

Foetal echoencephalography and Doppler ultrasonography of the middle cerebral artery in canine foetuses

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OBJECTIVES: This study aimed to evaluate the characteristics of the brain and vascular indices of the middle cerebral artery of canine foetuses.

METHODS: Twenty-five bitches were selected. Tissue development, echogenicity, echotexture and brain echobiometric data were studied, and the major structures were identified between the 5th and 8th gestational weeks. The area and volume of the brain mass (BMA and BMV), cranial area and volume (AC and VC), brain mass index (BMI) and brain volume index (BVI) were determined. A single ultrasound examination was performed during each studied week (6th, 7th and 8th). Doppler ultrasonography was performed to assess the maximum and minimum velocity, resistance and pulsatility index of middle cerebral artery of the foetuses.

RESULTS: Echoencephalography was performed to evaluate the morphological characteristics of the central nervous system. Cerebral echobiometry indicated an increase in area and volume of the hemispheres and cranium ($P < 0.001$) but no changes in BMI or BVI over the gestational period studied. Doppler ultrasonography identified increases in peak systolic velocity ($P = 0.0188$) and end diastolic velocity ($P = 0.0274$) and decreases in resistance index ($P = 0.0002$) and pulsatility index ($P < 0.001$).

CLINICAL SIGNIFICANCE: Echoencephalography and spectral Doppler ultrasonography of the middle cerebral artery in canine foetuses might be a useful technique for prenatal care.

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INTRODUCTION

Echoencephalography in veterinary medicine is used for the diagnosis of brain changes such as haemorrhage, ischaemia, oedema, hydrocephalus, abscesses and tumours in puppies younger than four weeks (Carvalho *et al.* 2004). However, there are few studies on the use of echoencephalography in veterinary obstetrics. According to Cruz *et al.* (2003), the small size of foetuses and their neurological tissues make it difficult to define intracranial anatomy for prenatal diagnoses.

In pregnant women, Doppler ultrasonography is used to investigate maternal (uterine artery), utero-placental (umbilical

arteries) and foetal circulation. Doppler imaging allows non-invasive diagnosis in cases of placental insufficiency and foetal evaluation of haemodynamic changes that occur in response to lack of oxygen to the foetus (Miranda *et al.* 2010).

In humans, the middle cerebral artery is the main vessel of interest in prenatal diagnoses. The most important variables for diagnoses are the vascular resistive index, pulsatility, peak systolic velocity and end diastolic velocity. The vascular indices changes of the middle cerebral artery of foetuses, such as increased peak systolic velocity (PSV) and pulsatility index (PI), may indicate anaemia, inadequate foetal growth and materno-foetal hydrops (Abdel-Fattah *et al.* 2001, Delle Chiaie *et al.* 2001, Mari *et al.*

2007). However, the importance of the vascular indices of the middle cerebral artery in animals has not been determined (Costa *et al.* 2003).

As echoencephalography and Doppler flowmetry of the middle cerebral artery are important imaging methods for prenatal monitoring and foetal diagnoses in human obstetrics, this study aimed to evaluate the sonographic characteristics of canine foetus brains and determine the vascular indices of the middle cerebral artery of foetuses in pregnant bitches using triplex Doppler ultrasonography.

MATERIAL AND METHODS

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the School of Agrarian and Veterinary Sciences of the Sao Paulo State University, Brazil (protocol No 017 314/10). Twenty-five healthy and multiparous shih-tzu, English bulldog, French bulldog and pug bitches from the same kennel weighing 5 to 25 kg and aged four to six years were selected for this study after physical and obstetric examinations.

The kennel owner was trained to detect the first signs of pro-oestrus to determine the ideal time for mating. Confirmation was determined by observing the signs of oestrus (i.e. acceptance of copulation by the female) and by vaginal cytology. The dogs were mated for 3 days or artificial insemination (n=13) was performed. In the case of natural mating, the acceptance to the male was observed for confirmation.

Pregnancy diagnosis was carried out 2 weeks after mating or insemination, according to the methodology employed in other studies (Feliciano *et al.* 2007) with 7.5 and 12.0 MHz linear transducers and MyLab VET 30 ultrasound equipment (ESAOTE). A single ultrasound examination was performed weekly using B-mode and Doppler between the 5th and 8th weeks of pregnancy.

The morphological characteristics of the central nervous system of foetuses were evaluated by B-mode ultrasound in sagittal and transverse views of the cranium. Tissue development (ratio of brain mass and cranium), echogenicity, echotexture and brain structures were studied between the 5th and 8th weeks of pregnancy.

Through transverse images were obtained as single images of the craniums representing the entire length of the skull and brain mass of the foetuses. After the visualization of the foetuses by abdominal ultrasonography in bitches, the transverse images of the foetal cranium and the extension of the skulls and the brain mass were determined by movement of angulation of the transducer. These were drawn using software that calculates the area and volume of the brain mass (BMA and BMV), cranial area and volume (CA and CV), the brain mass index (BMI) and the brain volume index (BVI). The BMI and BVI were determined by the ratios of BMA/CA and BMV/CV (Fig 1).

During the 6th, 7th and 8th gestational weeks, pulsed Doppler ultrasonography was performed to assess the PSV, end-diastolic velocity (EDV), resistance indice ($RI = [PSV - EDV] / PSV$) and pulsatility index ($PI = [PSV - EDV] / \text{mean velocity}$) of the middle cerebral

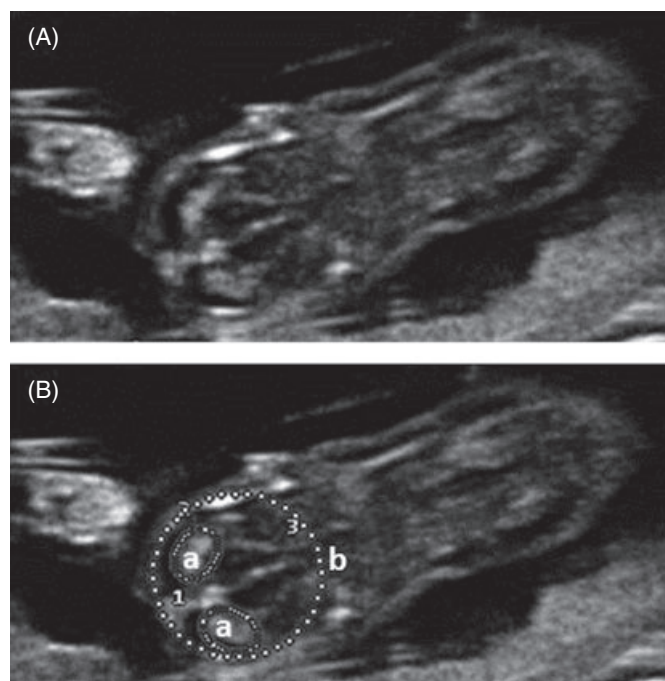


FIG 1. (A) Ultrasound image of a canine foetal cranium, in transverse view. (B) Note the brain echobiometry of the canine foetus, which is limited to the cerebral hemispheres (a) and cranium (b)

artery of the foetuses directly from Doppler waveforms using the ultrasound machine's electronic callipers (Hudson *et al.* 1997).

For vascular indices, Doppler ultrasonography was performed to determine vessel volume using the uniform insonation method according to Feliciano *et al.* (2012). Power Doppler ultrasonography was performed to increase the sensitivity in detecting the blood flow of the foetal middle cerebral artery and to transform the examination from angle-independent to insonation or incident angle (Drost 2007).

The data were analysed for normality of residuals and homogeneity of variances (F test). PROC MEANS-SASTM and GraphPad Prisma 4TM were used for analysis. The significance level was set $P < 0.05$ for all tests. Raw and transformed averages were evaluated using analysis of variance, and t-tests were applied for paired samples. Tukey's multiple comparison was performed to analyse significance for values between the weeks.

RESULTS

The mean pregnancy period in the 26 bitches was 58 days, and the mean number of puppies per pregnancy was four.

Foetal echoencephalography was performed to evaluate the morphological and quantitative characteristics of the central nervous system of the foetuses, and the tissue development during gestation was studied.

At the 5th week of pregnancy, a small amount of brain mass in the cranium was observed. The brain tissue mass increased progressively during the following weeks. Echogenicity and echotexture of brain tissue were constant over the period studied.

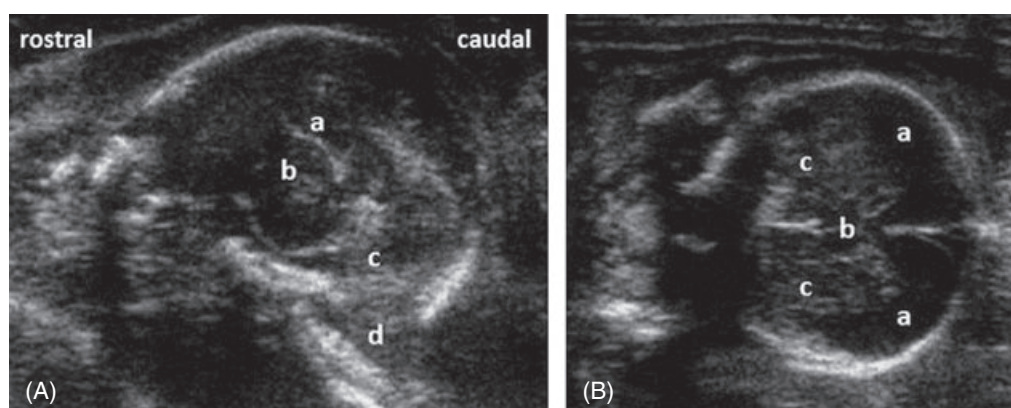


FIG 2. Echonocephalography of canine foetus at the 7th gestational week. (A) Sagittal view showing the following structures: (a) corpus callosum, (b) thalamus, (c) cerebral aqueduct and (d) cerebellum. (B) Transverse view showing the following structures: (a) lateral ventricles, (b) 3rd ventricle and (c) basal nuclei

Table 1. Mean values of the brain echobiometry of canine foetuses between the 5th and 8th gestational weeks

Index	Weeks of pregnancy				P (5%)
	5th	6th	7th	8th	
AC (cm ²)	0.96 ±0.31	1.84 ±0.50	3.06 ±0.63	4.37 ±1.00	<0.0001*
VC (cm ³)	0.73 ±0.34	1.90 ±0.73	3.94 ±1.27	6.93 ±2.25	<0.0001*
BMA (cm ²)	0.19 ±0.08	0.35 ±0.12	0.56 ±0.16	0.69 ±0.11	<0.0001*
BMV (cm ³)	0.04 ±0.02	0.09 ±0.05	0.20 ±0.16	0.22 ±0.05	<0.0001*
BMI	0.19 ±0.04	0.19 ±0.04	0.18 ±0.03	0.16 ±0.02	0.0957
BVI	0.04 ±0.01	0.04 ±0.01	0.04 ±0.01	0.04 ±0.01	0.2765

t-tests were performed for paired samples, with a 5% significance level. Area (AC) and volume (VC) of cranium, area (BMA) and volume (BMV) of brain mass, brain mass index (BMI) and brain volume index (BVI)

*Significant with P<0.05

At the 7th week of pregnancy, echonocephalography was performed to identify brain structures such as the corpus callosum, thalamus, cerebral aqueduct, cerebellum, lateral ventricles, 3rd ventricle, the cerebellar tentorium and basal nuclei (Fig 2). These structures were poorly identified in the 8th week of pregnancy due to ultrasound artefacts from the calcification of the foetal craniums at this stage of pregnancy. Echogenicity and echotexture of brain tissue were constant over the period studied.

Cerebral echobiometry identified increasing areas and volumes of the hemispheres and cranium (P<0.001) but not MBI or VBI over the gestational period studied. The values are summarised in Table 1.

Doppler ultrasonography was performed to determine vascular indices of the middle cerebral artery (Fig 3). Increasing PSV (P=0.0188) and EDV (P=0.0274) and decreasing PI (P<0.001) and RI (P=0.0002) were observed. The mean values of the middle cerebral artery in canine foetuses are shown in Table 2.

DISCUSSION

In veterinary obstetrics, ultrasonographic findings of the neurological system are used during prenatal care for detection of the cerebral hemispheres at day 37 of pregnancy, and the biparietal diameter is used for predicting the parturition date (Kustritz

Table 2. Mean values of the vascular index of the middle cerebral artery in canine foetuses at the indicated gestational weeks

Index	Weeks of pregnancy			P (5%)
	6th	7th	8th	
PSV (cm/s)	10.23 ±1.90	11.82 ±2.02	13.31 ±3.61	0.0188*
EDV (cm/s)	3.44 ±1.29	4.67 ±1.21	5.19 ±2.18	0.0274*
PI	1.42 ±0.27	1.31 ±0.18	1.02 ±0.13	<0.0001*
RI	0.76 ±0.06	0.69 ±0.04	0.63 ±0.10	0.0002*

t-tests were used for paired samples, with a 5% significance level. Peak systolic velocity (PSV), end diastolic velocity (EDV), vascular resistance (RI) and pulsatility index (PI)

*Significant with P<0.05

2005, Feliciano *et al.* 2007). The brain structures identified in this study have not yet been described in veterinary obstetrics and could be used for prenatal diagnoses of canine foetuses, similar to clinical evaluation in adult dogs described by Carvalho *et al.* (2004).

Foetal echonocephalography is important for the early diagnosis of foetal malformations, as hydrocephalia can compromise a pregnancy. This was demonstrated by Cruz *et al.* (2003) in two canine foetuses that presented with hydrocephalus, in which this diagnosis helped guide appropriate management of the pregnant female.

To the authors' knowledge, this study is the first to perform brain echobiometric studies and determine indices for monitoring the cranial and brain development of canine foetuses. Between the studied echobiometry values in canine foetuses, the MBI and VBI might have greater diagnostic importance because of the constant values (P>0.01) and the gestational age, skull size and breed independence depicted in this study. The MBI and VBI values presented can be used to detect foetal malformations such as hydrocephalus, as this may cause changes in these values. According to Carvalho and Chammas (2010) and Thomas (2010), ultrasound findings in dogs with hydrocephalus are typically ventriculomegaly and/or decreases in brain mass, two factors that may affect the BMI and BVI.

Studies performed by Miranda *et al.* (2010) and Blanco *et al.* (2011) depicted the importance of Doppler ultrasonography for pregnancy monitoring in bitches. This study determined the

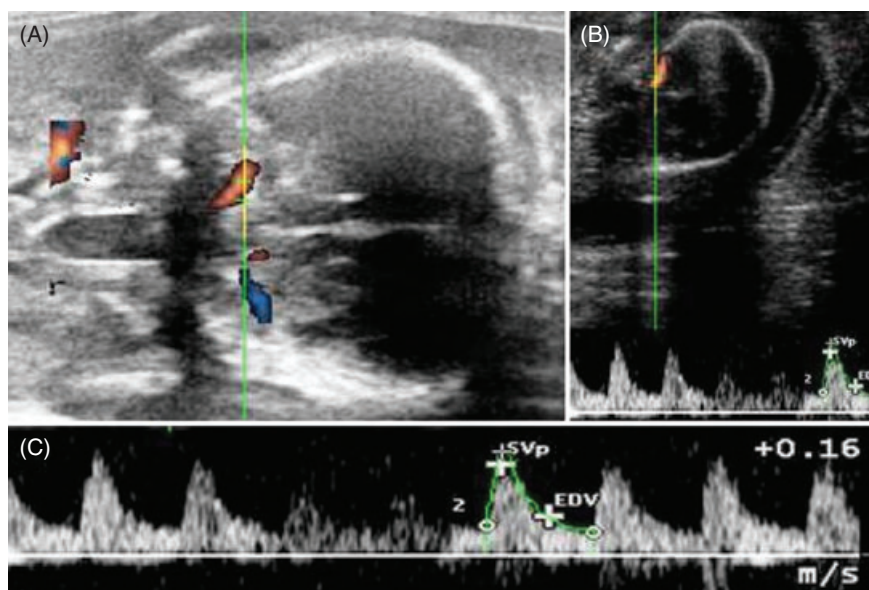


FIG 3. (A) Colour Doppler of the middle cerebral artery of fetuses. (B) Ultrasound spectral Doppler wave image that was used to determine vascular indexes of the middle cerebral artery. (C) Waveforms of vascularisation of the middle cerebral artery

normal vascular indices for the middle cerebral artery of canine fetuses, which will add to the utility of Doppler imaging.

The Doppler flowmetry of the middle cerebral artery in canine fetuses might be a suitable technique in routine examination. This technique requires a careful evaluation because of the influence of foetal movements, skull mineralisation degree and the possibility of repeating the Doppler examination in the same foetus. Vascular indices of foetal brains can be used in the early diagnosis of canine hydrocephalus and for prognosis in the treatment of neonates. This is supported by the findings of Carvalho and Chammas (2010) in adult dogs with hydrocephalus, who showed increased RI in the middle cerebral artery that decreased after treatment. This technique was not described in the evaluation of canine fetuses, however, accordingly the results of normal foetuses show the usefulness for prenatal examination in bitches.

CONCLUSION

In conclusion, echoencephalography and Doppler flowmetry of the middle cerebral artery in canine fetuses may be useful for prenatal diagnoses in dogs.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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