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Research Article

DOPPLER ULTRASONOGRAPHIC ASSESSMENT OF MATERNAL AND FETAL HAEMODYNAMICS IN NORMAL CANINE PREGNANCY

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ABSTRACT

The aim of this study was to assess the haemodynamic characteristics of maternal and fetal blood circulation during normal pregnancy period in she dogs using colour and pulsed wave Doppler ultrasonography. The blood flow waveforms of the uteroplacental arteries, umbilical arteries and fetal aorta were recorded and their respective blood flow velocities such as peak systolic velocity (PSV) and end diastolic velocity (EDV) and indices of resistive index (RI) and pulsatility index (PI) were recorded weekly from 3rd to 8th week in 20 pregnant she dogs. The results revealed that overall mean PSV, EDV of the uteroplacental arteries, umbilical arteries and fetal aorta increased significantly (P<0.01) between different weeks of pregnancy whereas overall mean resistive index (RI) of the uteroplacental arteries, umbilical arteries and fetal aorta were found to be significantly (P<0.01) lower at different weeks of pregnancy. The overall mean PI of the umbilical arteries and fetal aorta decreased significantly (P<0.01) in different weeks of pregnancy while it was not significant (P<0.01) in uteroplacental arteries. It is concluded that colour and pulsed wave Doppler ultrasonography can be a useful diagnostic tool to monitor the stage of pregnancy as well as for the prediction of adverse obstetric outcome in she dogs.

Keywords: Doppler ultrasonography, Female dog, Haemodynamics, Pregnancy, She dog

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The Doppler ultrasound is a simple and non-invasive method to determine maternal and fetal haemodynamics during pregnancy in female dogs (Nautrup, 1998). The Doppler examination displays the two dimensional flow information in colour superimposed on the B-mode image and detects the sound reflected from moving organ or blood vessels of pregnant animals such as uteroplacental arteries, umbilical arteries and fetal aorta arteries in which the altered frequency is converted to audible pulsed signal of pregnancy (Szatmari *et al.*, 2001).

Colour flow Doppler is a simple, rapid and functional method of evaluation which provides an immediate qualitative evaluation of the blood flow of the pregnant blood vessels. Pulsed wave Doppler has the ability to select the depth from which the Doppler information is received and helps to analyze the blood flow within a blood vessel (Blanco *et al.*, 2008). In pulsed wave Doppler technique, blood flow can be analyzed in 3 ways, viz., waveforms, resistive indices and flow velocity. The Doppler waveform represents haemodynamic changes in the blood flow during the cardiac cycle. The objective evaluation of the waveform is obtained by calculating velocity indices which describes the relationship between the systole and diastolic bloodstream. The velocity indices commonly used in human obstetrics are the resistance index (Pourcelot, 1974) and pulsatility index (Gosling and King, 1974). These would be extremely valuable in diagnosing early pregnancy and even preventing abnormal gestation in compromised canine pregnancies (Blanco *et al.*, 2008).

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MATERIALS AND METHODS

Experimental animals: A total of twenty pregnant she dogs, irrespective of breeds, age and body weight presented to the Veterinary Hospital, Teaching Veterinary Clinical Complex, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Aizawl, Mizoram, India were utilized for the present study.

Ultrasonographic evaluation: Ultrasonographic examination (Esoate MyLab 40 vet Genua, Netherland) was carried out using a 5-8 MHz microconvex transducer for detection of pregnancy from 3rd to 8th week at weekly interval after last day of successful mating (3 breeding allowed in between day 1 and day 6 of estrus). Prior to the examination, the abdomen area was clipped and cleaned thoroughly with an alcohol swab for proper skin contact. Conductive gel was used as a coupling media between the scan head and skin. The animals were restrained calmly and put in dorsal recumbency, which was changed to right or left lateral recumbency as required for optimal ultrasound scanning. The clipped area was thoroughly scanned starting from the inguinal region placing the probe in long axis to the body of the bitch towards the ventral midline for detection of the maternal and fetal blood vessels using colour flow mode and their blood flow

waveforms were later recorded by pulsed wave Doppler mode. The colour Doppler was applied near around the uterus to detect the blood vessels associated with pregnancy such as utero-placental arteries, umbilical arteries and fetal aorta arteries whereas, pulsed wave Doppler was applied to determine sample volume, using the uniform intonation angle method. All the examinations and measurements were disregarded if the angle of intonation was > 20º. The cursor was placed properly in the particular blood vessels to measure the trace of blood velocities and indices, which were obtained automatically following software identification of the ultrasonic scanner for each waveform. The measurements of PSV, EDV, PI and RI of the blood vessels were automatically calculated for each waveform after the waveforms of at least three consecutive cardiac cycles from the most caudal fetus of each uterine horn viewed (Scotti *et al.*, 2008).

Statistical analysis: The statistical analyses were performed using the software SPSS 20 and Microsoftexcel 2007. Two way ANOVA was used to compare the relation between weeks of pregnancy and principal haemodynamics parameters of all vessels examined after mating. Significance was accepted at a level of $P<0.05$.

RESULTS AND DISCUSSION

In the present study, the utero-placental artery was detected in the neighborhood of the gestational sac or between the two gestational sacs at 3rd week of mating (Fig. 1) which was also supported by previous workers (Feliciano *et al.*, 2014). The mean values (cm/s) of PSV of the utero-placental arteries in the present study showed a steep increase waveform from the 4th week and decreased later in the 8th week of pregnancy (Table 1). The present finding was analogous with the observation made by previous workers (Feliciano *et al.*, 2014). Conversely the mean values (cm/s) of EDV increased as the pregnancy progressed. As predictable, in this study, in all the animals, PSV and EDV of this artery increased progressively during gestation, as has been reported for utero-placental arteries by previous worker (Nautrup, 1998). The RI in the present study decreased from the 5th week until the last week of pregnancy which is supported by the previous workers (Feliciano *et al.*, 2014). RI of uterine artery increased in pathological or abnormal canine pregnancies and decreased in normal pregnancies. It was also reported that these differences could not be accredited by weight, age or litter size as they showed to have no effect on Doppler parameters (Blanco *et al.*, 2011). While in case of PI, it was increased from 4th week of pregnancy and then decreased until the end of the week. The present result was not in accordance with the results obtained by previous workers (Feliciano *et al.*, 2014). It might be due to difference in

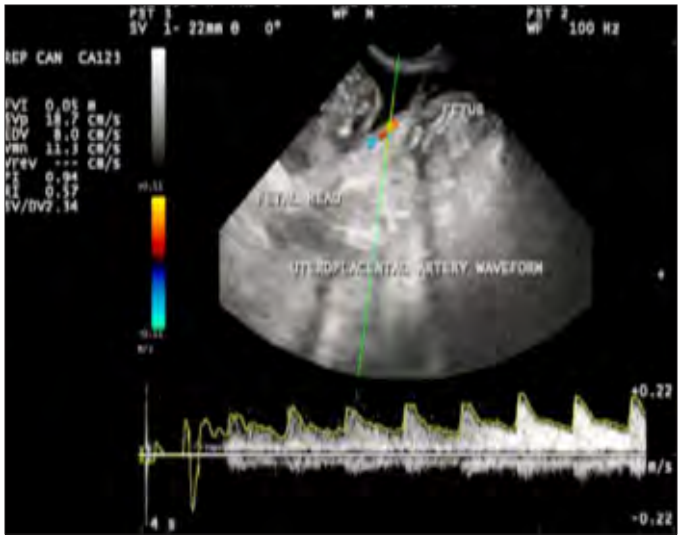

Fig. 1. Ultrasonographic image of utero-placental artery using colour flow Doppler mode (coloured area) and waveform visualized by pulsed wave Doppler mode (white arrow)

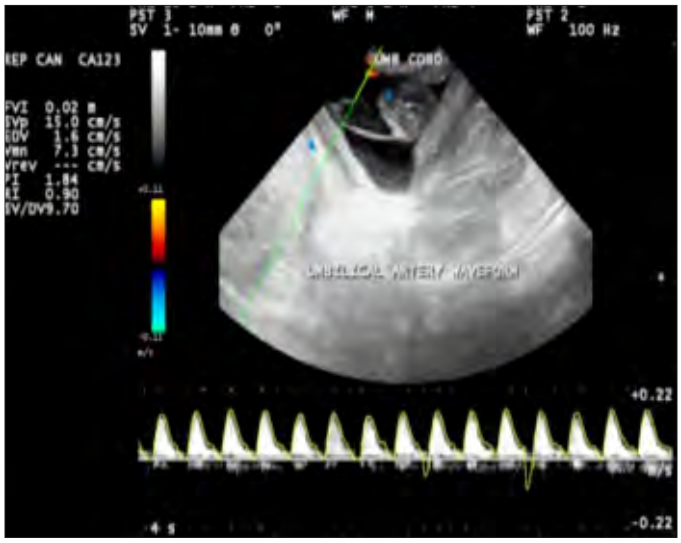

Fig. 2. Ultrasonographic image of umbilical artery using colour flow Doppler mode and wave form visualized by pulsed wave Doppler mode

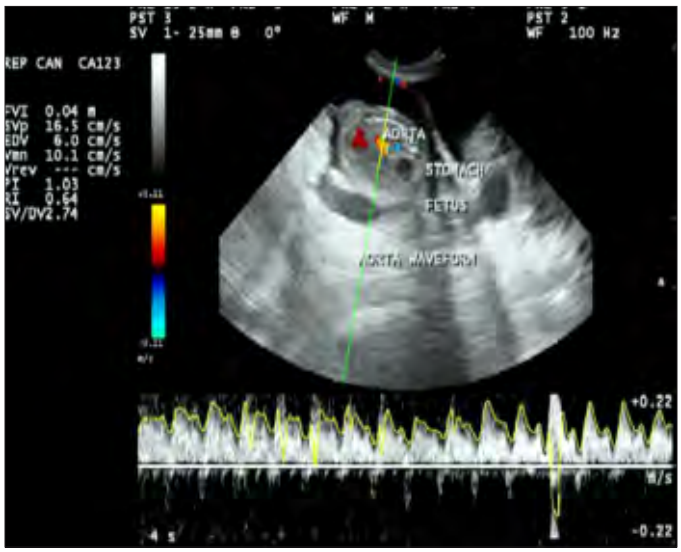

Fig. 3. Ultrasonographic image of fetal aorta artery using colour Doppler mode and waveform visualized by pulse wave Doppler mode

Table 1

Blood flow velocities and indices of uteroplacental arteries in female dogs by using colour Doppler ultrasound at different weeks of pregnancy (Mean ± SE)

Index	PSV (cm/s)	EDV(cm/s)	RI (cm/s)	PI (cm/s)
Week of pregnancy				
3rd	12.25± 0.73^{a}	3.68± 0.74^{a}	0.62 ± 0.06^{ab}	1.14 ± 0.13^{a}
4th	14.25± 0.50^{ab}	5.35 ± 0.51^{ab}	0.63 ± 0.03^{ab}	1.14± 0.10^{a}
5th	13.20 ± 0.93^{ab}	5.45 ± 0.27^{ab}	0.78 ± 0.10^{b}	1.26± 0.10^{a}
6th	15.55± 0.84^{b}	5.91 ± 0.73^{ab}	0.60 ± 0.06^{ab}	0.85 ± 0.06^{a}
7th	15.63 ± 0.51^{b}	7.47 ± 0.52^{b}	0.44 ± 0.02^{a}	0.70 ± 0.05^{a}
8th	11.77 ± 0.98^{a}	5.21 ± 0.81^{ab}	0.72 ± 0.09^{ab}	1.21 ± 0.28^{a}
F-cal value	4.501*	3.775*	2.867*	2.324^{NS}

Different superscript letters within a same week indicate differences (P < 0.05) within each column; *P < 0.05; NS Non significant.

Table 2

Blood flow velocities and indices of umbilical arteries in female dogs by using colour Doppler ultrasound at different weeks of pregnancy (Mean ± SE)

Index	PSV	EDV	RI	PI
Weeks of pregnancy				
4th	8.85 ± 1.17^{a}	0.01 ± 0.01^{a}	1.21 ± 0.13^{b}	2.78 ± 0.57^{b}
5th	8.95 ± 0.67^{a}	0.06 ± 0.06^{a}	1.00 ± 0.00^{ab}	2.53 ± 0.12^{b}
6th	11.45 ± 1.15^{a}	0.68 ± 0.31^{ab}	0.98 ± 0.18^{ab}	2.19 ± 0.16^{b}
7th	17.85 ± 1.15^{b}	1.67 ± 0.32^{b}	0.90 ± 0.02^{ab}	1.73 ± 0.07^{ab}
8th	17.47 ± 0.88^{b}	3.39 ± 0.68^{c}	0.60 ± 0.09a	0.69 ± 0.12^{a}
F-cal value	18.814*	14.663*	4.095*	8.568*

Different superscript letters within a same week indicate differences (P < 0.05) within each column; *P < 0.05

Table 3

Blood flow velocities and indices in fetal aorta arteries by using colour Doppler ultrasound at different weeks of pregnancy (Mean ± SE)

Index	PSV	EDV	RI	PI
Weeks of pregnancy				
4th	11.48 ± 0.95^{a}	0.60 ± 0.37^{a}	1.67 ± 0.36^{b}	2.50 ± 0.29^{b}
5th	13.98 ± 1.20^{ab}	2.74 ± 0.75^{b}	1.27 ± 0.24^{ab}	2.23 ± 0.45^{b}
6th	15.91 ± 0.87^{b}	0.80 ± 0.31^{ab}	1.03 ± 0.05^{ab}	2.05 ± 0.18^{ab}
7th	16.40 ± 1.25^{b}	2.13 ± 0.65^{ab}	0.94 ± 0.05^{ab}	1.91 ± 0.14^{ab}
8th	13.80 ± 2.82^{ab}	2.58 ± 0.40^{ab}	0.64 ± 0.10^{a}	0.95 ± 0.18^{a}
F-cal value	3.5528*	3.567*	3.501*	4.502*

Different superscript letters within a same week indicate differences (P < 0.05) within each column; *P < 0.05

breed, different portion of utero-placental artery examined and due to the angle difference of transducer at the time of observation (Blanco *et al.*, 2008).

Free floating umbilical cord was observed near the embryonic or fetal abdominal wall using color flow mode (Fig. 2) and identified from the 4th week onwards in normal pregnancies which was also supported by previous workers (Nautrup, 1998; Feliciano *et al.*, 2014) in canine.

In the present study, it was recorded that the flow velocity waveforms of umbilical cord have a characteristic saw-tooth appearance of arterial blood flow (Fig. 2). The umbilical arteries blood flow was characterized only by the systolic waveform until the 6th week of pregnancy and end diastolic waveform was observed after the 6th week of pregnancy which was in agreement with the observations made by previous workers (Di Salvo *et al.*, 2006). The umbilical vein was observed flat during all weeks of pregnancy.

The peak systolic velocity of the umbilical arteries increased significantly (P<0.05) at different weeks of pregnancy in female dogs (Table 2). Conversely the EDV increased significantly (P<0.05) from 4th week to 8th week of pregnancy which was also supported by previous workers (Feliciano *et al.*, 2014). The increase supply of

PSV and EDV indicates the necessity of the body to intensify the maternal and fetal circulation, with consequent nutrient infusion and tissue development (Nautrup,1998).

The fetal aorta was seen ventral to the vertebral column of the fetus using trans-abdominal B-mode ultrasonography and blood flow of the fetal aorta arteries was detected from the 4th week of pregnancy and more regularly from the 5th week onwards by applying colour flow mode (Fig. 3). The PSV mean values were significantly increased (P<0.05) from the 4th week until the 7th week of pregnancy in the present study which was in accordance with the findings of previous workers (Feliciano *et al.*, 2014).

The fetal aorta EDV mean values in the present study were significantly increased (P<0.05) from the 5th week and slightly decreased from the 6th week and increased again from the 7th week until the last week of pregnancy (Table 3). It was also supported by previous workers (Di Salvo *et al.*, 2006) and it was observed that the EDV mean values were significantly increased (P<0.05) from the 5th week until birth.

The present study confirmed that the velocities such as PSV and EDV of utero-placental, umbilical and fetal aorta arteries increased significantly with the evolution of pregnancy as a result of increasing demand of vital nutrients and oxygen to meet the fetal vital organs (brain and heart), organs of the abdominal cavity and the placental bed. Thus, the increasing demand of blood supply from the mother to the fetus helps in the development of the healthy pregnancy (Costa *et al.*, 2005).

During early pregnancy, the resistive index and pulsatility index showed higher vascular resistance due to immature development of the placenta. As the pregnancy progresses, the maturation of placental circulation takes place (Blanco *et al.*, 2011) and vascular resistance of the blood vessels reduces subsequently. The progressive and significant decrease of the RI and PI can be correlated with the placental development and probably with the histological changes of the spiral arteries. The higher vascular resistance of the blood vessels such as utero-placental, umbilical and fetal aorta arteries in late pregnancy stage has been correlated with the intra-uterine growth retardation (Owen and Ogston, 1977), maternal hypertension (Gudmundsson *et al.*, 1990) and perinatal death (Dubiel *et al.*, 2003). The present findings when compared with the literatures of different researchers showed no pregnancy was detected abnormal at the time of assessing the principal blood flow parameters such as PSV, EDV, RI and PI of the pregnant blood vessels. It is therefore, concluded that the Doppler ultrasonography can be a future diagnostic tool to monitor the stage of pregnancy to prevent any pregnancy related complications.

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