

## Triplex Doppler Ultrasonography in Prenatal of Pregnant Bitches

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### ABSTRACT

**Background:** The Dopplerfluxometry is a diagnostic technique that provides anatomical information and real-time dynamic tissue evaluation by determining the presence and direction of blood flow in a vessel and their hemodynamic features. Considering the importance of Doppler to pregnant women and the recent studies of vascular indices in pregnant animals and the application of news technologies for Doppler ultrasonography as spectral and power technology, the objective of this study was to determine the absolute values of the vascular indices from the uterine, umbilical and fetal aorta arteries in pregnant bitches, using new techniques of doppler ultrasonography.

**Materials, Methods & Results:** Twenty six multiparous bitches from the same kennel, weighting 5 - 25 kg, aged 4 - 6 years old, were selected to the current study. Doppler ultrasonography was performed to assess the maximum and minimum velocity, resistance and pulsatility indices of the uteroplacental, umbilical and fetal abdominal aorta arteries (5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> gestational week). The mean pregnancy period in twenty six bitches was 60 days and the mean number of puppies per pregnancy was four. Twenty five animals presented healthy pregnancies and one presented hydrallantois at the 7<sup>th</sup> and 8<sup>th</sup> gestational week. During Doppler exams in healthy pregnant bitches, there were progressive increase in PSV ( $P < 5\%$ ) to the uteroplacental, fetal aorta and umbilical arteries, constant values in the uteroplacental artery EDV ( $P > 5\%$ ) but increased EDV ( $P < 5\%$ ) in umbilical and fetal aorta and decreased PI and RI in the vessels evaluated ( $P < 5\%$ ). For the bitch with hydrallantois was observed EDV = 3 cm/s, PI and RI of 1.82 to 1.46; PSV was 11 cm/s, EDV = 2.1 cm/s, PI = 1.46 and RI = 0.76 at the 7<sup>th</sup> and 8<sup>th</sup> week of pregnancy, respectively, different values from those obtained in healthy bitches.

**Discussion:** In agreement with these authors and complementing the research carried with unpublished data for veterinary medicine and power technology (as the real values of PSV and EDV obtained between the 5<sup>th</sup> and 8<sup>th</sup> gestational week), the present study suggests that this method is useful for diagnosis of pregnancy pathologies, during different stages of pregnancy in bitches. It was verified by the results obtained that, in pregnant healthy bitches, the indices (PSV and EDV) of uteroplacental, umbilical and fetal aorta arteries increased progressively with the evolution of pregnancy and PI and RI decrease progressively. The progressive development of the fetal circulation coupled with the appearance of a diastolic peak velocity in these arteries, there is a drop the values of RI and PI decrease in the umbilical artery and RI decrease in the aorta artery. The increase of PSV and EDV values demonstrates the necessity of the body to intensify the maternal-fetal circulation, with consequent nutrient infusion and tissue development. In general, the physiological behavior of blood flow for the vascular indices observed is due to the development of gestational structures and maturation of placental and fetal circulation. Regarding pathological pregnancy. The vascular indices were different during the final stage of the gestation process. These findings reaffirm the idea that, in abnormal bitches pregnancies, there is a reduction in blood flow and higher resistance in the umbilical placental microcirculation. Thus, it was concluded that the maternal-fetal Doppler and power technology is a useful technique for prenatal diagnosis of pathological pregnancies in bitches.

**Keywords:** canine, pregnancy, vascular indices, Doppler.

## INTRODUCTION

In pregnant women, the Doppler allows evaluation of the maternal utero-placental circulation (uterine artery), (umbilical arteries) and fetal available, using a non-invasive diagnosis in cases of placental insufficiency and fetal assessment of hemodynamic changes that occur in response to deficit of oxygen to the fetus [10].

The Duplex Doppler for the study of pregnant bitches can help to detect canine fetal abnormalities, correlating its importance in medicine [5,12] and evaluating the vascular indices of the maternal fetal blood vessels: peak systolic (PSV) and end-diastolic velocity (EDV), vascular resistance (RI) and pulsatility index (PI). The spectral Doppler was used in veterinary to evaluate six animals and the values of PSV, EDV, PI and RI were determined in percentage values during pregnancy [1,11]. Although these studies have contributed to important advances in the evaluation of pregnant bitches, they were limited to partial search and relative vascular indices of the main vessels related to the gestational process.

Considering the importance of Doppler techniques for prenatal and obstetric care of pregnant women and the recent studies of vascular indices of pregnant animals in veterinary medicine and the application of new technologies for Doppler ultrasonography as spectral and power technology, the objective of this study was to determine the absolute values of the vascular indices from the uterine, umbilical and fetal aorta arteries in pregnant bitches using these new techniques of doppler ultrasonography.

## MATERIALS AND METHODS

Twenty six healthy multiparous Shi-tzu, English Bulldog, French Bulldog and Pug bitches from the same kennel, weighing 5 - 25 kg, aged 4 - 6 years old, were selected for the current study by physical and obstetric examinations.

The kennel owner was trained to detect the first signs of proestrus, in order to determinate the ideal time for mating. The confirmation of this period was carried out by observing the signs of estrus (i.e., acceptance of copulation by the female) and by vaginal cytology. The female in estrus and the male were put together during three days for mating or artificial insemination was performed. In case of natural mating, the acceptance to the male was inspected and assured.

Pregnancy diagnosis was carried out following two weeks of the mating or insemination, according to methodology employed in other trial [7]. Ultrasound (US) was performed with a 7.5 and 12.0 MHz linear transducer using MyLab™30 VET machine<sup>1</sup>. The US examinations were carried out by a single examiner.

During the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> gestational week, pulsed Doppler ultrasonography was performed to assess the PSV, end-diastolic velocity (EDV), resistance indice ( $RI = [PSV - EDV] / PSV$ ) and pulsatility index ( $PI = [PSV - EDV] / \text{mean velocity}$ ) [8] of the uteroplacental and umbilicals and abdominal aorta fetal arteries (Figure 1).

For vascular indices, Doppler ultrasonography was performed to determine vessel volume using the uniform insonation method [9]. *Power* Doppler ultrasonography was performed to increase the sensitivity in detecting the blood flow of the vessels and to transform the examination from angle-independent to insonation or incident angle [6]. Data were previously tested for normality of residuals and homogeneity of variances (F test).

### *Statistical analysis*

The PROC MEANS-SAS™ and the GraphPad Prisma 4™ softwares were used. Significance level was 5% for all tests. Raw and transformed averages were evaluated using analysis of variance and, additionally, the t-test for paired samples was applied and Tukey's multiple comparison test between the weeks was employed. Significance level was expressed as a *P*-value.

## RESULTS

The mean pregnancy period in twenty six bitches was 60 days and the mean number of puppies per pregnancy was four. Twenty five animals presented normal gestation and there was no harm to neonates during the pregnancy ultrasound monitoring. A Pug bitch had sonographic findings compatible with hydrallantois at the 7<sup>th</sup> gestational week. This animal was monitored by ultrasound during pregnancy until indicated time for the cesarean.

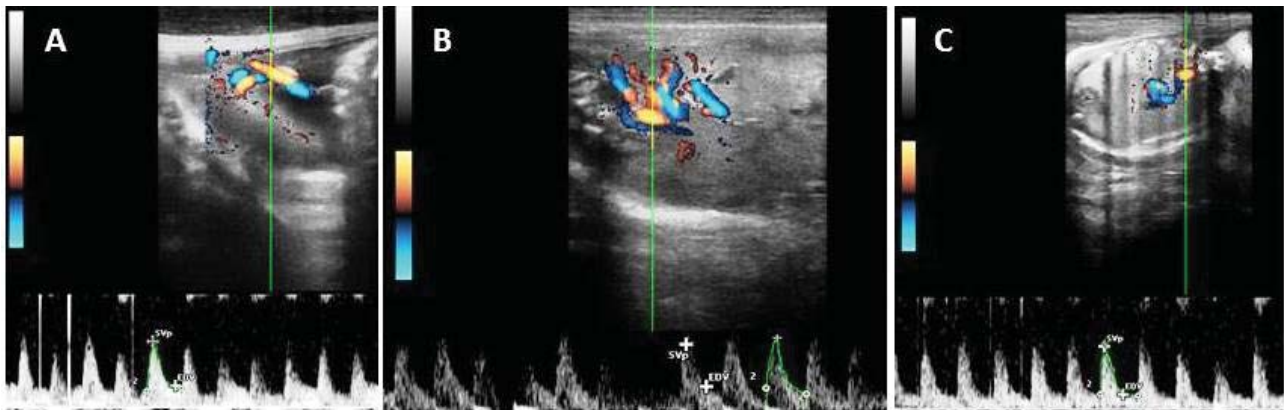
By Doppler ultrasonography evaluating the vascular indices of the uteroplacental artery of physiological pregnancy bitches presented a progressive increase for PSV ( $P < 0.001$ ), constant values for EDV and decrease of PI and RI, between the 5<sup>th</sup> and 8<sup>th</sup> gestational weeks. The mean values of uterine artery in bitches are shown in Table 1.

For the vascular indices of the umbilical artery of healthy bitches, there were progressive increase for PSV ( $P = 0.0048$ ) and EDV ( $P < 0.001$ ) and decrease for PI ( $P < 0.001$ ) and RI ( $P < 0.001$ ) during gestational development. The mean values of umbilical artery in bitches are shown in the Table 2.

Regarding to abdominal aorta of the fetuses were showed progressive increase for PSV ( $P = 0.0093$ ) and EDV ( $P < 0.001$ ) and decrease for RI ( $P < 0.001$ ) and PI ( $P < 0.001$ ), between 5<sup>th</sup> and 8<sup>th</sup> pregnancy weeks for healthy animals. The mean

values of umbilical artery in bitches are shown in Table 3.

By comparing the values obtained in bitches with normal pregnancies and pathological pregnancy, it was possible to detect changes in the behavior of vascular indices of the uteroplacental artery, during late pregnancy of the bitches. It were observed EDV= 3 cm/s, PI = 1.82 and RI = 1.46 at the 7<sup>th</sup> gestational week and PSV = 11 cm/s, EDV = 2.1 cm/s, PI = 1.46 and RI = 0.76 at the 8<sup>th</sup> week of pregnancy in the bitche with hydrallantois, different values fromthose obtained in healthy bitches.



**Figure 1.** Spectral and *Power* Doppler ultrasound images in pregnant bitches. Note in (A), (B) and (C) Doppler assessments to determinate of vascular indices in the umbilical, uteroplacental and fetal aorta arteries.

**Table 1.** Mean values of the vascular indices of uterine artery in pregnant bitches.

Index	Weeks of pregnancy				<i>P</i> (5%)
	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	
PSV (cm/s)	15.1 ± 3.9	16.5 ± 4.1	18.9 ± 4.4	22.4 ± 6.6	< 0.05*
EDV (cm/s)	7.2 ± 3.0	8.1 ± 3.1	8.9 ± 3.4	9.6 ± 4.7	0.1
PI	1.0 ± 0.3	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	< 0.05*
RI	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	< 0.05*

The *t*-test for paired samples, 5% significance level. PSV: Peak systolic velocity; EDV: End diastolic velocity; RI: vascular resistance; PI: pulsatility index. \*Significant if  $P < 0.05$ .

**Table 2.** Mean values of the vascular indices of umbilical artery in pregnant bitches.

Index	Weeks of pregnancy				<i>P</i> (5%)
	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	
PSV (cm/s)	14.3 ± 3.1	16.5 ± 4.4	18.0 ± 4.0	19.3 ± 6.3	< 0.05*
EDV (cm/s)	2.1 ± 0.6	3.5 ± 1.6	4.1 ± 1.0	6.2 ± 2.3	< 0.05*
PI	2.1 ± 0.2	2.0 ± 0.2	1.8 ± 0.4	1.6 ± 0.4	< 0.05*
RI	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	< 0.05*

The *t*-test for paired samples, 5% significance level. PSV: Peak systolic velocity; EDV: End diastolic velocity; PI: pulsatility index; RI: vascular resistance. \*Significant if  $P < 0.05$ .

**Table 3.** Mean values of the vascular indices of abdominal aorta fetal in pregnant bitches.

Index	Weeks of pregnancy				P (5%)
	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	
PSV (cm/s)	13.4 ± 4.2	15.1 ± 3.1	15.5 ± 3.4	17.9 ± 4.6	< 0.05*
EDV (cm/s)	1.9 ± 0.6	2.9 ± 0.9	3.8 ± 1.1	4.4 ± 1.1	< 0.05*
PI	2.0 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	1.8 ± 0.1	< 0.05*
RI	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	< 0.05*

The *t*-test for paired samples, 5% significance level. PSV: Peak systolic velocity; EDV: End diastolic velocity; PI: Pulsatility index; RI: Vascular resistance. \*Significant if  $P < 0.05$ .

## DISCUSSION

Studies using the Triplex Doppler [1,11] and using Duplex Doppler [5,12] have demonstrated the importance of these techniques for pregnancy monitoring in bitches. In agreement with these authors and complementing the research carried with unpublished data for veterinary medicine and power technology (as the real values of PSV and EDV obtained between the 5<sup>th</sup> and 8<sup>th</sup> gestational week), the present study suggests that this method is useful for diagnosis of pregnancy pathologies, during different stages of pregnancy in bitches.

In pregnant healthy bitches the indices (PSV and EDV) of uteroplacental, umbilical and fetal aorta arteries increased progressively with the evolution of pregnancy according with the literature [1], likewise the PI and RI decrease progressively [11]. In early pregnancy, the simultaneous presence of high values of RI and PI and the absence of diastolic flow in the aorta and umbilical arteries characterize a blood flow of high resistance. The progressive development of the fetal circulation coupled with the appearance of a diastolic peak velocity in these arteries, the values of RI and PI decrease in the umbilical artery and RI decrease in the aorta artery [13].

The reduction of RI and PI of maternal fetal blood flow is secondary to the perfusion increase of the vessels and fetal size increase [11]. The increase of PSV and EDV values demonstrates the necessity of the body to intensify the maternal-fetal circulation, with consequent nutrient infusion and tissue development [12]. In other words, there is an increasing demand to meet the vital organs (brain and heart), organs of the abdominal cavity and the placental bed [4].

In general, the physiological behavior of blood flow observed is due to the development of gestational

structures and maturation of placental and fetal circulation [3,12].

Regarding to pathological pregnancy, the values of vascular indices were different at the final stage of gestation process. These findings reaffirm the idea that, in abnormal bitches pregnancies, there is a reduction in blood flow and higher resistance in the umbilical placental microcirculation [2]. It is expected, because of the inadequate trophoblastic invasion and the evident changes in the parameters of the mode Doppler for uteroplacental, umbilical and fetal arteries reflect on image findings such as the increase in placental resistance (abnormal dopplerfluxometry of vessels) and in the fetal hemodynamic response (decrease in tissue oxygen supply) [5].

## CONCLUSION

It was concluded that the maternal-fetal Doppler (associated with power technology) is a useful technique for the diagnosis of pathological pregnancies and hemodynamic changes in the main vessels involved in pregnancy of bitches.

### SOURCE AND MANUFACTURER

<sup>1</sup>MyLabTM30 VET - ESAOTE, Genoa, Italy.

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**Ethical approval.** The trial was conducted following approval (process no 017314/10) by the Animal Welfare and Ethics Committee of the School of Agrarian and Veterinary Sciences of the São Paulo State University (FCAV/UNESP - Jaboticabal).

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



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