	0.0000 (0.0000 to 0.8419)	0.7778 (0.3999 to 0.9719)	0.0000 (0.0000 to 0.8419)	0.7778 (0.3999 to 0.9719)	0.0000	1.0000
Combined RI < 0.7 and *HR Variation* 27.92 - 30.67%	0.5000 (0.0126 to 0.9874)	0.8750 (0.4735 to 0.9968)	0.5000 (0.0126 to 0.9874)	0.8750 (0.4735 to 0.9968)	4.000	0.3778
Combined RI < 0.7 and *HR Variation* > 30.67%	0.9545 (0.7716 to 0.9988)	0.8000 (0.2836 to 0.9949)	0.9545 (0.7716 to 0.9988)	0.8000 (0.2836 to 0.9949)	4.773	0.0014

The best-fit regression equation for the correlation between the RI and the HR Gradient was documented when a logarithmic model was used, while for the RI and HR Variation correlation the best-fit was found with a semilog model, which required the log transformation of HR Variation. In both cases the best-fit provided a R>0.50. Scatterplots showing the regression models and the best-fit line are shown in Figures 2.6 and 2.7, as well as the mathematical data of the regression equations. These models indicate an opposing behavior of the medians of the RI and HR Gradient and HR Variation. In summary, as delivery time approaches the RI of the umbilical artery decreases, and both the HR Gradient (Figure 2.8A) and the HR Variation (Figure 2.8B) increase.



Model	n	Slope	Slope 95% CI	Intercept	Intercept 95% CI	R	SD residual
Log-log	34	-0.07030	-0.1113 to -0.02927	-0.02749	-0.09680 to 0.04183	0.5209	0.03953

FIGURE 2.6 – Logarithmic correlation of resistive index and HR Gradient during the antepartum periods in the 11 bitches with normal delivery. HR, heart rate; CI, confidence interval; SD, standard deviation.



Model	n	Slope	Slope 95% CI	Intercept	Intercept 95% CI	R	SD residual
Semilog	34	-0.1287	-0.1953 to -0.06199	0.8837	0.7950 to 0.9725	0.5708	0.03803

FIGURE 2.7 – Semi-logarithmic correlation of resistive index and HR Variation during the antepartum periods in the 11 bitches with normal delivery. HR, heart rate; CI, confidence interval; SD, standard deviation.

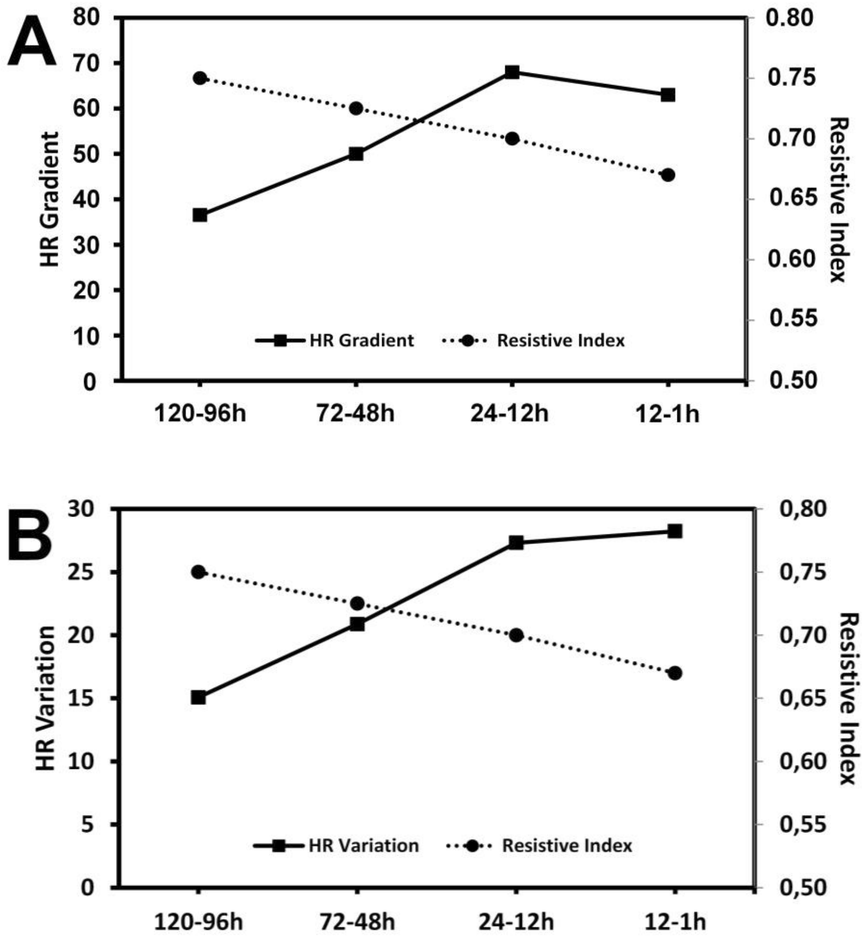


FIGURE 2.8 – Medians of the resistive index and HR Gradient (A) and the resistive index and HR Variation (B) showing the opposing trends of these parameters during the antepartum periods. HR Gradient in beats and HR Variation in percentage. HR, heart rate.

2.4 DISCUSSION

Reduction in prenatal mortality by prevention of premature delivery is one of the great challenges in obstetric veterinary medicine. In this study, we show that the combined analysis of oscillations in fetal HR and the umbilical artery RI assist in determining the time of delivery.

It is known that in normal delivery oscillation in fetal HR begins to increase five days before delivery, intensifying as delivery approaches. Such oscillations in fetal HR result in maximum HR above 200 bpm with the greatest amplitudes of acceleration and deceleration occurring as delivery approaches (GIL et al., 2014). The increasing range of variation in fetal HR is demonstrated by the coefficient of variation (Table 2.1). This finding was confirmed in this study and quantitative analysis was possible, improving the reliability of our data.

In agreement with an earlier study, we also demonstrated the reduction in the umbilical artery RI value as delivery approached (GIANNICO et al., 2015), confirming that lower values (0.7) occur in some fetuses 72 hours before delivery and in all fetuses by 12 hours before delivery. Despite the variation in the RI between the fetuses the coefficient of variation for RI was similar in each antepartum period, showing that there is no great variation among the fetuses or between the same fetus at different times of data acquisition.

To improve assessment of fetal HR oscillation two other forms of analysis (HR Gradient and HR Variation) (Figure 2.2) were created in this study. When compared to the median of fetal HR oscillation (Table 2.1, Figure 2.3), HR Gradient (and consequently HR Variation) are shown to be better parameters for determining time of delivery. It is therefore advisable to monitor HR Variation in routine pregnancy ultrasound examinations.

Figure 2.5 shows that, with a 95% confidence interval for each prepartum period, some values of HR Variation may be located in two or even three prepartum periods. Thus, the percentage of HR Variation could not be used to determine the exact prepartum phase due to overlapping of HR Variation values. However, when the HR Variation is greater than 30.67% there is a high chance of delivery occurring within 12 hours.

Despite the HR Variation, especially when greater than 30.67%, may be a valuable parameter to predict delivery, the combinations of HR Variation and RI provided more accurate estimations of the expected delivery date. The analysis of sensitivity and specificity of each parameter, either isolated or in combination (Table 2.2) clearly shows that the concomitant use of HR Variation and RI of umbilical artery results in greater accuracy to predict the correct delivery time. There is a negative correlation between parameters i.e. as delivery approaches the HR Gradient and the HR Variation increase (range of the fetal heart beats increases) and RI decreases.

Following visualization of phase 4 of the fetal intestine (GIL et al., 2015) the analysis of HR variation and the RI may be rewarding, since after this stage the normal delivery is expected to occur between 1 and 4 days. In this research, the use of these parameters allowed to determine whether the normal delivery was approaching. Previous articles comment about the necessity of serial ultrasound exams to get the correct delivery time. Nonetheless, our results point toward the ability of a single assessment, performed after the 8th week of gestation, to document the fetal HR and RI of umbilical artery as surrogates of the impending delivery (GIL et al., 2014; GIANNICO et al., 2015). Since oscillation of fetal HR occurs for many days before delivery, whilst RI values below 0.7 only occur a few hours before delivery, we recommend that the concomitant assessment of umbilical artery RI begin whenever the HR Variation becomes greater than 24% is detected. In such situation, delivery is likely to occur within 12 hours once values of 0.7 are present in all fetuses (Table 2.1, Figure 2.3A).

In both human beings and dogs the acceleration and deceleration of fetal HR, have been described as a physiological phenomena during uterine contractions (CALDEYRO-BARCIA et al., 1957; HON, 1958; SCHNETTLER et al., 2012; TRANQUILLI, 2012; GIL et al., 2014). In canine fetuses, the RI of the umbilical artery progressively decreases throughout normal gestation, with lowest values in the terminal phase of gestation (NAUTRUP, 1998; DI SALVO et al., 2006; MIRANDA; DOMINGUES, 2010; GIANNICO et al., 2015). The reduction in the umbilical artery RI as pregnancy advances is related to the reduced vascular resistance to promote perfusion of the placenta and fetal viscera. This vascular alteration occurs with the maturation of placento-fetal circulation, cardiovascular development of the fetus and other maternal factors (DI SALVO et al., 2006; DILMEN et al., 1994; CHARNOCK-JONES; BURTON, 2000; COAN et al., 2004; MU; ADAMSON, 2006; BLANCO et al., 2014).

The limitation of this study is that only a small number of bitches were included. Studies including a larger number of bitches experiencing normal delivery are required to validate the model. Additionally, further division of the prepartum periods might be helpful to produce guidelines of fetal HR variation for more specific prepartum times and the inclusion of different breeds might help to establish breed-related values if natural breed variations exist.

In conclusion the quantitative analysis of the oscillation of fetal HR may be used as an auxiliary method for estimating the delivery time in dogs. HR Variation greater than 27.92% with umbilical artery RI less than 0.7 in all fetuses indicates that delivery should occur within 12 hours. There is a correlation between fetal HR oscillation and the umbilical artery flow RI and the concomitant use of these two measurements might provide a more accurate prediction of the impending delivery in the bitch.

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3 CHAPTER 3

CANINE FETAL ECHOCARDIOGRAPHY: CORRELATIONS FOR THE ANALYSIS OF CARDIAC DIMENSIONS

ABSTRACT

The aim of this study was to develop regression models for correlation of canine fetal heart development with body size to characterize normal development or suggest cardiac anomalies. Twenty clinically healthy pregnant bitches, either brachycephalic and non-brachycephalic, were examined ultrasonographically. Transabdominal fetal echocardiography was conducted every four days from the beginning of cardiac chambers differentiation until parturition. Ten cardiac parameters were measured: length, width and diameter of the heart; heart area; left and right ventricular dimensions; left and right atrial dimensions; and aortic and pulmonary artery diameter. Femoral length, biparietal diameter and abdominal cross-sectional area were also recorded. Regression equations were developed for each parameter of fetal body size, and linear and logarithmic models were compared. The model with the highest correlation coefficient was chosen to produce equations to calculate relative dimensions based on the correlations. Only the left-ventricular chamber differed between the two racial groups. Biparietal diameter was the independent parameter that produced the highest correlation coefficient for the most fetal cardiac dimensions, although good correlations were also observed using femoral length and abdominal cross-sectional area. Heart width and heart diameter were used as surrogates of cardiac development, as these measurements showed the best statistical correlation. Quantitative evaluation of fetal cardiac structures can be used to monitor normal and abnormal cardiac development.

Key-words: fetal ultrasonography, pregnancy, gestational ultrasound, cardiac parameters, fetal heart

ECOCARDIOGRAFIA FETAL EM CÃES: CORRELAÇÕES PARA A ANÁLISE DAS DIMENSÕES CARDÍACAS

RESUMO

O objetivo deste estudo foi desenvolver modelos de regressão para correlacionar o desenvolvimento do coração fetal canino com o tamanho do corpo fetal, caracterizando o desenvolvimento normal ou sugerindo anomalias cardíacas. Vinte cadelas gestantes clinicamente saudáveis, braquicefálicas e não braquicefálicas, foram examinadas pelo exame ultrassonográfico. O ecocardiograma fetal transabdominal foi realizado a cada quatro dias desde o início da diferenciação das câmaras cardíacas até o parto. Foram mensurados dez parâmetros cardíacos: comprimento, largura e diâmetro cardíaco; área cardíaca; dimensões ventriculares esquerda e direita; dimensões do átrio esquerdo e direito; e diâmetro da aorta e tronco pulmonar. O comprimento femoral, o diâmetro biparietal e a área abdominal pelo corte transversal também foram registrados. As equações de regressão foram desenvolvidas para cada parâmetro de tamanho do corpo fetal e os modelos lineares e logarítmicos foram comparados. O modelo com o maior coeficiente de correlação foi escolhido para produzir equações e calcular as dimensões relativas. Somente a câmara ventricular esquerda diferiu entre os dois grupos raciais. O diâmetro biparietal foi o parâmetro independente que produziu o maior coeficiente de correlação para a maioria das dimensões cardíacas fetais, embora também tenham sido observadas boas correlações utilizando o comprimento femoral e a área da seção transversal abdominal. A largura do coração e o diâmetro cardíaco foram os parâmetros escolhidos para acompanhar o desenvolvimento cardíaco, pois essas medidas apresentaram a melhor correlação estatística. A avaliação quantitativa das estruturas cardíacas fetais pode ser usada para monitorar o desenvolvimento cardíaco normal e anormal.

Palavras-chave: ultrassonografia fetal, gestação, ultrassonografia gestacional, parâmetros cardíacos, coração fetal

3.1 INTRODUCTION

Sonographic measurements of fetal ultrasound parameters are the basis for detection of fetal abnormalities (DEGANI, 2001). In human medicine, biometric changes in the cardiac structure are evaluated using parameters of fetal size when congenital heart defects or cardiac growth alterations unrelated to congenital heart disease (especially intrauterine growth restriction) are suspected (RYCHIK et al., 2004; SCHNEIDER et al., 2005; LEE et al., 2008; WOOD et al., 2009).

In human fetuses the Z-score is used to quantify the degree to which an individual measurement lies above or below the mean value for a given population (SCHNEIDER et al., 2005; PETTERSEN et al., 2008; LEE et al., 2010). The quantification of growth of cardiac structures compared to overall somatic growth in the prenate can be compared to values predicted by parameters such as fetal femoral length (FL) and biparietal diameter (BPD) (DEVORE, 2005; SCHNEIDER et al., 2005; LEE et al., 2010; LI et al. 2015).

Research shows that in both dogs and human beings a relationship exists between cardiac size and body structures (GUTGESELL; REMBOLD, 1990; MORRISON et al., 1992; BATTERHAM et al., 1999; CORNELL et al., 2004; DE SIMONE; GALDERISI, 2014). Also during intrauterine life, the correlation between cardiac and non-cardiac fetal structures, both in children and human fetuses, facilitates the detection of pathological increases in cardiac dimensions (SCHNEIDER et al., 2005; LEE et al., 2010; CHUBB; SIMPSON, 2012). To date, there is no published data on indices of canine fetal cardiac size obtained by echocardiography nor equations describing relationships between these indices and fetal size in dogs. Our hypothesis is that the correlation between cardiac and non-cardiac fetal structures can be used to analyze the cardiac development in dogs. The aim of this study was to apply techniques from human medicine to develop regression equations that describe the relationship between fetal cardiac size and FL, BPD and abdominal cross-sectional area (AC) in healthy fetuses of healthy bitches. Using this information quantitative cardiac evaluation can be performed to characterize normal cardiac development.

3.2 MATERIALS AND METHODS

A prospective observational study was conducted on 20 clinically healthy pregnant bitches. Several breeds were enrolled, including brachycephalic dog breeds as English Bulldog (5), Pug (2), Pekingese (1), Chihuahua (1), French Bulldog (1) and American Staffordshire Terrier (1), and non-brachycephalic dog breeds as Schnauzer (4), Yorkshire Terrier (1), Chinese Crested (1), English Cocker Spaniel (1), X-breed (1), Siberian Husky (1). These bitches ranged from 1 to 7 years of age, weighing 2.7 to 26 kg and were carrying between 3 and 11 fetuses. Fetuses were excluded if any structural abnormality (either cardiac or non-cardiac) was detected ultrasongraphically. All procedures were conducted in accordance with the institutional Animal Use Committee guidelines.

Two-dimensional and Doppler ultrasonographic evaluations were carried out using ultrasound equipment (MyLab 30 – Esaote, Genova, Italy) with a 7.5 to 12 MHz linear multifrequency transducer (LA523 reference – Esaote, Genova, Italy). The bitches were positioned in dorsal recumbency using a sponge trough to better approach the abdomen and perform the ultrasonographic examination. Abdominal hair was clipped to optimize ultrasonographic image acquisition and acoustic gel was applied to the transducer. Image quality was maximized by adjusting the gain, focus and depth penetration for each fetus during examination.

The pregnant bitches were examined ultrasonographically twice a week throughout pregnancy, from the estimated 20th day of gestation, to determine the onset of cardiac chamber differentiation, which occurs between the 37th and 40th days of gestation (YEAGER; CONCANNON, 1990). After detection of cardiac chambers ultrasound examination was carried out every four days until parturition to evaluate and measure cardiac structures.

Evaluations were performed on as many fetuses as possible on each occasion but, evaluation was not performed if fetal positioning was sub-optimal for echocardiographic measurements. The same protocol was used to evaluate all fetuses in the bitch, and the sonographer images were acquired in a clockwise circle, starting with the fetuses of the left uterine horn cranial to caudal followed by the right uterine horn caudal to cranial.

Recordings of cross-sectional fetal echocardiographic studies were acquired using images similar to those obtained for B-mode echocardiography in dogs (Boon, 2011). A single experienced cardiologist assisted by a sonographer who was a member of the Brazilian College of Veterinary Radiology, was responsible for image acquisition throughout the study. The transducer was oriented to produce longitudinal and transversal sections of the fetus, and subtle rotations were made until images similar to those obtained in canine echocardiography could be recognized. The intrauterine fetal positioning during sonographic examination precluded the acquisition of some images and certain views on all fetuses.

For each scanned fetus, after identification of all heart chambers and the great vessels, ten cardiac parameters were measured using two-dimensional fetal echocardiography by transabdominal ultrasonography of the pregnant bitch: length, width and diameter of the heart; heart area; left and right ventricular dimensions; left and right atrial dimensions; and aortic and pulmonary artery diameter (Figure 3.1). Cardiac chambers were recorded during their largest diameter, suggesting end-diastole, aortic diameter was measured in the transverse plane of the fetal heart, and the pulmonary artery diameter was measured at the level of the valve annulus. All measurements were taken from inner-edge to inner-edge. Means were taken from three values obtained from separate frames.

Femoral length (FL) was measured in either femur, depending on fetal positioning and quality of image, from the origin to the distal end of the femoral shaft (Figure 3.2A). Biparietal diameter (BPD) was measured in the sagittal plane and the markers placed at the parietal bones symmetrically on either side of the fetal skull, with the central location of an echogenic line produced by the falx cerebri. Measurements were made from outer to outer edges of the fetal skull (Figure 3.2B). Abdominal cross-sectional area (AC) was obtained from a transverse image of the abdomen as the largest cross-section diameter of the body of the fetus at the level of the liver and stomach, where the entire abdominal circumference was contoured and the area bounded by this circumference was calculated by the ultrasound software (Figure 3.2C).

Measurements were made in as many fetuses as possible, and at least three fetuses from each bitch were examined at each time point. For any given cardiac dimension to be recorded, excellent views of the cardiac structure were required so it was essential the fetus was in the correct position. For any given cardiac dimension to be recorded, images similar to the standard echocardiographic views proposed for dogs were necessary, therefore the fetus needed to be in the correct position.

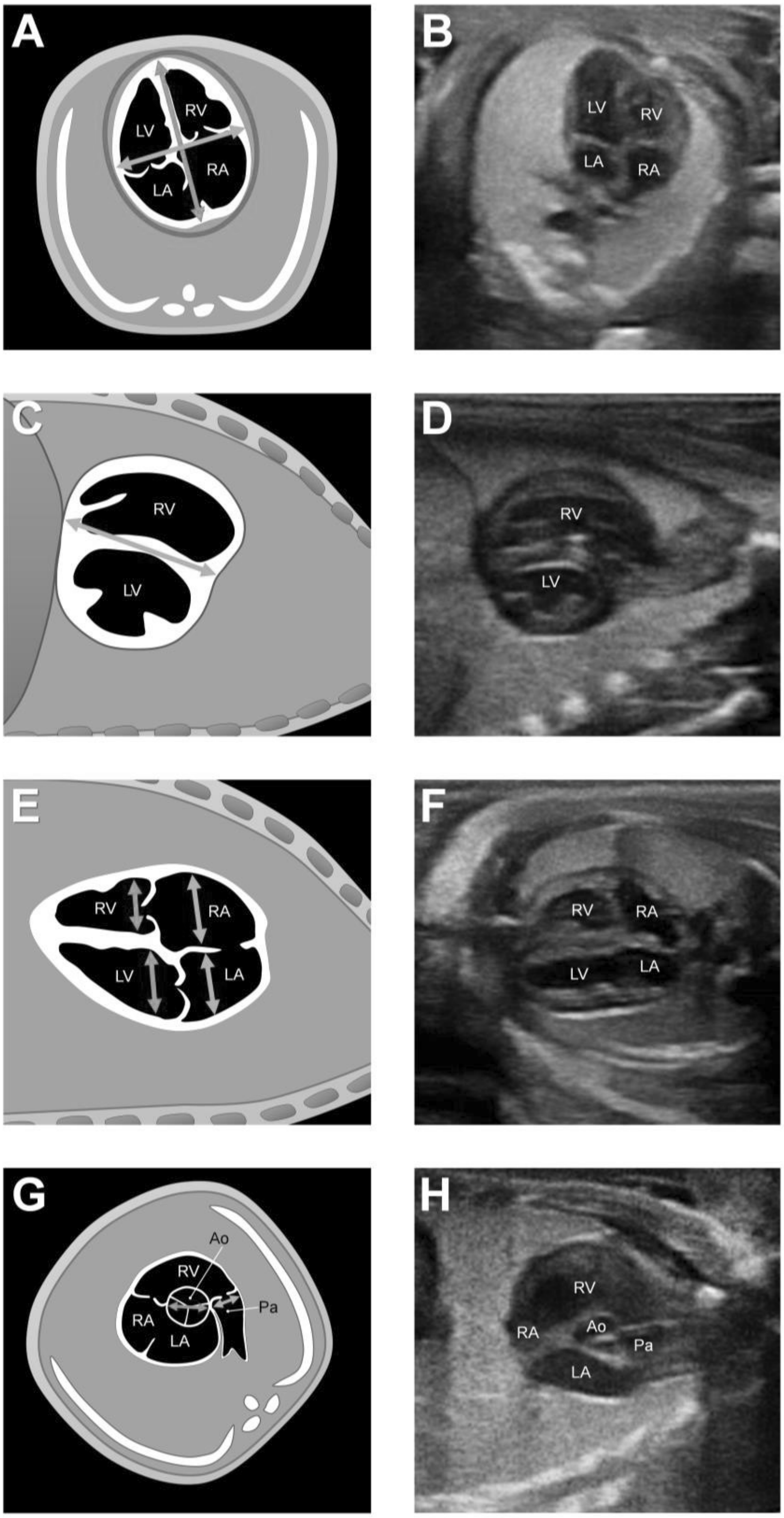

FIGURE 3.1 – Schematic drawing and its image of ultrasonographic examination of fetal cardiac structures and dimensions. A) and B) Four-chamber view showing the measurement of length (blue arrow), width (orange arrow) and cross-sectional heart area (red circle). C) and D) Short-axis view showing the measurement of heart diameter (orange arrow). E) and F) Long-axis view showing the measurement of ventricular chambers (blue arrows) and atrial chambers (orange arrows). G) and H) Short-axis view showing the measurement of aortic (blue arrow) and pulmonary artery (orange arrow) diameter. Ao, aorta; LA, left atrium; LV, left ventricle; Pa, pulmonary artery; RA, right atrium; RV, right ventricle.

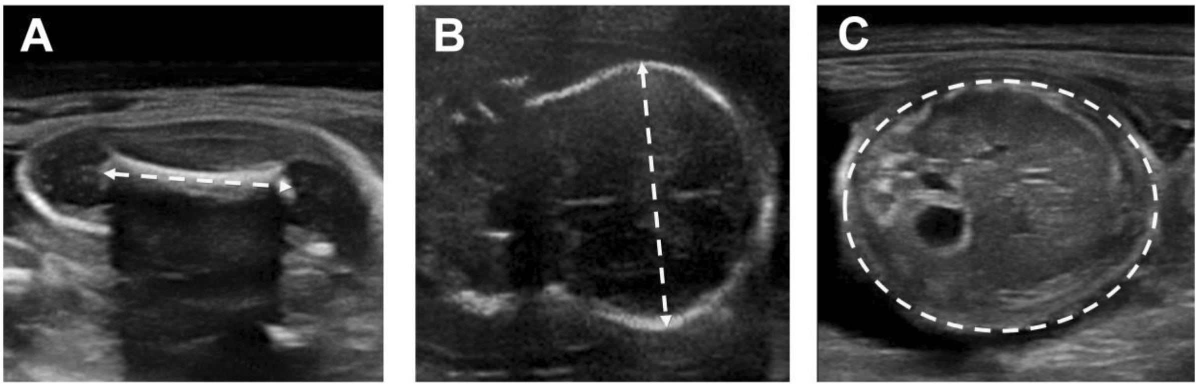

FIGURE 3.2 – Ultrasound images illustrating the performance of fetal body measurements. A) Femoral length. B) Biparietal diameter. C) Abdominal cross-sectional area.

Cardiac parameters were evaluated in 20 bitches every four days (from the formation of the heart chambers until delivery), thus a total of five serial evaluations (period of 40-44, 44-48, 48-52, 52-56, 56-60 days of gestation) were obtained, totaling a maximum of 100 measurements for cardiac parameter. However, because of fetal positioning, some parameters could not be measured in a given day, resulting in less than 100 measurements.

Regression equations were developed for each structure by relating the natural logarithm of the measured structure to the natural logarithm of the normalizing variable. After plotting the data, we compared linear and logarithmic models. In this study, the FL, BPD and AC were correlated and separate regressions were developed for each. Prior to this, an analysis was performed to check for differences between brachycephalic and non-brachycephalic dogs. When differences existed between these two populations, they were considered separately for the purpose of constructing the regression equations, whose slopes and intercepts were further compared using an analysis of covariance (ANCOVA) for the linear ones, while the extra sum-of-squares was used for the non-linear regressions. Plots of the regressions were made, together with 95% CIs, and the original data were then superimposed. All analyses were performed using the software GraphPad Prism (Version 5.0 - San Diego, CA, USA) using default settings.

3.3 RESULTS

Table 3.1 gives the results of the comparison between brachycephalic and non-brachycephalic dogs. Only the measurements of “left ventricle” attained statistical difference (P=0.0239) between groups. Therefore, separate regression equations were proposed for this parameter.

Linear and logarithmic regression models were performed for each independent variable (FL, BPD, and AC) and the model with the highest correlation coefficient, in other words, that which best described the data for each cardiac parameter, was chosen (Table 3.2 to 3.4). For the fetal cardiac dimensions (linear model), the following equation was used:

$$Y = \text{intercept} + \text{slope} \cdot X$$

For fetal cardiac dimensions (logarithmic model), the following equation was used:

$$Y = 10^{(\text{slope} \cdot \log(X) + \text{intercept})}$$

where “Y” represents the fetal cardiac parameter and “X” the independent variable (FL, BPD, or AC). The fetal cardiac dimensions and fetal body measurements (FL, BPD, or AC) are expressed in centimeters or square centimeters. The intercept and slope values for each cardiac dimension, as well as their respective 95% confidence intervals, are shown in Tables 3.2, 3.3 and 3.4 for the parameters FL, BPD and AC, respectively.

Interestingly, the comparison of non-linear regression equations for the brachycephalic and non-brachycephalic groups relating the FL and CA, respectively, with the parameter “left ventricle” showed no statistical difference between slopes (P=0.5441 and P=0.8753, respectively) and intercepts (P=0.0532 and P=0.7178, respectively). Although no statistical difference between slopes (P=0.6777) was found, when the linear regression equations relating left ventricle and the BPD were compared, a significant difference was documented between intercepts (P=0.0114). Thus, when the left ventricle is to be correlated with either FL or CA, a pooled slope and intercept can be calculated instead, therefore replacing the two group equations by an equation that treats the population as a whole (Table 3.2 and 3.4). On the contrary, the correlation of left-ventricular chamber with BPD requires the two populations to be considered separately in accordance with the breed standard (Table 3.3).

TABLE 3.1 – Means and standard deviations of the morphometric parameters obtained in brachycephalic and non-brachycephalic fetuses during pregnancy. Significant difference was attained only for the comparison of left-ventricular chamber in both groups (in bold). Medians and interquartile range is shown for parameters that did not attain a normal distribution.

Structure	*Brachycephalic breeds*			*Non-brachycephalic breeds*			*P*
	n	*Mean (Median)*	*SD (IQR)*	***n***	*Mean (Median)*	*SD (IQR)*	
Femur length	42	1.288	0.3808	35	1.394	0.4333	0.2597
Biparietal diameter	51	2.076	0.4612	42	1.990	0.4724	0.3777
Abdominal cross-sectional area	40	9.283 (7.225)	5.662 (5.105- 13.39)	33	9.185 (8.730)	4.536 (5.425- 12.25)	0.7605*
Heart length	49	1.614	0.4025	40	1.537	0.3920	0.3655
Heart width	49	1.262	0.3096	40	1.178	0.2775	0.1852
Heart diameter	48	1.064	0.2587	42	1.013	0.2433	0.3394
Heart área	42	1.673 (1.435)	0.9361 (1.040- 2.000)	36	1.581 (1.485)	0.7326 (0.9125- 2.118)	0.9441*
Left ventricle	**51**	**0.3284**	**0.0901**	**40**	**0.2855**	**0.0862**	**0.0239**
Right ventricle	51	0.3420	0.0764	40	0.3198	0.0862	0.1967
Left atrium	51	0.4375	0.1076	41	0.4073	0.1115	0.1923
Right atrium	51	0.4506	0.1209	41	0.4102	0.1112	0.1028
Pulmonary artery	33	0.3182	0.0865	33	0.3021	0.0948	0.4747
Aorta	33	0.3133	0.0868	32	0.2916	0.0958	0.3404

n represents the number of cases in which the cardiac dimension was measured; SD, standard deviation; IQR, interquartile range
P was calculated using either the Student T (for parametric data) or the Mann-Whitney test (non-parametric data)
* Non-parametric data

TABLE 3.2 – Regression equations relating cardiac dimensions and femoral length showing, in addition to the intercept and slope, confidence intervals (CI), regression coefficient (R^2) and root mean square error (RMSE). The only parameter in which a statistical difference was documented between the group of brachycephalic and non-brachycephalic dogs is shown separately at the bottom.

Structure	*n*	*Intercept*	*Intercept 95% CI*	*Slope*	*Slope 95% CI*	*RMSE*	*R^2*	*Best model*
Heart length	75	0.5755	0.3999 to 0.7511	0.7729	0.6469 to 0.8988	0.2201	0.6725	linear
Heart width	72	0.4410	0.3168 to 0.5651	0.5924	0.5041 to 0.6806	0.1516	0.7195	linear
Heart diameter	76	0.4139	0.2981 to 0.5297	0.4800	0.3974 to 0.5625	0.1429	0.6451	linear
Heart area	63	-0.1021	-0.4523 to 0.2482	1.2700	1.018 to 1.5210	0.4094	0.6261	linear
Right ventricle	76	-0.5280	-0.5470 to -0.5090	0.5259	0.4290 to 0.6229	0.0433	0.6366	logarithmic
Left atrium	77	-0.4312	-0.4555 to -0.4069	0.6067	0.4844 to 0.7290	0.0691	0.5948	logarithmic
Right atrium	77	-0.4313	-0.4552 to -0.4073	0.6580	0.5387 to 0.7772	0.0679	0.6458	logarithmic
Pulmonary artery	56	-0.5991	-0.6359 to -0.5623	0.7114	0.5392 to 0.8837	0.0566	0.6001	logarithmic
Aorta	55	0.0773	0.02232 to 0.1323	0.1656	0.1275 to 0.2036	0.0571	0.5905	linear
Left ventricle (all dogs)	76	-0.5695	-0.5977 to -0.5413	0.6192	0.4779 to 0.7605	0.0582	0.5397	logarithmic
Left ventricle (brachy)	42	-0.5510	-0.5870 to -0.5149	0.6950	0.5009 to 0.8891	0.0579	0.5989	logarithmic
Left ventricle (non-brachy)	34	-0.6036	-0.6428 to -0.5645	0.6139	0.4331 to 0.7947	0.0471	0.6289	logarithmic

n represents the number of cases in which the cardiac dimension was measured

TABLE 3.3 – Regression equations relating cardiac dimensions and biparietal diameter showing, in addition to the intercept and slope, confidence intervals (CI), regression coefficient (R^2) and root mean square error (RMSE). The only parameter that attained a statistical difference between the brachycephalic and non-brachycephalic dogs is shown at the bottom.

Structure	*n*	*Intercept*	*Intercept 95% CI*	*Slope*	*Slope 95% CI*	*RMSE*	*R^2*	*Best model*
Heart length	88	0.0141	-0.1877 to 0.2158	0.7594	0.6635 to 0.8554	0.2025	0.7428	linear
Heart width	85	0.0289	-0.1153 to 0.1731	0.5763	0.5080 to 0.6446	0.1422	0.7730	linear
Heart diameter	88	0.0504	-0.0774 to 0.1782	0.4757	0.4153 to 0.5362	0.1280	0.7406	linear
Heart area	74	-0.3489	-0.4618 to -0.2359	1.7040	1.3970 to 2.0120	0.3900	0.6703	logarithmic
Right ventricle	90	0.0246	-0.0170 to 0.0663	0.1487	0.1289 to 0.1685	0.0426	0.7174	linear
Left atrium	91	0.0101	-0.0515 to 0.0716	0.2009	0.1717 to 0.2301	0.0629	0.6776	linear
Right atrium	91	-0.0207	-0.0822 to 0.0408	0.2195	0.1903 to 0.2488	0.0628	0.7153	linear
Pulmonary artery	65	-0.0182	-0.0831 to 0.0466	0.1572	0.1266 to 0.1877	0.0551	0.6272	linear
Aorta	64	-0.0205	-0.0886 to 0.0477	0.1551	0.1229 to 0.1872	0.0578	0.5998	linear
Left ventricle (all dogs)	90	-0.0370	-0.0851 to 0.0110	0.1681	0.1453 to 0.1909	0.0490	0.7096	linear
Left ventricle (brachy)	50	-0.0287	-0.1014 to 0.0439	0.1696	0.1357 to 0.2035	0.0518	0.6784	linear
Left ventricle (non-brachy)	40	-0.0357	-0.0953 to 0.0238	0.1602	0.1313 to 0.1892	0.0421	0.7679	linear

n represents the number of cases in which the cardiac dimension was measured

TABLE 3.4 – Regression equations relating cardiac dimensions and abdominal cross-sectional area showing, in addition to the intercept and slope, confidence intervals (CI), regression coefficient (R^2) and root mean square error (RMSE). The only parameter that attained a statistical difference between brachycephalic and non-brachycephalic breeds is shown at the bottom.

Structure	*n*	*Intercept*	*Intercept 95% CI*	*Slope*	*Slope 95% CI*	*RMSE*	*R^2*	*Best model*
Heart length	72	-0.1194	0.9193 to 1.1700	0.3385	0.0438 to 0.0674	0.2414	0.6112	logarithmic
Heart width	69	-0.2735	0.6815 to 0.8158	0.3828	0.0428 to 0.0555"	0.1218	0.8268	logarithmic
Heart diameter	70	-0.3088	0.5996 to 0.7555	0.3369	0.0280 to 0.0428	0.1450	0.6393	logarithmic
Heart area	61	-0.4174	-0.5609 to -0.2740	0.6505	0.5133 to 0.7877	0.3946	0.6410	logarithmic
Right ventricle	73	-0.7773	0.2025 to 0.2539	0.3182	0.0083 to 0.0132	0.0479	0.6139	logarithmic
Left atrium	73	-0.7046	0.2382 to 0.3113	0.3499	0.0117 to 0.0186	0.0696	0.5937	logarithmic
Right atrium	73	-0.7401	0.2248 to 0.2911	0.3930	0.0144 to 0.0207"	0.0643	0.6830	logarithmic
Pulmonary artery	55	-0.9128	0.1351 to 0.2070	0.4226	0.0108 to 0.0175"	0.0583	0.6109	logarithmic
Aorta	54	-0.9431	0.1218 to 0.1979	0.4485	0.0113 to 0.0186	0.0617	0.5930	logarithmic
Left ventricle (all dogs)	90	-0.0370	-0.0851 to 0.0110	0.1681	0.1453 to 0.1909	0.0490	0.7096	logarithmic
Left ventricle (brachy)	50	-0.0287	-0.1014 to 0.0439	0.1696	0.1357 to 0.2035	0.0518	0.6784	logarithmic
Left ventricle (non-brachy)	40	-0.0357	-0.0953 to 0.0238	0.1602	0.1313 to 0.1892	0.0421	0.7679	logarithmic

n represents the number of cases in which the cardiac dimension was measured

Figure 3.3 shows sample scatterplots of the individual data points depicting the relationship of the cardiac parameters with each fetal body measurement (FL, BPD and AC), representing the regression equation that best described the data. For the sake of brevity, additional scatterplots are available in “annexes and appendices”.

All puppies were born and grew without apparent clinical signs of disease. There was no mortality either during fetal life or post partum.

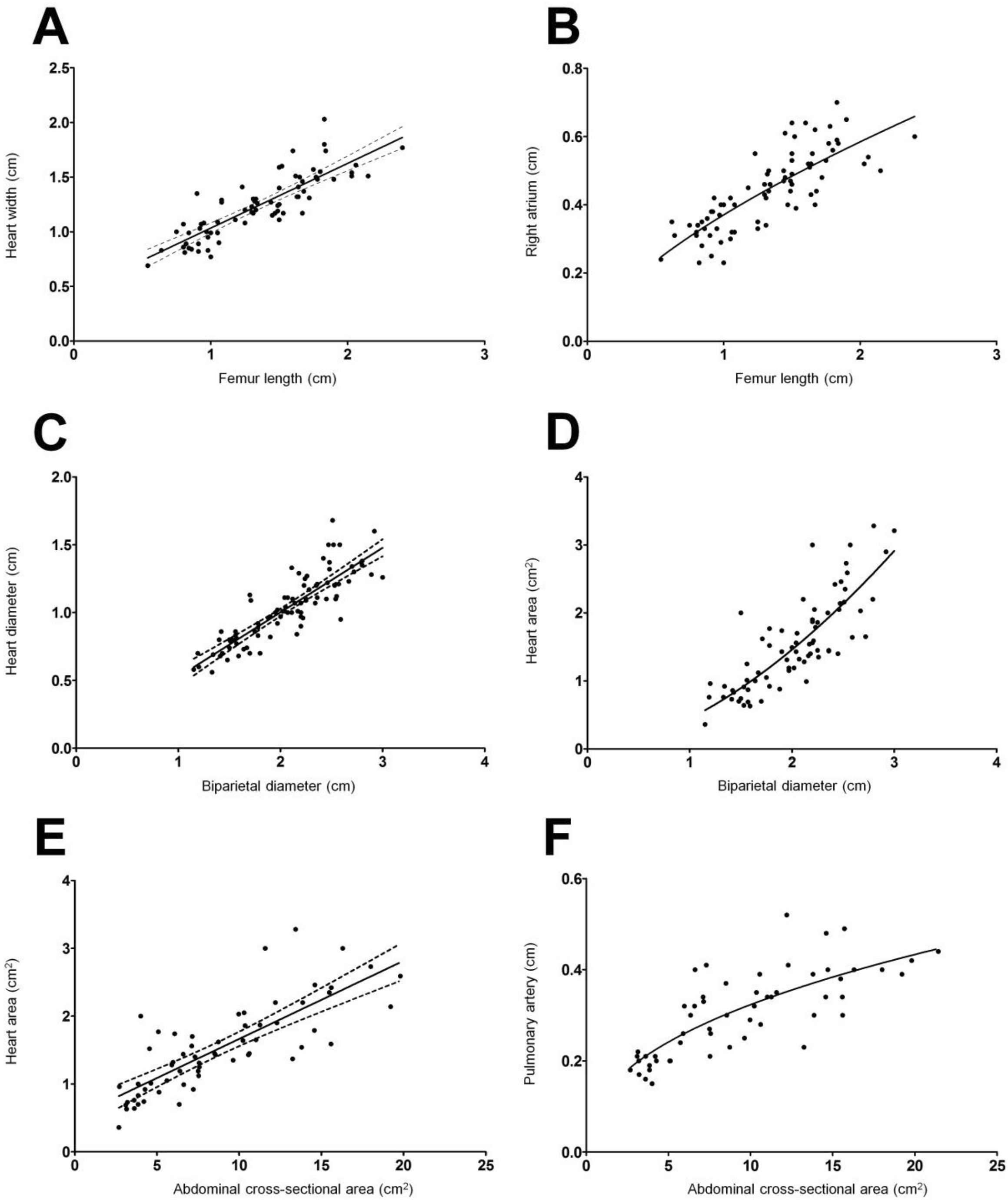

FIGURE 3.3 – Sample scatterplots showing the linear (left) and logarithmic (right) relationships between fetal body size and cardiac structures and dimensions. A) and B) Relationships between femoral length and heart width (linear) and right atrium (logarithmic). C) and D) Relationships between biparietal diameter and heart diameter (linear) and heart area (logarithmic). E) and F) Relationships between abdominal cross-sectional area and heart area (linear) and pulmonary artery diameter (logarithmic). ________, correlation average; _ _ _ _, 95% confidence interval for correlation.

Example calculating the expected range of heart size values (heart width, 95% confidence) based on BPD

For a fetus with a biparietal diameter (BPD) of 2.2 cm: the calculation of the predicted fetal heart size requires the equation, appropriate intercept and slope values suggested above (described in Table 3.3).

$$Y = \text{intercept} + \text{slope} \cdot X$$
$$\text{Heart width} = 0.0289 + 0.5763 \cdot 2.2$$
$$\text{Heart width} = 1.2968 \approx 1.3$$

In this case, a fetus with BPD of 2.2 cm should have a mean heart width of 1.3 cm.

To verify that the value of the measured heart width (as shown in Fig. 1A) is within the confidence intervals provided, we must calculate the minimum and maximum values for this heart parameter. Thus the same formula is used with the minimum and maximum values of the confidence interval of the intercept and the slope as follows:

For minimum confidence interval:

$$Y = \text{intercept} + \text{slope} \cdot X$$
$$\text{Heart width} = -0.1153 + 0.5080 \cdot 2.2$$
$$\text{Heart width} = 1.0023 \approx 1$$

For maximum confidence interval:

$$Y = \text{intercept} + \text{slope} \cdot X$$
$$\text{Heart width} = 0.1731 + 0.6446 \cdot 2.2$$
$$\text{Heart width} = 1.5912 \approx 1.6$$

In this case, the measured value of the heart width should be between 1 and 1.6 cm (95% confidence interval).

3.4 DISCUSSION

This study shows, for the first time in veterinary medicine, ten correlations between dimensions of fetal cardiac structures and measurements of fetal size (FL, BPD and AC) made using transabdominal fetal ultrasonography. The analysis of correlations between these basic parameters show us the results of normal hearts, but we can hypothesize that, by extrapolation from human studies, these parameters might be used to detect possible fetal cardiac abnormalities.

In medicine, it is known that abnormal fetal heart size may suggest cardiac abnormalities, both congenital and secondary to maternal systemic diseases (CHAOUI et al., 1994; WUTTIKONSAMMAKIT et al., 2011). We believe that it occurs in dogs, and identifying an increased fetal heart would potentially indicate, still in intrauterine life, either a congenital heart disease or systemic diseases of the bitch, including endocrine and infectious diseases. Also, little information exists regarding the fading puppy syndrome, which is characterized by apparently healthy born puppies that usually die in the first days due (BLUNDEN, 1986; BLUNDEN, 1998). For many of these puppies no obvious cause of death is found, and causes such as crushing by the mother, infection, congenital anomalies, premature birth and parasites can be an explanation (BLUNDEN, 1998). Perhaps fetal echocardiography could clarify some cases of puppies presenting the fading puppy syndrome. Also, the identification of cardiac abnormalities could help the clinician to determine treatment strategies for puppies after birth, including surgical correction.

There are many statistical methods for studying the association between two or more variables, of which regression and correlation is in widespread use, and this method has been used to develop the normal ranges for many fetal variables (ROYSTON; WRIGHT, 1998; SILVERWOOD; COLE, 2007). In this study we used linear and logarithmic regression models and selected the one that best represented the data from each cardiac parameter. For BPD this was the linear model. Thus, for ease of analysis, this measure of fetal size proved to be the most straightforward of the three variables analyzed in this study.

Linear and logarithmic models have also been used in previous studies in human fetuses and children but other groups have described different models (polynomial cubic or quadratic) that best described the relationship between human fetal heart dimensions and fetal size (HATA et al., 1997; DAUBENEY et al., 1999; TRAISRISILP et al., 2001; LEE et al., 2010; LUEWAN et al., 2011; LI et al., 2015).

These discrepancies between studies may result from the different methods used, and the inclusion of individuals from different species and populations.

For accurate data interpretation the correlation coefficient (R^2) and root mean square error (RMSE) must be taken into consideration. The higher the value of R^2, the stronger the association (EVANS, 1996). The RMSE is a measure often used to describe the differences between values predicted by a model and the values actually observed. Basically, the RMSE is the sample standard deviation, and indicates the magnitudes of errors in estimates (HYNDMAN; KOEHLER, 2006). Thus, a good correlation parameter for fetal heart dimensions must provide a high value of R^2 and low value of RMSE.

In this investigation we divided the bitches into two groups in accordance with the breed standards. It is known that brachycephalic dogs have different body conformation, and this factor could alter our heart analysis since the correlation included body structures. Among the ten cardiac parameters that we studied, only one (left ventricle) was different between these two groups. Nevertheless, the analysis of the regression equations showed that only BPD required the use of specific equations for brachycephalic and non-brachycephalic breeds. Except for the left ventricle, all other cardiac structures behaved similarly in these two groups.

BPD was the independent variable that produced the highest correlation coefficient with the majority of fetal cardiac dimensions, although good correlations were also observed using FL and AC. In addition, BPD was preferred as this was the measurement of fetal size for which the linear model produced the best data fit for most parameters (since the formula for calculation was simpler). For this reason measurement of BPD seems most appropriate for correlation with the development of fetal cardiac structures. In addition, BDP is routinely measured in pregnancy ultrasound (YEAGER et al., 1992; LUVONI; GRIONI, 2000).

Cardiac parameters that showed the highest R^2 and lower RMSE with BPD were heart width (R^2=0.7730 and RMSE=0.1422) and heart diameter (R^2=0.7406 and RMSE=0.1280). We suggest that these two cardiac parameters should be added to routine ultrasonographic pregnancy examination, and correlated to fetal size since these two parameters have suitable values for R^2 and RMSE, and are cardiac parameters that can be measured either by echocardiographers or sonographers.

This paper demonstrates how to calculate the size of certain heart structures and the confidence interval for this calculation. Values outwith the confidence intervals may suggest cardiac anomalies that should be monitored. In this study we were able to characterize the relationship between an observed value and a reference standard, which contributes to the interpretation of possible cardiac abnormalities. Based on these results, correlations can be calculated from a measurement of fetal size and fetal echocardiographic parameters.

One limitation of this study is that our data did not demonstrate whether these correlations could be used to detect fetal cardiac abnormalities. In addition, we made the assumption that the FL, BPD and AC are normal based on the clinical aspect to the birth and development of the puppy, and we could not confirm that it would not be altered by or in association with fetal cardiac disease. For this, all this information should be validated and tested in clinical cases. Furthermore, we did not study the intra-observer variability and within day variability, which could possibly interfere with results. Therefore, further research is required to validate this new methodology.

This study proposes a mathematical and quantitative evaluation of the growth of fetal cardiac structures in dogs. Although future studies are needed to validate the method, so that these data can be used to benchmark predicted outcomes, some of the parameters discussed here are simple to measure, and can easily be incorporated into routine fetal sonographic scans. These examinations can provide more information about the normal and abnormal development of canine hearts. Finally, this study may be a cornerstone for future echocardiographic studies aimed at investigating fetal cardiac development in veterinary patients.

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4 CHAPTER 4

EVALUATION OF CARDIAC FUNCTION IN CANINE FETUSES

ABSTRACT

The objective of this study was to describe the cardiac function values of canine fetuses, demonstrating the course of the parameters of systolic, diastolic and global cardiac function. These parameters were also analyzed to verify a possible relation with the moment of delivery. Twenty bitches with a total of 70 fetuses were evaluated, and a fetal echocardiogram was performed every four days, starting at 40 days of gestation and ending at delivery, representing a total of five periods. Parameters such as mitral atrioventricular inflow, the outflow tracts, fractional shortening, myocardial performance index and cardiac output were assessed, and their evolution in the five periods was documented. The ROC curve and cut-off values showed that the E-wave and E/A ratio, parameters of diastolic function, are the echocardiographic parameters in fetuses that best corelate with the time of delivery. This research adds novel data to the studies in canine fetal cardiology and can also contribute to fetal cardiac evaluation for the diagnosis, monitoring or prediction of various fetal conditions and cardiac diseases.

Key-words: canine, fetal ultrasonography, fetal echocardiography, Doppler ultrasonography, pregnancy

AVALIAÇÃO DA FUNÇÃO CARDÍACA EM FETOS CANINOS

RESUMO

O objetivo deste estudo foi descrever valores de função cardíaca de fetos caninos, demonstrando o curso dos parâmetros da função sistólica, diastólica e global durante a gestação. Esses parâmetros também foram analisados para verificar uma possível relação com o momento do parto. Foram avaliadas vinte cadelas e 70 fetos. O exame ecocardiográfico fetal foi realizado a cada quatro dias, com início aos 40 dias de gestação e término no momento do parto, representando um total de cinco períodos. Foram avaliados parâmetros como o fluxo atrioventricular da valva mitral, vias de saída (fluxo aórtico e pulmonar), fração de encurtamento, índice de desempenho miocárdico e débito cardíaco, com posterior análise da evolução de todos os parâmetros nos cinco períodos pré-parto. A curva ROC e os valores de corte revelaram que a onda E e a relação E/A, parâmetros de função diastólica, são os parâmetros ecocardiográficos principais da análise da função cardíaca fetal que melhor se correlacionam ao momento do parto. Esta pesquisa colabora com os estudos em cardiologia fetal canina e também pode contribuir para a avaliação cardíaca fetal para o diagnóstico, monitoramento ou prognóstico em várias condições fetais e doenças cardíacas.

Palavras-chave: cães, ultrassonografia fetal, ecocardiografia fetal, ultrassonografia Doppler, gestação

4.1 INTRODUCTION

Human fetal echocardiography has been developed in order to evaluate cardiac abnormality in both structure and function, especially with the use of pulsed Doppler in the assessment of fetal hemodynamics (VAN MIEGHEM et al., 2009a). The major focus of fetal echocardiography has been the identification of congenital heart defects but, recently, the evaluation of cardiac function has drawn even more attention in medicine (DEVORE, 2005; MÄKIKALLIO et al., 2005; RUSSELL; McAULIFFE, 2008; VAN MIEGHEM et al., 2009a).

A number of fetal diseases, as well as structural defects, may alter the hemodynamics of the atrial and/or ventricular chambers, resulting in changes in chamber size, shape, contractility, filling properties, stroke volume and cardiac output (DEVORE, 2005). Fetal cardiac function assessment has been proposed in medicine, and some grade of dysfunction may be due to an intrinsic myocardial disease or to a secondary adaptive mechanism, which could be helpful in the diagnosis and monitoring of several systemic fetal conditions. In human fetuses, cardiac function may be a marker of disease severity and prognosis of a number of prenatal conditions, such as intrauterine growth restriction, sacrococcygeal teratomas, congenital heart defects, hydrops, fetal anemia and fetal arrhythmia (VEILLE; COVITZ, 1994; HOFSTAETTER et al., 2006; CRISPI et al., 2008; GARDINER et al., 2008; WILSON et al., 2008).

The heart, when facing several diseases, primary or secondary to systemic alterations, responds with changes in systolic function, diastolic function or both. This can result in modulation of the primary function of the heart, which is to eject blood to provide adequate perfusion of the organs (Guyton and Hall, 2006). The role of the heart is to contract its muscular walls with sufficient pressure to eject blood into the aorta/pulmonary artery (systole). Immediately after this event, there is an adequate filling of the ventricles from the atria (diastole) (GUYTON; HALL, 2006, BIJNENS et al., 2012).

To maintain normal cardiac function, both phases (systole and diastole) must be preserved and need to occur in a synchronized manner (CRISPI; GRATACÓS, 2012). This specific cardiac function can be measured by means of previously described parameters, the most important being fractional shortening and the flow from ventricular outflow tract (to assess systolic function), mitral atrioventricular inflow (E-wave, A-wave and E/A ratio) to assess diastolic function, and myocardial performance index (MPI) as a global function index. In addition, cardiac output can also be evaluated (BOON, 2011).

In veterinary medicine, little is known about fetal echocardiography and only one study in the area has been published so far (GIANNICO et al., 2016b). There is no published data about fetal cardiac function. In this article, we documented for the first time values of cardiac function of canine fetuses. Special focus was given to the atrioventricular mitral inflow, the outflow tracts, the MPI and cardiac output. Finally, we describe some parameters that aid in determining the proximity of delivery.

4.2 MATERIALS AND METHODS

Twenty clinically healthy (primiparous or pluriparous) pregnant bitches, of different breeds (5 English Bulldog, 4 Schnauzer, 2 Pug, 1 Pekingese, 1 Chihuahua, 1 American Staffordshire Terrier, 1 Chinese Crested, 1 English Cocker Spaniel, 1 French Bulldog, 1 Siberian Husky, 1 X-breed, 1 Yorkshire Terrier) ranging from 2 to 7 years of age where evaluated in this study. Two-dimensional and Doppler ultrasonographic evaluations were performed by a cardiologist assisted by a sonographer, with bitches in dorsal recumbency using ultrasonographic equipment (MyLab 30 – Esaote, Genova, Italy) with a 7.5 to 12 MHz linear multifrequency transducer (LA523 reference – Esaote, Genova, Italy).

After determining cardiac chamber differentiation, which occurs between the 37th and 40th days of gestation (YEAGER; CONCANNON, 1990), ultrasound examination was carried out every four days until parturition to evaluate and measure cardiac parameters. Thus, a total of consecutive serial evaluations (period of 41–44, 45–48, 49–52, 53–56, 57–60 days of gestation) were obtained. For this study, only fetal cardiac evaluation was assessed in these periods. Analyses were performed on as many fetuses as possible, since echocardiographic measurements require correct positioning. Recordings of cross-sectional fetal echocardiographic were acquired as suggested by Giannico et al. (2016b).

The echocardiographic parameters were obtained in their respective views, and videos and images were saved in DICOM format for further analysis. The duration of the ultrasound examination varied according to the amount of fetuses, and the approximate time of evaluation in each fetus was five minutes. For each fetal heart evaluated, parameters of systolic, diastolic and global function were acquired and are discussed in detail below.

For statistical analysis, all the parameters of systolic, diastolic and global function were analyzed at the prepartum periods and their minimum and maximum values, interquartile range (IQR), median and mean, as well as standard deviation (SD) and coefficient of variation (CV) for each of these periods. Nonparametric data were analyzed using the Krukall-Wallis test, followed by the post hoc Dunn's test. Data with normal distribution was analyzed by ANOVA (analysis of variance), followed by the Tukey post-test. A P-value lower than 0.05 was considered significant.

The parameters that showed difference in analysis of variance or Kruskal-Wallis over the three weeks prior to delivery were posteriorly used to analyse the sensitivity and specificity of fetal echocardiographic parameters in predicting delivery. Receiver operating characteristic (ROC) curves were calculated from fetal echocardiographic data to differentiate bitches in which delivery occurred within the next 4 days from those that needed more than 4 days (up to 20 days) to start delivery. Based on this last analysis and considering the fetal echocardiographic parameters that presented area under the curve (AUC) greater than 0.75 of the ROC curve, the cut-off values with the best combination of sensitivity and specificity for predicting delivery within 4 days where determined.

4.2.1 Systolic function

One of the parameters for systolic function is left ventricular contraction. For this measurement, the diameter of the left ventricular cavity during diastole (LVd) and during systole (LVs) were acquired in centimeters, and fractional shortening (FS%) was calculated by means of the formula (LVd – LVs) ÷ LVd and shown as a percentage value. These two parameters were acquired through short-axis view at the level of the papillary muscles, being the left ventricular cavity measured from the interventricular septum to the free wall in its largest diameter observed (thus, considering the diastole) and, similarly, the left ventricular cavity in its smaller diameter (thus, considering the systole). Figure 4.1 illustrates this measurement.

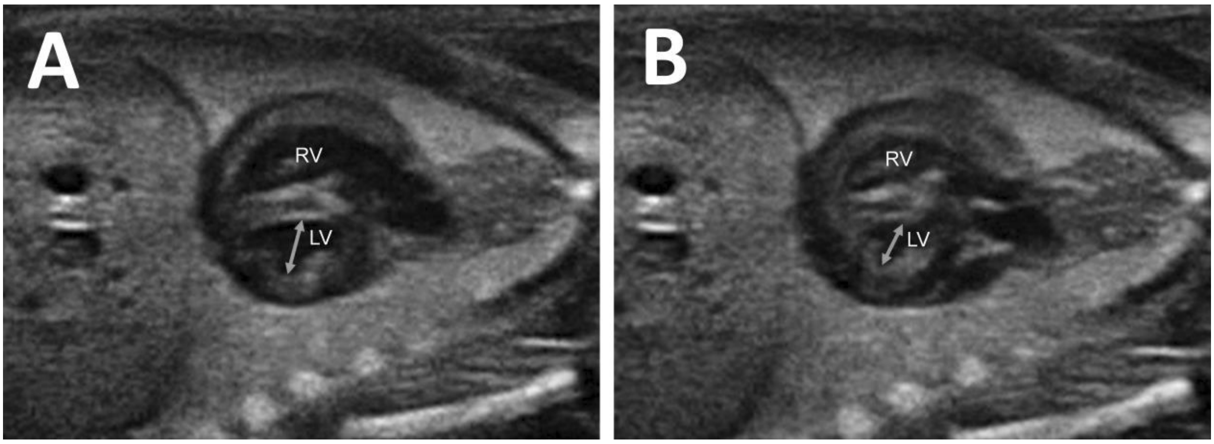

FIGURE 4.1 – Images of ultrasonographic examination of a fetal heart. This image is a short-axis view at the level of the papillary muscles and shows the measurement of the left ventricular cavity from the interventricular septum to the free wall to analyze the systolic function of the left ventricle by fractional shortening (FS%). A) Diameter of the ventricular cavity during diastole (LVd, orange arrow). B) Diameter of the ventricular cavity during systole (LVs, green arrow). LV, left ventricle; RV, right ventricle.

In addition to this data, the parameters of the outflow tracts were acquired and quantified in cm/s. The flow velocity in the outflow tract of the left ventricle (aortic flow; Ao Vmax) was measured at the longitudinal five chambers view (Figure 4.2A and 4.2B). The flow velocity in the outflow tract of the right ventricle (pulmonary flow, P Vmax) was acquired by cross-section at the level of the papillary muscles, at the time of opening of the pulmonary valve (Figure 4.2C and 4.2D).

The last two parameters to assess systolic function were the isovolumic contraction time (IVCT) and left ventricular ejection time (LVET). For this measurement, the longitudinal five chambers view was acquired, and the Doppler sample volume was placed in order to include both the lateral wall of the ascending aorta and the mitral valve, as shown in Figure 4.2G. These parameters are best explained in the schematic drawing of Figure 4.3 and the waves formed in this view are shown in Figure 4.2H and Figure 4.3.

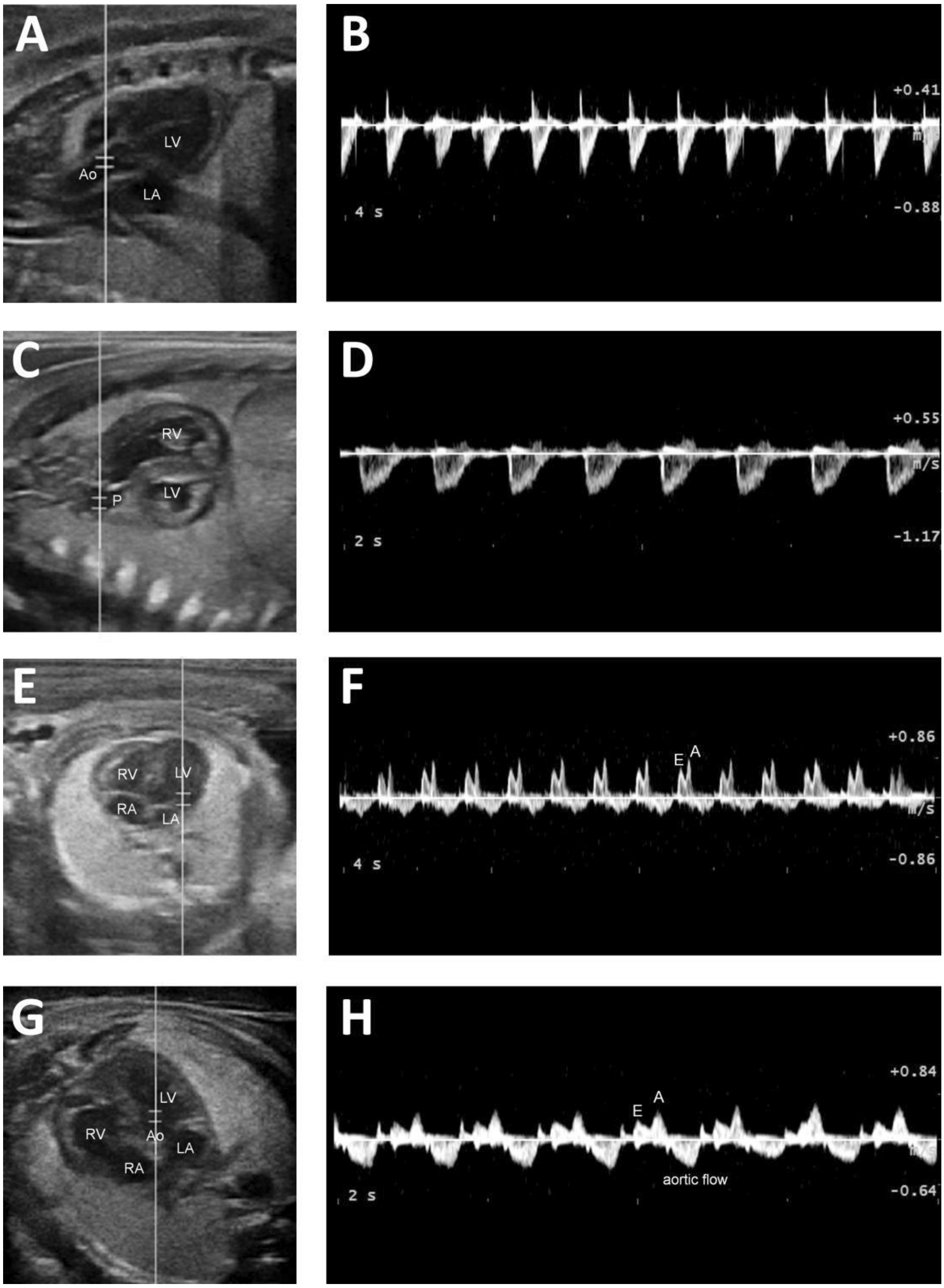

FIGURE 4.2 – Images of ultrasonographic examination of fetal heart and the respective views to acquire the transvalvular flows. A) and B) Longitudinal apical five chambers view (the Doppler sample volume positioned under the valve) and the flow in the outflow tract of left ventricle (aortic flow; Ao Vmax). C) and D) Short-axis view at the level of the papillary muscles, at the time of opening of the pulmonary valve (the Doppler sample volume positioned under the valve) and the flow in the outflow tract of right ventricle (pulmonary flow, P Vmax). E) and F) Longitudinal four chambers view (the Doppler sample volume positioned at the opening of mitral valve) and the flow of inflow tract of left ventricle, forming the E-wave and A-wave. G) and H) Longitudinal apical five chambers view (the Doppler sample volume positioned between lateral wall of the ascending aorta and the mitral valve) and the flow formed by E-wave and A-wave and the aortic flow. Ao, aorta; LA, left atrium; LV, left ventricle; P, pulmonary artery; RA, right atrium; RV, right ventricle.

4.2.2 Diastolic function

To estimate the diastolic fetal function, the parameters of the inflow tract (E-wave and A-wave of mitral flow, and E/A ratio) were analyzed. In order to do this, a longitudinal four chambers view was acquired, with the cursor of the pulsed Doppler positioned at the opening of the mitral valve (Figure 4.2E). Figure 4.2F shows the two waves evaluated. In addition, the isovolumic relaxation time (IVRT), another parameter of diastolic function, was also obtained. For the IVRT, the same longitudinal apical five chambers view previously shown was acquired (Figure 2G). The waves formed in this view are shown in Figure 4.2H and Figure 4.3, where the IVRT is explained in detail.

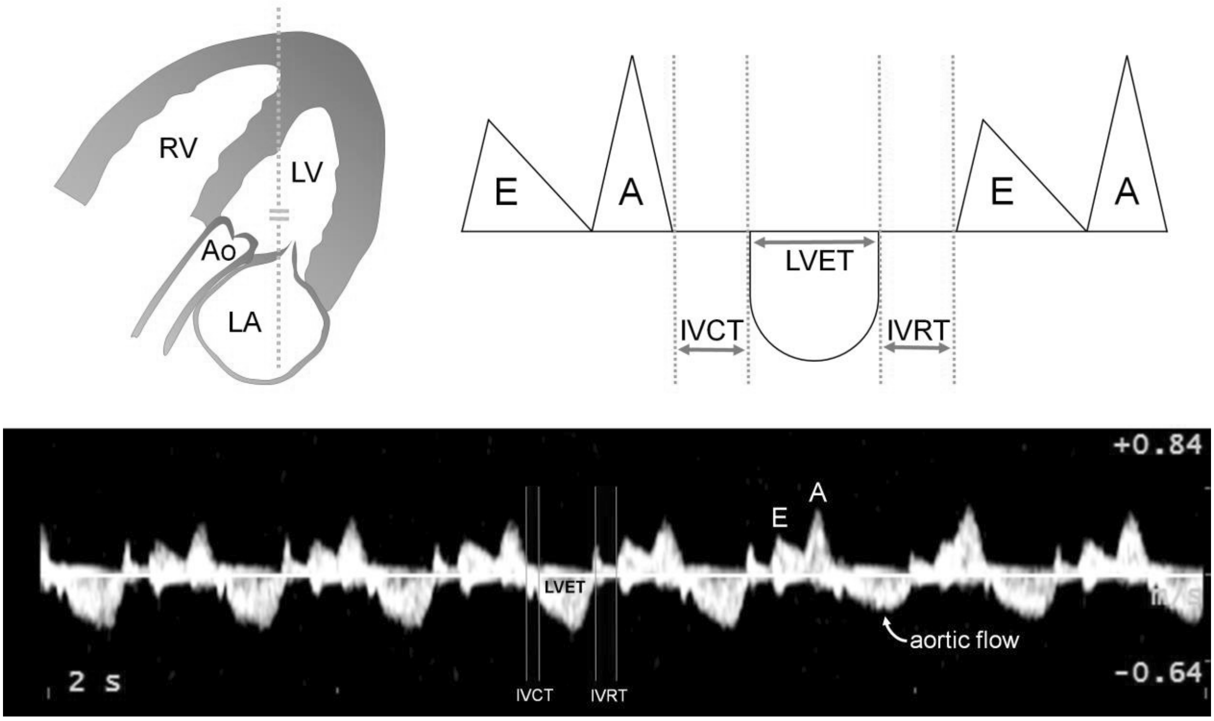

FIGURE 4.3 – Schematic drawing with the acquired flow for the measurement of myocardial performance index (MPI). The schematic drawing of the longitudinal apical five chambers view in the upper left corner demonstrates the positioning of the Doppler sample volume (between the outflow and inflow tract of the left ventricle to obtain the aortic and mitral flow simultaneously). In the upper right corner there is a schematic drawing of the flow formed demonstrating the positive E-wave and A-wave and negative aortic flow. In this same scheme and in the flow located at the bottom of the image there is the demonstration of the place of measurement of the three parameters necessary to calculate the MPI (isovolumic contraction time, IVCT; left ventricular ejection time, LVET; isovolumic relaxation time, IVRT).

4.2.3 Global cardiac function

One of the analyzed parameters that reveals global cardiac function is cardiac output. To estimate it, other parameters were measured. The time-velocity integral (TVI) from the aorta or pulmonary outflows was multiplied by the respective vessel area (aorta or pulmonary artery) to estimate the stroke volume (SV). Left and right SV multiplied by the heart rate (HR) yield the right cardiac output (RCO) and left cardiac output (LCO) which, together, provided the combined cardiac output (CCO). The TVI was acquired by the wave formed by the outflow tract of the left and right ventricle, as explained above and shown in Figure 4.2A, 4.2B, 4.2C and 4.2D. In these same cardiac views, the diameter of aortic and pulmonary valve were acquired. Finally, to obtain the value of the area of the vessel, these diameters were used in the following formula: $\pi \cdot (\text{diameter} \div 2)^2$. Table 4.1 summarizes the data obtained for the CCO estimative and formulas used.

TABLE 4.1 – Summary of parameters for the estimation of combined cardiac output of canine fetuses with abbreviations and formulas.

Abbreviation	*Parameter*	*Formula*
TVI	time-velocity integral	
Ao area	Aorta area	$\pi \cdot (\text{aortic diameter} \div 2)^2$
P area	Pulmonary area	$\pi \cdot (\text{pulmonary diameter} \div 2)^2$
Left SV	Left Stroke Volume	TVI aorta · Ao area
Right SV	Right Stroke Volume	TVI pulmonary · P area
LCO	Left Cardiac Output	Left SV · HR
RCO	Right Cardiac Output	Right SV · HR
CCO	Combined Cardiac Output	LCO + RCO

HR, heart rate

Another way to assess global cardiac function is by means of the myocardial performance index (MPI). The image for the measurement of the parameters used for MPI of the left ventricle was performed in the apical five chambers view, as previously described (Figure 4.2G). Images used for measuring the MPI were obtained by placing the sample volume at the outflow tract of the left ventricle, to obtain the aortic and mitral flow simultaneously (Figure 4.2H). To calculate this index, three parameters were measured: IVCT, IVRT and LVET (Figure 4.3). The MPI is given by the formula (IVCT + IVRT) ÷ LVET.

4.3 RESULTS

All puppies of all bitches were born healthy, either by normal delivery or cesarean section remained alive and developed appropriatley. The number of fetuses evaluated in each bitch depended on the number of fetuses held during the gestational period. Pregnancies of one, two and three fetuses were all evaluated, and in bitches with more than four fetuses, at least three fetuses were evaluated. Also, the echocardiographic data of cardiac function assessed varied in each period by the number of fetuses correctly positioned for the acquisition of a determined parameter. In this study, pregnant bitches gave birth to a total of 70 fetuses, but the number of fetuses with a given echocardiographic parameter may vary due to these reasons.

We denominate the pre-delivery periods analyzed as follows: Period 5 (41–44 days of gestation or 20–17 days for delivery); Period 4 (45–48 days of gestation or 16–13 days for delivery); Period 3 (49–52 days of gestation or 12–9 days for delivery); Period 2 (53–56 days of gestation or 8–5 days for delivery); Period 1 (57–60 days of gestation or 4–1 days for delivery).

When analyzing the parameters of systolic function between the prepartum periods, no statistical difference was observed for FS%. Despite this data, there was a significant increase in LVd and LVs; variables used to determine this index. Other parameters of systolic function that also showed statistical difference were Ao Vmax and P Vmax, where a significant increase in velocity between the periods was observed. However, the IVCT and LVET parameters did not differ statistically from the time of birth. Table 4.2 presents all the data from this analysis.

TABLE 4.2 – Variation of canine fetal echocardiographic parameters of systolic function in the prepartum periods.

		Period 5	*Period 4*	*Period 3*	*Period 2*	*Period 1*	*P*
	N	15	17	17	19	20	
	Min-Max	0.15-0.34	0.14-0.43	0.22-0.48	0.27-0.53	0.22-0.59	
	IQR	0.17-0.25	0.22-0.33	0.28-0.40	0.33-0.47	0.34-0.49	
LVd (mm)	Median	0.21	0.27	0.32	0.38	0.41	<0.0001
	Mean	0.22^{A}	0.28^{AB}	0.34^{BC}	0.39^{C}	0.41^{C}	
	SD	0.051	0.079	0.077	0.085	0.099	
	CV	23.37%	28.23%	22.46%	21.37%	23.85%	
	N	15	17	17	19	20	
	Min-Max	0.07-0.20	0.06-0.28	0.11-0.37	0.12-0.40	0.18-0.37	
	IQR	0.11-0.14	0.13-0.20	0.16-0.25	0.22-0.29	0.23-0.33	
LVs (mm)	Median	0.12	0.17	0.22	0.26	0.27	<0.0001
	Mean	0.12^{A}	0.17^{AB}	0.21^{BC}	0.26^{CD}	0.27^{D}	
	SD	0.031	0.054	0.065	0.064	0.057	
	CV	25.02%	32.76%	30.41%	25.07%	21.03%	
	N	15	17	17	19	20	
	Min-Max	20.0-56.0	19.0-62.0	14.0-62.0	22.0-63.0	16.0-57.0	
	IQR	38.0-50.0	35.5-47.5	26.0-44.5	29.0-40.0	30.5-41.5	
FS (%)	Median	47.0	41.0	40.0	36.0	35.5	0.2233
	Mean	42.9	41.1	37.2	36.8	35.2	
	SD	10.83	11.10	12.48	10.42	10.35	
	CV	25.23%	27.03%	33.57%	28.31%	29.40%	
	N	10	10	13	12	16	
	Min-Max	0.35-0.60	0.37-0.71	0.32-0.67	0.40-0.77	0.43-0.75	
	IQR	0.41-0.52	0.42-0.54	0.43-0.60	0.52-0.58	0.50-0.67	
Ao Vmax (m/s)	Median	0.47	0.51	0.47	0.55	0.59	0.0270
	Mean	0.47^{A}	0.49^{AB}	0.49^{AB}	0.56^{AB}	0.58^{B}	
	SD	0.078	0.101	0.115	0.092	0.097	
	CV	16.70%	20.29%	23.29%	16.52%	16.61%	
	N	9	12	11	12	13	
	Min-Max	0.26-0.45	0.34-0.60	0.37-0.65	0.30-0.66	0.35-0.76	
	IQR	0.29-0.43	0.38-0.50	0.47-0.55	0.49-0.60	0.46-0.71	
P Vmax (m/s)	Median	0.36	0.45	0.50	0.52	0.56	<0.0001
	Mean	0.36^{A}	0.44^{AB}	0.51^{BC}	0.53^{BC}	0.57^{C}	
	SD	0.070	0.076	0.079	0.095	0.135	
	CV	19.60%	17.13%	15.52%	17.97%	23.71%	
	N	10	10	9	9	13	
	Min-Max	6.0-32.0	6.0-32.0	13.0-25.0	13.0-33.0	13.0-45.0	
	IQR	13.0-19.0	12.2-20.5	13.0-20.5	13.0-19.0	16.0-25.0	
IVCT (ms)	Median	13	19	19	16	19	0.2240*
	Mean	15.4	17.6	17.7	17.7	21.7	
	SD	6.867	7.427	4.272	6.344	8.596	
	CV	44.59%	42.20%	24.18%	35.91%	39.63%	
	N	10	10	9	9	13	
	Min-Max	83.0-127.0	89.0-114.0	100.0-124.0	83.0-134.0	92.0-127.0	
	IQR	87.5-114.3	95.0-109.5	102.0-114.5	90.5-109.0	103.5-114.0	
LVET (ms)	Median	102	105	102	108	108	0.6205*
	Mean	102.5	103.5	107.3	104.1	109.7	
	SD	14.93	8.436	8.930	15.130	9.241	
	CV	14.57%	8.15%	8.32%	14.53%	8.42%	

*Nonparametric data. Capital letters after the means (parametric data) or after the medians (non-parametric data) show the differences identified in the multiple comparison test.

N, number; Min-Max, minimum and maximum; IQR, interquartile range; SD, standard deviation; CV, coefficient of variation.

LVd; left ventricular cavity during diástole; LVs; left ventricular cavity during systole; fractional shortening (FS%); Ao Vmax, outflow tract of left ventricle (aortic flow); P Vmax, outflow tract of right ventricle (pulmonary flow); IVCT, isovolumic contraction time; LVET, left ventricular ejection time.

When measuring diastolic function, the observation of mitral flow waves showed that the E-wave was statisticaly different between the periods, showing an increase of its velocity with the near delivery. On the other hand, the A-wave showed no difference between the periods. Thus, it was possible to verify an increase in the relation between these two waves (E/A ratio), being this parameter statistically significant. Another parameter of a diastolic function, IVRT, also showed a difference in some periods, where it was possible to observe an increase in its duration in the last periods before delivery. The values from the analysis of these parameters of diastolic function are described in Table 4.3.

TABLE 4.3 – Variation of canine fetal echocardiographic parameters of diastolic function in the prepartum periods.

		Period 5	*Period 4*	*Period 3*	*Period 2*	*Period 1*	*P*
E-wave (m/s)	N	10	13	12	12	16	
	Min-Max	0.16-0.27	0.20-0.31	0.22-0.35	0.19-0.37	0.24-0.48	
	IQR	0.17-0.24	0.21-0.27	0.24-0.31	0.24-0.30	0.27-0.38	
	Median	0.21	0.24	0.26	0.29	0.34	<0.0001
	Mean	0.21[A]	0.24[AB]	0.27[B]	0.28[B]	0.34[C]	
	SD	0.039	0.038	0.045	0.051	0.063	
	CV	18.66%	15.39%	16.37%	18.41%	18.69%	
A-wave (m/s)	N	10	13	12	12	16	
	Min-Max	0.30-0.56	0.32-0.52	0.33-0.54	0.25-0.56	0.29-0.53	
	IQR	0.32-0.49	0.35-0.48	0.43-0.50	0.38-0.48	0.38-0.47	
	Median	0.38	0.41	0.44	0.43	0.44	0.5962
	Mean	0.40	0.41	0.45	0.43	0.42	
	SD	0.087	0.066	0.058	0.082	0.073	
	CV	21.59%	16.14%	12.86%	19.34%	17.20%	
E/A ratio	N	10	13	12	12	16	
	Min-Max	0.44-0.65	0.49-0.68	0.49-0.77	0.54-0.78	0.51-0.91	
	IQR	0.50-0.55	0.58-0.62	0.55-0.66	0.59-0.72	0.78-0.86	
	Median	0.52[A]	0.60[A]	0.60[AB]	0.68[BC]	0.83[C]	<0.0001*
	Mean	0.53	0.59	0.61	0.67	0.80	
	SD	0.058	0.050	0.076	0.074	0.098	
	CV	11.14%	8.46%	12.48%	11.18%	12.20%	
IVRT (ms)	N	10	10	10	9	13	
	Min-Max	13.0-32.0	13.0-32.0	19.0-35.0	19.0-38.0	19.0-51.0	
	IQR	17.5-25.0	15.2-26.0	24.2-32.0	20.5-35.0	23.5-38.0	
	Median	22[A]	19[A]	25[AB]	25[AB]	32[B]	0.0054*
	Mean	21.50	20.40	26.50	26.89	31.85	
	SD	5.986	6.381	4.950	7.390	8.601	
	CV	27.84%	31.28%	18.68%	27.48%	27.01%	

*Nonparametric data. Capital letters after the means (parametric data) or after the medians (non-parametric data) show the differences identified in the multiple comparison test.
N, number; Min-Max, minimum and maximum; IQR, interquartile range; SD, standard deviation; CV, coefficient of variation
E-wave and A-wave, mitral atrioventricular inflow; IVRT, isovolumic relaxation time.

In regards to the parameters that together are used to calculate the CCO (Table 4.1), it was observed that only the aortic and pulmonary TVI remained similar during all analyzed periods. On the other hand, other parameters such as Ao area, P area, Left SV, Right SV, LCO, RCO, and the final value of the cardiac output (CCO) presented statistical difference between the periods, all of them showing a significant increase with the proximity of delivery. The mean RH used to estimate CCO also showed a statistical difference, evidencing a significant decrease with the proximity of delivery. Table 4.4 shows the statistical analysis of these parameters used to calculate the CCO.

Still regarding global cardiac function, statistical analysis showed that MPI presented a significant difference between the periods, evidencing an increase of this index with near delivery. This fact was due to the significant increase in IVRT, already mentioned previously, since the other parameters that interfere with MPI did not present statistical difference (IVCT and LVET). Table 4.4 also exposes the MPI data. The data of the parameters used for the calculation of this index have already been commented and are presented in Table 4.2 and 4.3.

Table 4.5 shows the data of receiver operating characteristic (ROC) curves. This analysis was calculated to differentiate bitches in which delivery occurred in Period 1 (up to 4 days to delivery) from those which presented time to delivery greater than 4 days (Period 2 to 5). It is possible to notice that two parameters stand out, presenting area under the curve (AUC) with greater value when compared to the other analyzed parameters. The E-wave and the E/A ratio presented AUC of 0.8564 and 0.9309 with 95% confidence interval from 0.7547 to 0.9581 and 0.8264 to 1.035, respectively. Figure 4.4 shows the ROC curve plot of the two parameters that showed the highest accuracy inpredicting delivery.

TABLE 4.4 – Variation of canine fetal echocardiographic parameters of cardiac global function in the prepartum periods.

		Period 5	*Period 4*	*Period 3*	*Period 2*	*Period 1*	*P*
TVI aorta (cm)	N	9	8	12	13	14	0.1601*
	Min-Max	2.9-6.0	2.0-5.0	2.0-6.0	3.0-7.0	3.0-7.0	
	IQR	3.0-4.1	2.8-4.0	3.0-5.0	3.5-4.8	3.8-5.2	
	Median	3.8	3.0	4.0	4.0	4.0	
	Mean	3.8	3.3	3.9	4.2	4.5	
	SD	0.946	0.930	1.322	1.096	1.207	
	CV	24.61%	27.77%	33.40%	25.94%	26.74%	
Ao area (cm^2)	N	11	12	14	15	16	<0.0001
	Min-Max	0.01-0.05	0.02-0.10	0.06-0.13	0.03-0.19	0.07-0.20	
	IQR	0.02-0.04	0.03-0.06	0.07-0.12	0.07-0.17	0.09-0.15	
	Median	0.03	0.03	0.09	0.09	0.11	
	Mean	0.03^{A}	0.05^{A}	0.09^{B}	0.11^{B}	0.12^{B}	
	SD	0.0122	0.025	0.024	0.049	0.036	
	CV	39.50%	55.40%	25.82%	47.14%	29.49%	
Left SV (mL)	N	6	6	11	11	14	<0.0001
	Min-Max	0.05-0.15	0.07-0.14	0.11-0.72	0.23-0.87	0.24-1.43	
	IQR	0.06-0.14	0.08-0.13	0.24-0.45	0.28-0.57	0.36-0.87	
	Median	0.12	0.12	0.36	0.36	0.49	
	Mean	0.11^{A}	0.11^{A}	0.36^{AB}	0.43^{B}	0.60^{B}	
	SD	0.041	0.029	0.166	0.205	0.323	
	CV	38.47%	26.35%	45.61%	47.22%	53.62%	
LCO (mL/min)	N	6	6	11	11	14	<0.0001
	Min-Max	11.0-36.0	16.0-32.0	26.0-178.0	57.0-201.0	51.0-300.0	
	IQR	15.5-34.5	17.5-30.5	53.0-104.0	67.0-119.0	77.7-184.3	
	Median	28.5	27.0	85.0	86.0	106.5	
	Mean	25.8^{A}	25.0^{A}	85.2^{AB}	97.7^{B}	127.4^{B}	
	SD	10.07	6.63	40.69	43.68	68.32	
	CV	38.97%	26.53%	47.76%	44.70%	53.62%	
TVI pulmonary (cm)	N	6	11	11	12	14	0.0501*
	Min-Max	2.0-4.0	2.0-5.0	3.0-5.0	3.0-5.0	2.0-7.0	
	IQR	2.6-4.0	3.0-4.0	4.0-4.4	3.0-4.0	3.7-5.2	
	Median	3	4	4	4	4	
	Mean	3.1	3.5	4.1	3.8	4.3	
	SD	0.765	0.820	0.545	0.718	1.326	
	CV	24.44%	23.13%	13.14%	18.72%	30.94%	
P area (cm^2)	N	11	12	14	15	16	<0.0001*
	Min-Max	0.02-0.07	0.03-0.12	0.05-0.14	0.05-0.15	0.07-0.21	
	IQR	0.02-0.03	0.03-0.05	0.07-0.13	0.08-0.13	0.09-0.13	
	Median	0.03^{A}	0.04^{A}	0.09^{B}	0.10^{B}	0.12^{B}	
	Mean	0.03	0.05	0.09	0.10	0.12	
	SD	0.014	0.025	0.030	0.032	0.036	
	CV	47.14%	52.81%	31.93%	30.63%	30.28%	
Right SV (mL)	N	5	8	10	10	14	<0.0001*
	Min-Max	0.04-0.14	0.13-0.48	0.23-0.50	0.18-0.53	0.24-0.85	
	IQR	0.06-0.12	0.14-0.20	0.29-0.46	0.21-0.50	0.31-0.73	
	Median	0.08^{A}	0.16^{AB}	0.37^{BC}	0,33^{BC}	0.49^{C}	
	Mean	0.09	0.20	0.37	0.35	0.52	
	SD	0.036	0.116	0.089	0.137	0.218	
	CV	41.29%	57.94%	23.93%	39.07%	41.56%	
RCO (mL/min)	N	5	8	10	10	13	<0.0001*
	Min-Max	9.0-33.0	30.0-102.0	53.0-118.0	40.0-119.0	53.0-178.0	
	IQR	13.5-29.0	31.7-46.0	71.5-105.3	49.0-112.3	75.5-159.0	
	Median	19.0^{A}	36.0^{AB}	88.0^{BC}	80.5^{BC}	103.0^{C}	
	Mean	20.8	44.6	86.6	82.0	115.0	
	SD	8.89	23.89	20.07	31.01	44.81	
	CV	42.79%	53.53%	23.17%	37.81%	38.97%	
CCO (mL/min)	N	3	4	8	6	12	0.0002
	Min-Max	20.0-69.0	57.0-64.0	79.0-248.0	108.0-221.0	138.0-478.0	
	IQR	20.0-69.0	57.5-63.0	159.0-208.0	123.0-200.0	170.5-351.3	
	Median	50.0	59.5	183.5	146.0	209.0	
	Mean	46.3^{A}	60.0^{A}	179.0^{AB}	157.0^{AB}	253.5^{B}	
	SD	24.70	2.94	49.90	42.70	108.20	
	CV	53.32%	4.91%	27.88%	27.20%	42.67%	
MPI	N	10	10	9	9	13	0.0072
	Min-Max	0.21-0.46	0.24-0.45	0.31-0.47	0.28-0.63	0.33-0.83	
	IQR	0.29-0.44	0.31-0.43	0.37-0.46	0.37-0.50	0.41-0.53	
	Median	0.38	0.36	0.43	0.41	0.47	
	Mean	0.36^{A}	0.36^{A}	0.41^{AB}	0.44^{AB}	0.49^{B}	
	SD	0.082	0.072	0.055	0.104	0.126	
	CV	22.90%	19.71%	13.33%	23.68%	25.42%	
HR (bpm)	N	16	18	18	18	19	<0.0001
	Min-Max	219-248	214-248	200-262	201-257	200-230	
	IQR	236-246	225-242	218-245	218-236	210-210	
	Median	236^{A}	233^{A}	233^{A}	236^{A}	210^{B}	
	Mean	238	232	232	229	211	
	SD	7.99	10.16	16.63	15.23	6.65	
	CV	3.36%	4.37%	7.16%	6.65%	3.15%	

*Nonparametric data. Capital letters after the means (parametric data) or after the medians (non-parametric data) show the differences identified in the multiple comparison test.

N, number; Min-Max, minimum and maximum; IQR, interquartile range; SD, standard deviation; CV, coefficient of variation

TABLE 4.5 – Receiver operating characteristic (ROC) curves calculated from fetal echocardiography data to differentiate bitches in which delivery occurred within the next 4 days (Period 1) from those that needed more than 4 days (Period 2 to 5) to start delivery.

	AUC	*SE*	*95% CI*	*N Bitches*	*N Fetuses*	*P*
LV_d	0.7570	0.0564	0.6464 - 0.8675	20	68	0.0005
LV_s	0.7982	0.0507	0.6987 - 0.8977	20	68	<0.0001
Ao Vmax	0.7007	0.0771	0.5496 - 0.8518	16	45	0.0179
P Vmax	0.7255	0.0864	0.5562 - 0.8948	13	44	0.0142
E-wave	0.8564	0.0519	0.7547 - 0.9581	16	47	<0.0001
E/A ratio	0.9309	0.0533	0.8264 - 1.035	16	47	<0.0001
IVRT	0.7673	0.0781	0.6141 - 0.9204	13	39	0.0042
Ao area	0.8059	0.0525	0.7029 - 0.9088	16	52	0.0002
Left SV	0.8151	0.0616	0.6943 - 0.9359	14	34	0.0007
LCO	0.7794	0.0681	0.6460 - 0.9128	14	34	0.0026
P area	0.7782	0.0556	0.6692 - 0.8873	16	52	0.0008
Right SV	0.8162	0.0650	0.6888 - 0.9436	14	34	0.0006
RCO	0.7986	0.0685	0.6643 - 0.9330	13	34	0.0017
CCO	0.8254	0.0720	0.6843 - 0.9665	12	21	0.0022
MPI	0.7783	0.0764	0.6286 - 0.9281	13	38	0.0030

AUC, Area under the curve; SE, standard error; CI, confidence interval

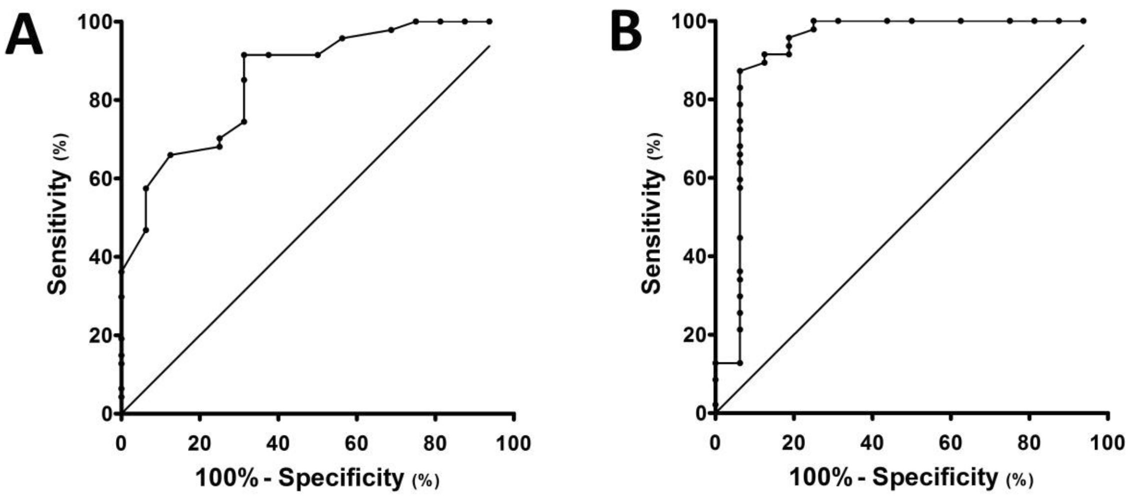

FIGURE 4.4 – Receiver operating characteristic (ROC) curves of the two fetal echocardiographic parameters that presented largest area under the curve (AUC) when compared to the other evaluated parameters. A) ROC curve of E-wave. B) ROC curve of E/A ratio.

The cut-off values of the fetal echocardiographic parameters that presented AUC of ROC curve greater than 0.75 are described in Table 4.6, as well as their sensitivity and specificity indices and parameters for their interpretation. The E-wave and E/A ratio were again emphasized, but mainly the E/A ratio, which presented a high accuracy. Table 4.6 shows that there is an accuracy of 92% with specificity of 0.98 for an E/A ratio greater than 0.78, showing strong evidence that labor will occur within 4 days.

TABLE 4.6 – Cut-off values of fetal echocardiographic parameters (only parameters that had an AUC >0.75) and their corresponding sensitivity and specificity indices obtained when differentiating bitches in which delivery occurred within the next 4 days from bitches in which delivery took more than 4 days to start (up to 20 days).

	Cut-off	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*LR (+)*	*LR (-)*	*Pretest probability (%)*	*Pretest odds*	*Posttest odds*	*Posttest probability (%)*	*Accuracy*	*Odds Ratio*
Ao area (cm^2)	**>0.08**	0.94	0.58	0.41	0.97	2.22	0.11	23.53	0.31	0.68	40.54	66.18	20.45
	>0.09	0.81	0.65	0.42	0.92	2.35	0.29	23.53	0.31	0.72	41.94	69.12	8.19
	>0.10	0.75	0.71	0.44	0.90	2.60	0.35	23.53	0.31	0.80	44.44	72.06	7.40
Left SV (mL)	**>0.37**	0.79	0.71	0.52	0.89	2.67	0.30	29.17	0.41	1.10	52.38	72.92	8.80
	>0.40	0.71	0.74	0.53	0.86	2.70	0.39	29.17	0.41	1.11	52.63	72.92	6.94
	>0.44	0.64	0.76	0.53	0.84	2.73	0.47	29.17	0.41	1.13	52.94	72.92	5.85
LCO (mL/min)	**>70.0**	0.79	0.62	0.46	0.88	2.05	0.35	29.17	0.41	0.85	45.83	66.67	5.92
	>85.0	0.64	0.65	0.43	0.81	1.82	0.55	29.17	0.41	0.75	42.86	64.58	3.30
	>90.0	0.64	0.71	0.47	0.83	2.19	0.51	29.17	0.41	0.90	47.37	68.75	4.32
P area (cm^2)	**>0.08**	0.94	0.56	0.39	0.97	2.12	0.11	23.53	0.31	0.65	39.47	64.71	18.91
	>0.09	0.88	0.62	0.41	0.94	2.28	0.20	23.53	0.31	0.70	41.18	67.65	11.20
	>0.10	0.69	0.71	0.42	0.88	2.38	0.44	23.53	0.31	0.73	42.31	70.59	5.43
RSV (mL)	**>0.34**	0.71	0.62	0.43	0.84	1.87	0.46	29.17	0.41	0.77	43.48	64.58	4.04
	>0.39	0.71	0.71	0.50	0.86	2.43	0.40	29.17	0.41	1.00	50.00	70.83	6.00
	>0.44	0.64	0.76	0.53	0.84	2.73	0.47	29.17	0.41	1.13	52.94	72.92	5.85
RCO (mL/min)	**>72.0**	0.77	0.59	0.42	0.87	1.87	0.39	27.66	0.38	0.71	41.67	63.83	4.76
	>80.0	0.77	0.65	0.45	0.88	2.18	0.36	27.66	0.38	0.83	45.45	68.09	6.11
	>93.0	0.69	0.76	0.53	0.87	2.94	0.40	27.66	0.38	1.13	52.94	74.47	7.31
CCO (mL/min)	**>161.0**	0.83	0.62	0.56	0.87	2.19	0.27	36.36	0.57	1.25	55.56	69.70	8.13
	>175.0	0.75	0.71	0.60	0.83	2.63	0.35	36.36	0.57	1.50	60.00	72.73	7.50
	>179.0	0.67	0.71	0.57	0.79	2.33	0.47	36.36	0.57	1.33	57.14	69.70	5.00
E-wave (m/s)	**>0.26**	0.94	0.57	0.43	0.96	2.20	0.11	25.40	0.34	0.75	42.86	66.67	20.25
	>0.28	0.75	0.68	0.44	0.89	2.35	0.37	25.40	0.34	0.80	44.44	69.84	6.40
	>0.31	0.69	0.85	0.61	0.89	4.62	0.37	25.40	0.34	1.57	61.11	80.95	12.57
E/A	**>0.69**	0.94	0.83	0.65	0.98	5.51	0.08	25.40	0.34	1.88	65.22	85.71	73.13
	>0.74	0.81	0.94	0.81	0.94	12.73	0.20	25.40	0.34	4.33	81.25	90.48	63.56
	>0.78	0.75	0.98	0.92	0.92	35.25	0.26	25.40	0.34	12.00	92.31	92.06	138.00
IVRT (ms)	**>24.0**	0.77	0.44	0.31	0.85	1.36	0.53	25.00	0.33	0.45	31.25	51.92	2.58
	>27.0	0.69	0.77	0.50	0.88	3.00	0.40	25.00	0.33	1.00	50.00	75.00	7.50
	>30.0	0.69	0.79	0.53	0.89	3.38	0.39	25.00	0.33	1.13	52.94	76.92	8.72
MPI	**>0.40**	0.85	0.53	0.38	0.91	1.79	0.29	25.49	0.34	0.61	37.93	60.78	6.11
	>0.43	0.69	0.61	0.38	0.85	1.75	0.51	25.49	0.34	0.60	37.50	62.75	3.45
	>0.46	0.62	0.79	0.50	0.86	2.92	0.49	25.49	0.34	1.00	50.00	74.51	6.00
LV_d (cm)	**>0.33**	0.85	0.56	0.36	0.93	1.93	0.27	22.73	0.29	0.57	36.17	62.50	7.18
	>0.37	0.70	0.71	0.41	0.89	2.38	0.43	22.73	0.29	0.70	41.18	70.45	5.60
	>0.40	0.60	0.76	0.43	0.87	2.55	0.52	22.73	0.29	0.75	42.86	72.73	4.88
LV_s (cm)	**>0.22**	0.85	0.59	0.38	0.93	2.06	0.26	22.73	0.29	0.61	37.78	64.77	8.10
	>0.25	0.65	0.76	0.45	0.88	2.76	0.46	22.73	0.29	0.81	44.83	73.86	6.04
	>0.27	0.55	0.82	0.48	0.86	3.12	0.55	22.73	0.29	0.92	47.83	76.14	5.70

PPV, positive predictive value; NPV, negative predictive value; LR (+), positive likelihood ratio; LR (-), negative likelihood ratio

4.4 DISCUSSION

Fetal heart function has been studied for some years in humans, enabling cardiac analysis in the intrauterine life, in order to diagnose congenital cardiac diseases and analyze systemic disorders of the fetus and/or the mother that cause fetal heart damage (REED et al., 1986; FERNANDEZ PINEDA et al., 2000; DEVORE, 2005; MÄKIKALLIO et al., 2005; GARDINER et al., 2006; CUI et al., 2008). In veterinary medicine there are no studies in this area and, therefore, this research presents for the first time the description of cardiac function (systolic, diastolic and global) in dog fetuses, possibly allowing intrauterine diagnosis of congenital heart diseases. The correction of several congenital heart defects is already a reality in dogs (SCHROPE, 2015; BIRETTONI et al., 2016; WEDER et al., 2016; DOBAK et al., 2017; TRESEDER; JUNG, 2017). The early diagnosis of these diseases may aid in better planning of the procedure, which can be carried out before the appearance of clinical signs and consequently the presence of obvious cardiac remodeling.

The echocardiographic planes of this study were performed according to a methodology described by Giannico et al., 2016b, which, in turn, was based on the examination of adult dogs (BOON, 2011). In the fetal echocardiographic examination, there are some limitations, such as fetal positioning that impairs the acquisition of the correct view. In routine echocardiographic examination in dogs, the imaging planes are performed by means of the operator's maneuvers with the transducer. However, in the fetuses, these maneuvers were limited because the operator depended on the correct positioning of the fetus inside the uterus. For this reason, some echocardiographic parameters of some fetuses were not measured. This data is represented in Table 4.5, where for different echocardiographic parameters there are the same number of bitches, but with different numbers of fetuses evaluated.

The systolic function parameter, FS%, which shows the percentage of myocardial contraction performed to eject blood, was acquired by the two-dimensional image, not using the M-mode. This method may present flaws, since the time of diastole and systole is determined by a qualitative analysis of the image, in which the maximum possible filling represents diastole, and the smallest measure is the contraction (systole). Some researchs in human fetuses use the M-mode measurement, proving that the reliability of the data is better (ALLAN et al.,1982; CARVALHO et al., 2001; DEVORE, 2005). This technique was not used in the present study because, in many cases, the perfect alignment of the M-mode was not possible. In addition, this technique would only be possible a few days before delivery, due to fetal cardiac size. It should be emphasized that this parameter showed great variation among the studied fetuses, with large amplitudes between the minimum and maximum values and high values of standard deviation and coefficient of variation. Perhaps, the method used here to verify the percentage of myocardial shortening is not ideal, but qualitative visualization can provide information about myocardial contraction.

The mean heart rate was used to calculate the CCO. This parameter decreased as delivery approached, becoming more evident in the last days of pregnancy (Period 5). This fact occurs secondarily to an acceleration and deceleration of fetal heart beat near delivery, which has been previously described (GIL et al., 2014; GIANNICO et al., 2016a). The fetal heart rate oscillation was not analyzed in this study and is not necessary for the acquisition of fetal heart function parameters. The CCO shows the amount of blood ejected by the heart in one minute (cardiac output). Although a reduction in the mean heart rate in Period 5 was observed, this fact did not cause a reduction in cardiac output, but rather a higher ejection volume (higher CCO in Period 5). This occurred because, at this moment, the heart presents a larger size of cavities as well as the diameter of the semilunar valves annulus, secondary to fetal growth.

The MPI is the sum of the isovolumetric contraction and relaxation times, divided by the ejection time. Because this index uses only time intervals, it is independent of heart rate and ventricular structure, but it is strongly influenced by systemic arterial and venous pressures and atrial function (PELLETet al., 2004). In this present study, this index was higher with the proximity of delivery, occurring secondary to the IVRT increase, since the other components (IVCT and LVET) did not change during the analyzed periods (Table 4). Knowing fetal MPI in each period of intrauterine life can aid in the diagnosis of fetal and systemic diseases of the pregnant bitch. Increased values of the MPI in human fetuses correlated with signs of systematic inflammation response syndrome (LETTI MÜLLER et al., 2010), besides being elevated in fetal hydrops and fetuses of diabetic mothers (ICHIZUKA et al., 2005; CRISPI et al., 2008; CHAO et al., 2009). Therefore, it would be indicated in the evaluation of cardiac function of all the fetuses of bitches that carry fetuses with anasarca, in dogs with a history of systemic or infectious diseases and in females with a history of reabsorption or fetal death.

Although this research shows data on gestational ultrasound, it is likely that an ultrasonographer will find it more difficult to acquire the correct echocardiographic sections and to measure and evaluate the suggested cardiac parameters. The ultrasonographer should be able to recognize cardiac changes of size/morphology and contractility, in addition recommend to the clinician a joint evaluation with an echocardiographer with a fetal echocardiogram to better define the diagnosis.

The complete fetal echocardiographic examination performed by an echocardiographer has, as main objective, to diagnose congenital alterations in the intrauterine phase. Thus, we believe that the technique should be implemented in fetal ultrasound routine, specialy by dog breeders. Another option would be the teaching and training of the sonographer to obtain the fetal echocardiographic parameters, so that global and specific parameters of fetal heart function can be implemented as an additional aid to determine the time of delivery and to diagnose intrauterine heart disease.

Considering the data from this research, we suggest that further studies should be performed regarding fetal heart function. The detection of alterations in the normal parameters of canine fetal heart function may help in the diagnosis of systemic diseases, indirectly. In human fetuses, it is known that changes such as anemia, placental insufficiency, intrauterine growth restriction, twin-to-twin transfusion syndrome, hydrops, congenital heart defects and fetal arrhythmia may cause cardiac dysfunction (VEILLE; COVITZ, 1994; RABOISSON et al., 2004; HOFSTAETTER et al., 2006; MÄKIKALLIO et al., 2006; RYCHIK et al., 2007; CRISPI et al., 2008; MÄKIKALLIO et al., 2008, VAN MIEGHEM et al., 2009b).

After analyzing all the data, we verified that the main echocardiographic parameters of fetal heart function in dogs that are related to the moment of delivery (pre-partum periods) are the E-wave and E/A ratio. The E-wave of the mitral flow increases as the delivery approaches and when it is divided by A-wave, consequently, this relation also increases. When there is more significant ventricular relaxation, there is a decrease in the intracavitary pressure and, thus, the blood moves from the left atrium to the left ventricle with a higher velocity, increasing the velocity of E-wave (CARCELLER-BLANCHARD; FOURON, 1993). This greater capacity of relaxation can be observed through the values of the IVRT in this research, that suffer an increase with the proximity of the delivery.

Based on the results of the ROC curve and the values of the area under the curve, the cut-off values were given, and we observed that an E/A ratio greater than 0.78 has an accuracy of 92% to predict that delivery will occur in up to four days. These parameters mark the last days for delivery, but should be used with caution, since a four days preterm birth could lead to fetal death.

Failure to perform echocardiographic examination in puppies for postpartum evaluation of possible congenital heart disease is one of the limitations of this study. Nevertheless, significant increases in transvalvar flow velocities and qualitative morphological alterations of cardiac chambers and valves that would characterize an abnormality were not observed on fetal echocardiography. In addition, all puppies grew healthy after birth. Another limiting factor of this research is that there was no evaluation of the parameters in the last hours nearest to delivery. Probably the mitral E-wave and the E/A ratio provide good results for the determination of the exact moment of delivery.

The need to know the moment of delivery is a constant search for obstetrical ultrasonographers. Parameters such as fetal heart rate oscillation analysis and reduction of umbilical artery flow resistivity index have proved to be useful associations and are able to estimate the time of delivery in up to 12 hours (GIL et al., 2014; GIANNICO et al., 2015; GIANNICO et al., 2016a). More specific research about the parameters of fetal heart function described in this study (mitral E wave and E/A ratio) should be considered for analysis of the last days and prepartum hours. We believe that these parameters, combined with data on fetal heart rate variation and umbilical artery resistivity index, can provide even greater accuracy in determining of the last prepartum moments.

In summary, this study demonstrated cardiac function values of canine fetuses at different gestational stages and after fetal cardiac formation. Considering the data of the described echocardiographic parameters, it is possible to consider that the IQR values presented in Table 4.2 possibly are expected for each gestational period. Thus, these values can be used for prenatal diagnosis of congenital heart disease and systemic alterations of the fetus and pregnant bitch. Besides, this research revealed that some parameters are related to the moment of delivery, emphasizing the mitral E-wave and the E/A ratio as being the best for this purpose. E-wave values greater than 0.31 m/s and E/A ratio greater than 0.78 strongly suggest that labor will occur in up to four days.

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5. ANNEXES AND APPENDICES

5.1 Approval in the Ethics Committee of the Department of Agricultural Sciences in the Federal University of Paraná

Universidade Federal do Paraná
Setor de Ciências Agrárias
Comissão de Ética no Uso de Animais – CEUA SCA

CERTIFICADO

Certificamos que o protocolo no. 043/2014, referente ao projeto "Avaliação imaginológica cardiovascular intrauterina em fetos caninos: ecocardiografia", sob a responsabilidade de Amália Turner Giannico, na forma em que foi apresentado (uso de 15 cães), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias, em reunião realizada dia 31 de julho de 2014.

CERTIFICATE

We certify that the protocol number 043/2014, regarding the project "Assessment of cardiovascular imaging in canine fetuses: echocardiography and doppler", under Amália Turner Giannico's supervision, in the terms it was presented (use of 15 dogs), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil) during session on July 31, 2014.

Curitiba, 31 de Julho de 2014.

Ricardo Guilherme D'Otaviano de Castro Vilani
Presidente

Ananda Portella Félix
Vice-Presidente

Comissão de Ética no Uso de Animais
Setor de Ciências Agrárias
Universidade Federal do Paraná.

5.2 Article published in *Animal Reproduction Science* (Chapter 1):

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The use of Doppler evaluation of the canine umbilical artery in prediction of delivery time and fetal distress

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ABSTRACT

The aim of this study was to describe changes in umbilical artery blood flow in the later stages of canine pregnancy. Seventeen pregnant bitches were examined sonographically to evaluate umbilical artery blood flow at the following antepartum times: 120–96, 96–72, 72–48, 48–24, 24–12, 12–6 and 6–1 h. The peak systolic velocity and end diastolic velocity were measured to calculate the resistive index (RI). Bitches were classified into two groups according to delivery method: normal delivery (Group 1, $n = 11$) and Cesarean section, due to fetal distress, (Group 2, $n = 6$). During the study, the RI of the umbilical artery in bitches in Group 1 significantly declined in the time periods 72–48, 24–12, 12–6 and 6–1 h before delivery when compared to the reference RI (120–96 h antepartum period), with values below 0.7 in the 12–6 and 6–1 h periods. In Group 2, the RI decreased significantly in the antepartum periods 96–72, 72–48, 48–24 h with respect to the period 120–96 h, and increased in the periods from 24–12, 12–6 and 6–1 h (being significantly higher in this last period) until the time of Cesarean section. Therefore monitoring of changes in umbilical artery RI in the pre-partum period may provide information about time of delivery in bitches and also assist in the diagnosis of possible dystocia and fetal distress.

1. Introduction

Conventional two-dimensional ultrasonography is a non-invasive, safe and efficient technique for monitoring fetal development and viability in veterinary medicine (England and Edward, 1990; Yeager and Concannon, 1990). However, two-dimensional ultrasonography provides little information about blood flow and therefore has been used in conjunction with Doppler ultrasound for clinical assessment in obstetrics and gynecology in a number of animal species (Nautrup, 1998; Di Salvo et al., 2006; Domingues et al., 2007; Blanco et al., 2008). Doppler ultrasound can be used to evaluate anatomical and functional vascular information such as blood flow velocity, direction and type (Nicolaides et al., 2000).

Pulsed wave Doppler allows analysis of blood flow within a single vessel. The Doppler waveform represents changes in the velocity of the blood flow during the cardiac cycle and a deflection in the late systolic or early diastolic flow is characteristic of high-resistance arterial blood flow waveforms (Blanco et al., 2008). The main flow parameters measured using this technique are the peak systolic velocity (PSV) and end diastolic velocity (EDV). The first parameter, PSV, is formed by the opening of the semilunar valves and the forward ejection of blood. This forward blood flow starts to decelerate when cardiac contraction provides insufficient forward force to overcome the elastic properties of the downstream vascular bed and

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5.3 Article published in *Theriogenology* (Chapter 2)*:*

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Assessment of umbilical artery flow and fetal heart rate to predict delivery time in bitches

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ABSTRACT

The aim of this study was to quantitatively investigate the oscillation of the fetal heart rate (HR) in advance of normal delivery and whether this index could be used to indicate impending delivery. In addition, fetal HR oscillation and umbilical artery resistive index (RI) were correlated to determine if the combination of these parameters provided a more accurate prediction of the time of delivery. Sonographic evaluation was performed in 11 pregnant bitches to evaluate the fetal HR and umbilical artery RI at the following antepartum times: 120 to 96 hours, 72 to 48 hours, 24 to 12 hours, and 12 to 1 hours. Statistical analysis indicated a correlation between the oscillation of fetal HR and the umbilical artery RI. As delivery approached a considerable reduction in the umbilical artery RI was documented and greater oscillations between maximum and minimum HRs occurred. We conclude that the quantitative analysis of fetal HR oscillations may be used to predict the time of delivery in bitches. The combination of fetal HR and umbilical artery RI together may provide more accurate predictions of time of delivery.

1. Introduction

After pregnancy diagnosis, a successful outcome requires that the neonates can survive in the extrauterine environment [1–3]. For veterinarians, owners, and breeders, the major concern when planning cesarean delivery is to maximize neonatal survival forwarding the bitch for surgery with mature fetuses [4]. Ability to predict an appropriate time of delivery to maximize viability, before the onset of intrauterine fetal distress, would improve neonatal survival.

Ultrasonography can be used to estimate gestational age in dogs. A variety of methods have been described involving morphologic evaluations, for example, crown-rump length at the beginning of pregnancy and biparietal diameter or the evaluation of fetal organogenesis by sequential examinations [5–9]. However, these ultrasonographic methods are not sufficiently accurate so as to predict the exact date of parturition and can result in premature preparation for normal delivery or planning for cesarean delivery [7,8].

In a recent publication, we introduced new sonographic parameters, which provide more accurate estimation of the expected delivery date in dogs. These parameters include fetal heart rate (HR) acceleration and deceleration during the antepartum period [10] and changes in umbilical artery blood flow, more specifically the resistive index (RI), at the end of canine pregnancy [11].

Our hypothesis is that a combination of fetal HR oscillations and umbilical artery RI could provide more accurate predictions of delivery date. Therefore, the purpose of this study was 4-fold: (1) to provide quantitative definition of the oscillations in fetal HR in bitches with normal delivery; (2) to identify percentage (relative to maximum) of fetal HR variation at different times close to the delivery time; (3) to document any correlation between the fetal HR oscillations

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ORIGINAL ARTICLE

Canine fetal echocardiography: correlations for the analysis of cardiac dimensions

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Abstract The aim of this study was to develop regression models for correlation of canine fetal heart development with body size to characterize normal development or suggest cardiac anomalies. Twenty clinically healthy pregnant bitches, either brachycephalic and non-brachycephalic, were examined ultrasonographically. Transabdominal fetal echocardiography was conducted every 4 days from the beginning of cardiac chambers differentiation until parturition. Ten cardiac parameters were measured: length, width and diameter of the heart; heart area; left and right ventricular dimensions; left and right atrial dimensions; and aortic and pulmonary artery diameter. Femoral length, biparietal diameter and abdominal cross-sectional area were also recorded. Regression equations were developed for each parameter of fetal body size, and linear and logarithmic models were compared. The model with the highest correlation coefficient was chosen to produce equations to calculate relative dimensions based on the correlations. Only the left-ventricular chamber differed between the two racial groups. Biparietal diameter was the independent parameter that produced the highest correlation coefficient for the most fetal cardiac dimensions, although good correlations were also observed using femoral length and abdominal cross-sectional area. Heart width and heart diameter were used as surrogates of cardiac development, as these measurements showed the best statistical correlation. Quantitative evaluation of fetal cardiac structures can be used to monitor normal and abnormal cardiac development.

Keywords Fetal ultrasonography · Pregnancy · Gestational ultrasound · Cardiac parameters · Fetal heart

Introduction

Sonographic measurements of fetal ultrasound parameters are the basis for detection of fetal abnormalities (Degani 2001). In human medicine, biometric changes in the cardiac structure are evaluated using parameters of fetal size when congenital heart defects or cardiac growth alterations unrelated to congenital heart disease (especially intrauterine growth restriction) are suspected (Rychik et al. 2004; Schneider et al. 2005; Lee et al. 2008; Wood et al. 2009).

In human fetuses the Z-score is used to quantify the degree to which an individual measurement lies above or below the mean value for a given population (Schneider et al. 2005; Pettersen et al. 2008; Lee et al. 2010). The quantification of growth of cardiac structures compared to overall somatic growth in the prenate can be compared to values predicted by parameters such as fetal femoral length (FL) and biparietal diameter (BPD) (DeVore 2005; Schneider et al. 2005; Lee et al. 2010; Li et al. 2015).

Research shows that in both dogs and human beings a relationship exists between cardiac size and body structures (Gutgesell and Rembold 1990; Morrison et al. 1992; Batterham et al. 1999; Cornell et al. 2004; de Simone and Galderisi 2014). Also during intrauterine life, the correlation between cardiac and non-cardiac fetal structures, both in children and human fetuses, facilitates the detection of pathological increases in cardiac dimensions (Schneider et al. 2005; Lee et al. 2010; Chubb and Simpson 2012). To date, there is

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5.5 Additional scatterplots – Supplementary figure 1 (Chapter 3)

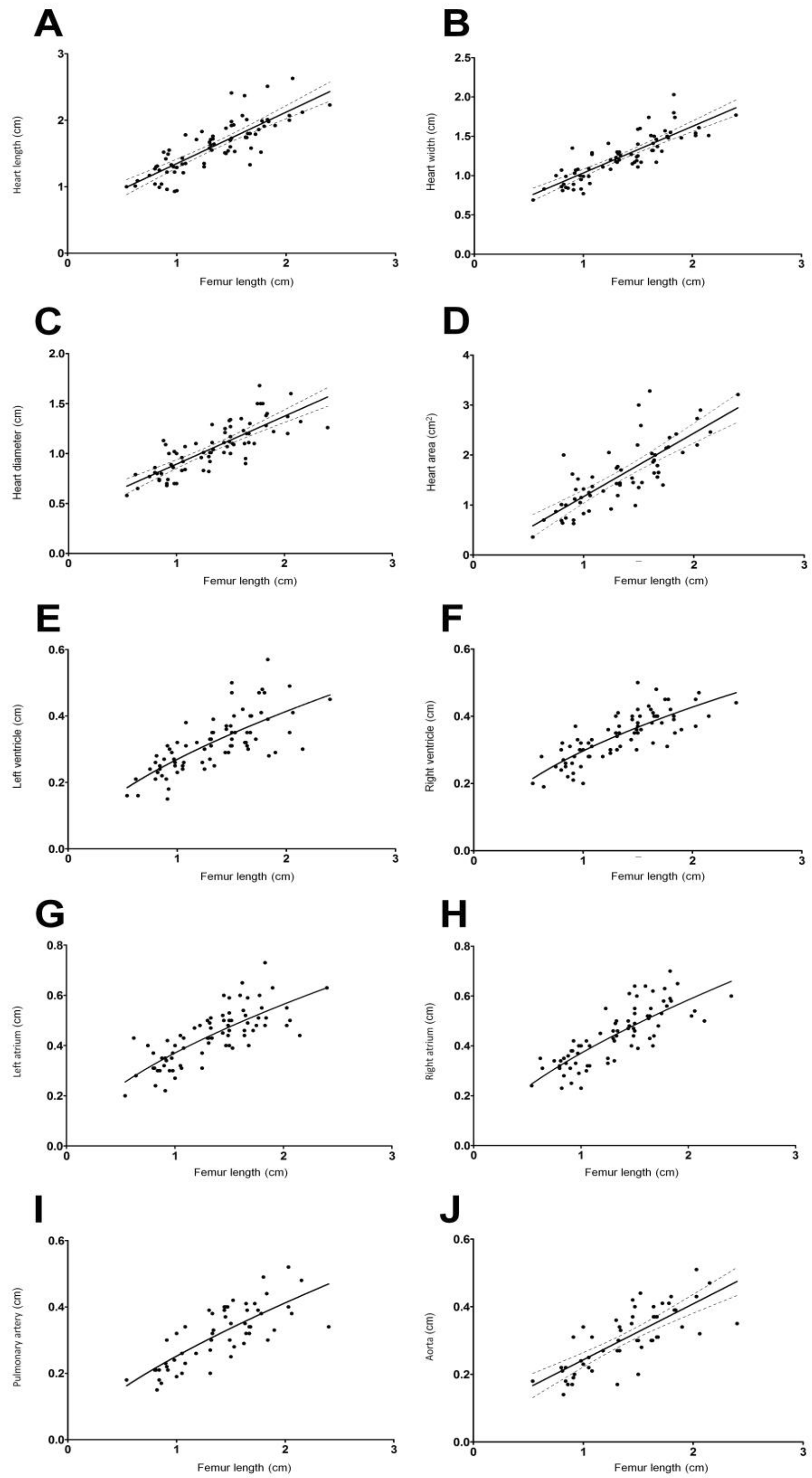

FIGURE S1 – Sample scatterplots showing the regression model that best describes the data (linear or logarithmic) and their relationships between the femur length and cardiac structures. A) Heart length (linear). B) Heart width (linear). C) Heart diameter (linear). D) Heart area (linear). E) Left ventricle (logarithmic). F) Right ventricle (logarithmic). G) Left atrium (logarithmic). H) Right atrium (logarithmic). I) Pulmonary artery (logarithmic). J) Aorta (linear). ________, correlation average; _ _ _ _, 95% confidence interval for correlation.

5.6 Additional scatterplots – Supplementary figure 2 (Chapter 3)

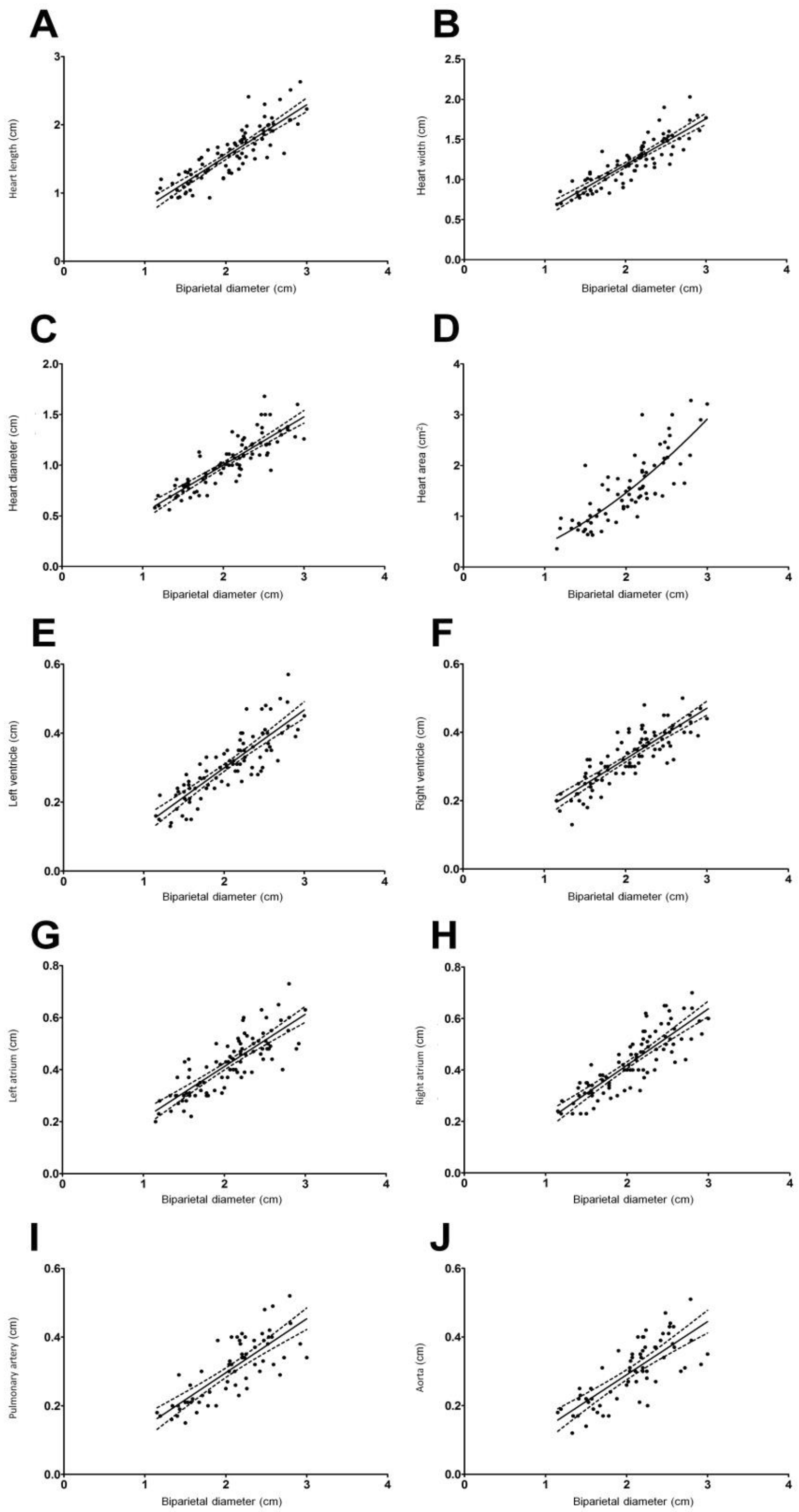

FIGURE S2 – Sample scatterplots showing the regression model that best describes the data (linear or logarithmic) and their relationships between the biparietal diameter and cardiac structures. A) Heart length (linear). B) Heart width (linear). C) Heart diameter (linear). D) Heart area (logarithmic). E) Left ventricle (linear). F) Right ventricle (linear). G) Left atrium (linear). H) Right atrium (linear). I) Pulmonary artery (linear). J) Aorta (linear). ________, correlation average; _ _ _ _, 95% confidence interval for correlation.

5.7 Additional scatterplots – Supplementary figure 3 (Chapter 3)

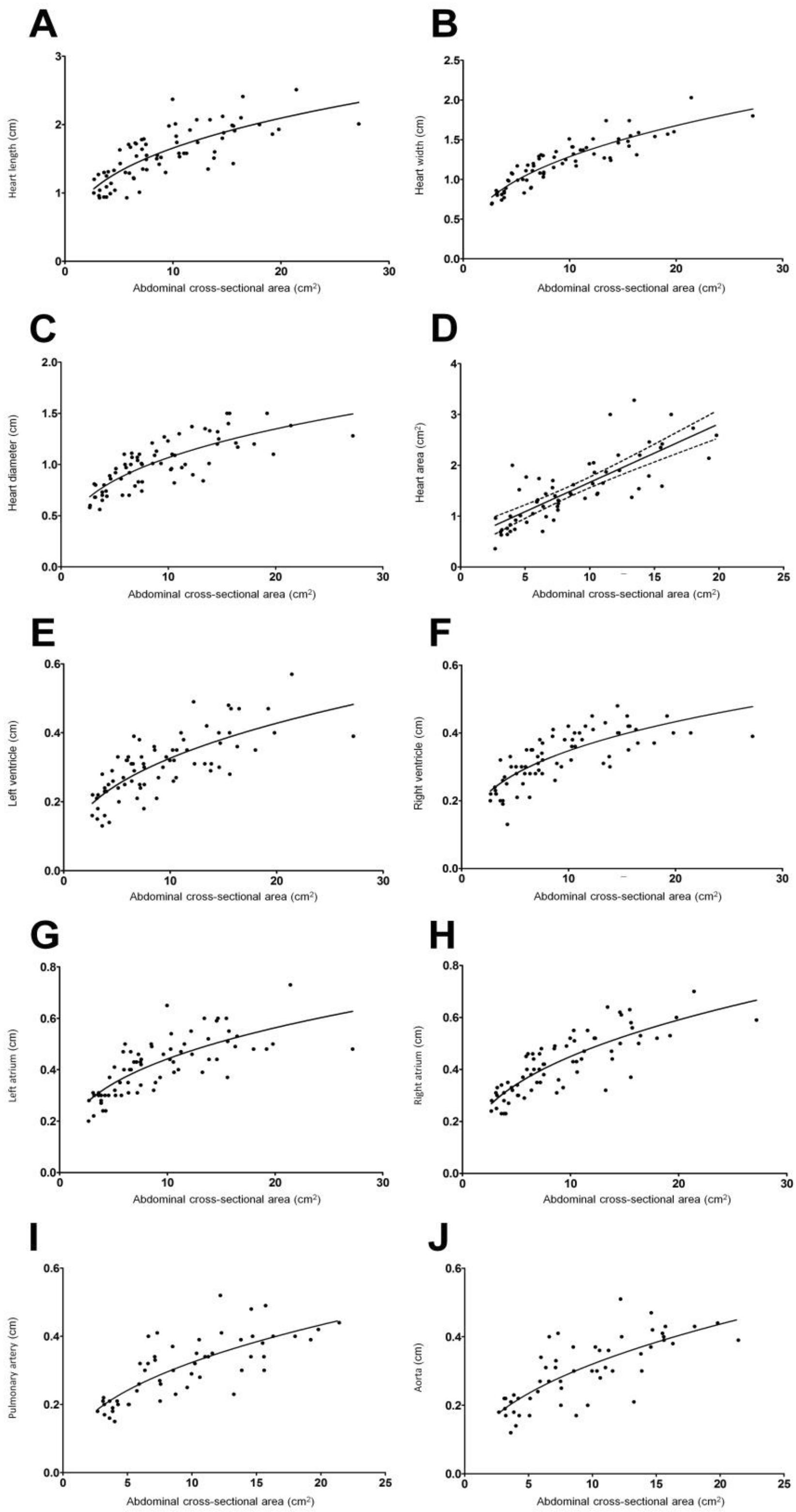

FIGURE S3 – Sample scatterplots showing the regression model that best describes the data (linear or logarithmic) and their relationships between the abdominal circumference area and cardiac structures. A) Heart length (logarithmic). B) Heart width (logarithmic). C) Heart diameter (logarithmic). D) Heart area (linear). E) Left ventricle (logarithmic). F) Right ventricle (logarithmic). G) Left atrium (logarithmic). H) Right atrium (logarithmic). I) Pulmonary artery (logarithmic). J) Aorta (logarithmic). ________, correlation average; _ _ _ _, 95% confidence interval for correlation.

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