Doppler ultrasonographic evaluation of ductus venosus blood flow in 55 canine fetuses

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Abstract

Purpose The ductus venosus (DV) blood flow has been studied in fetal lambs and in humans. This study aims to describe the velocities, the Doppler indices and the morphological patterns of the venous blood flow in the DV of canine fetuses during physiological pregnancy.

Methods The DV of 55 canine fetuses has been evaluated and the waveforms described using B-mode, color and pulsed-wave Doppler ultrasound technology.

Results We found 48 diphasic waves and 7 threephasic waves. No monophasic waveform was found. Six of seven threephasic waveforms belonged to litters in which perinatal mortality occurred. The peak velocity during ventricular systole S (cm/s), the peak velocity during the ventricular diastole D (cm/s), the velocity during atrial contraction aV (cm/s), the S/D index, the pulsatility index (PI) and the resistance index were measured.

Conclusions All Doppler indices and velocities were significantly correlated with each other ($p < 0.05$). The number of newborn puppies and the age of bitches were not related to DV vascular indices or flow rate ($p > 0.05$). Gestational age was proportional to the PI ($p < 0.02$). Doppler ultrasonography allows the assessment of DV blood flow in canine fetuses during pregnancy.

Keywords Sonography · Ductus venosus · Canine fetuses

SOMMARIO

Scopo Il flusso del dotto venoso (DV) è stato studiato in feti di agnelli e negli esseri umani. Questo studio intende descrivere le velocità, gli indici Doppler e i modelli morfologici nel DV di feti di cane, durante la gravidanza fisiologica.

Metodi E' stato valutato il DV di 55 feti di cane e descritte le forme d'onda utilizzando B-mode, Color Doppler e Doppler pulsato.

Risultati Abbiamo rilevato 48 onde difasiche e 7 onde trifasiche. Nessuna forma monofasica è stata rilevata. Sei delle sette forme d'onda trifasica appartenevano a cucciolate in cui si è verificata mortalità perinatale. Sono stati misurati il picco di velocità durante la sistole ventricolare S (cm/s), la velocità di picco durante la diastole ventricolare D (cm/s), la velocità durante la contrazione atriale aV (cm/s), l'indice S/D (S/D), l'indice di pulsatilità (PI) e l'indice di resistenza (RI).

Conclusioni Tutti gli indici Doppler e le velocità erano significativamente correlati tra loro ($P < 0.05$). Il numero di cuccioli appena nati e l'età delle femmine non erano correlati con gli indici vascolari del DV o con la portata ($p > 0,05$). L'età gestazionale era proporzionale alla PI ($p < 0,02$). Il Doppler permette di valutare il flusso sanguigno del DV nei feti di cane durante la gravidanza.

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Introduction

The ductus venosus Arantii (DV) [1] is a short vessel that, during fetal and neonatal life, connects the umbilical vein and the caudal vena cava [2]. DV arises from the left

branch of the portal vein and, together with the left hepatic vein, enters an “aneurismatic” dilation just before joining the caudal vena cava [3].

Because of this vascular communication the oxygenated blood, originating from the placenta, can reach the heart shortly without passing through the hepatic sinusoids. In this way oxygen can distribute preferentially to the fetal cardiac tissue and brain [4]. It was postulated that, during fetal hypoxia, over the 70 % of blood flow passing through the umbilical vein can shunt in the DV [5].

In dogs DV closes within 10 days after birth [6] through thrombotic obliteration and fibrotic transformation in the venous ligament [7].

The patency of DV in dogs and cats has been associated with congenital portosystemic shunts [2].

Fetal lambs have been used as animal model to study DV in humans [4, 5]. In humans, Doppler evaluation of DV is believed to be a relevant indicator of fetal well being [8, 9].

The ultrasonographic assessment of blood flow in the DV is performed in human medicine during evaluation of fetal hemodynamics [10].

The DV waveform and velocimetry have never been described in dogs.

The aim of this study is to evaluate the Doppler ultrasonographic patterns of the DV and to measure the DV flow velocities and waveform indices in canine fetuses.

Materials and methods

The study was performed in accordance with Italian legal animal welfare regulations. Informed consent was obtained from each owner. Dogs were deemed normal based upon physical exam, serum chemistry and urinalysis results.

Twenty-nine clinically healthy pregnant bitches (primiparous or multiparous) were recruited. The group included mixed and pure breeds, such as: American Staffordshire, Dogo Argentino, Bernese Mountain Dog, Boston Terrier, Chihuahua, Dogue de Bordeaux, English Bulldog, French Bulldog, Golden Retriever, Jack Russell Terrier, Labrador Retriever, Pinscher, Rhodesian Ridgeback, Shetland Sheepdog, and Yorkshire Terrier.

All bitches underwent an accurate reproductive cycle monitoring by vaginal cytology and plasma progesterone measurement in order to deduce the LH surge and the optimal time for mating [11, 12]. Only bitches where the initial progesterone sample at proestrus was <2 ng/mL were included in the study. The day of the LH peak was regarded as the first day on which the serum progesterone was ≥ 2 ng/mL [11, 12]. Gestational age was calculated from the estimated LH surge (D 0). Plasma progesterone concentration was determined using a quantitative test

based on ELFA technique (Enzyme Linked Fluorescent Assay; MiniVidas, bioMérieux). Assay principle combines an enzyme immunoassay competition method with a final fluorescent detection [13].

Date of delivery and number of puppies were recorded in all bitches.

Bitches underwent ultrasonographic examination during pregnancy once. Ultrasonographic diagnosis of pregnancy was made using two-dimensional and Doppler ultrasound examination (Esaote MyLab 70, Genua, Italy) with a 7.5–13 MHz linear electronic multi-frequency probe. Before imaging, the subjects were placed in dorsal recumbency and hair clipped from the costal arch to the inguinal region. Then a conductive gel was applied to the skin. Pharmacological restraint was never required.

In order to avoid to repeatedly measure the same fetus, we evaluated only two fetuses for each pregnancy, except in case of singleton: on the basis of their localization, the most cranially and the most caudally into the uterus, we were sure that they were different.

The well-being of the canine fetus was checked through ultrasonographic evaluations of the fetal movements [14] and heartbeat [14, 15].

First, the fetus abdomen was scanned to identify the intra-abdominal course of the umbilical vein. This was achieved by directing the ultrasound beam transversely or longitudinally through the fetal abdomen at the level of the liver. Aorta and caudal vena cava were revealed using the color Doppler (velocity setting <0.25 m/s): the DV appeared as a short trumpet-shaped vessel with a “sparkling” appearance, arising from the umbilical vein and entering the caudal vena cava (Fig. 1). This color phenomenon, used to correctly identify the DV, is known as “color aliasing” and was also obtained after changing the pulse repetition frequency (PRF). A section of the DV long axis was obtained and pulsed-wave Doppler was subsequently used to acquire the waveforms.

The angle of the pulsed Doppler sample volume was adjusted for angle-dependent measurements between 20° and 60° .

The Doppler and color filters were set at 40–100 Hz, and the velocity was set at $8\text{--}12 \times$ (number of waveforms in one screen image). The sample volume was set to 1–2 mm, in order to avoid the signal from other vessels nearby.

The same operator conducted all examinations. A sequence of at least three successive and symmetric waves was required to register measurements during the cardiac cycle using the manual trace function. According to morphology pulsed Doppler waveforms were classified as monophasic, diphasic or threephasic [7]. Waveform was considered monophasic when no modulations were seen other than those attributable to respiration; diphasic when modulations did not reach the baseline; threephasic when

Fig. 1 B-mode and color Doppler image of a canine fetus. The umbilical vein (*thin arrow*) passes through the liver parenchyma and, becoming narrow, gives rise to the ductus venosus (trumpet-shaped vessel with sparkling appearance: *arrowhead*). The ductus venosus enters the caudal vena cava (*thick arrow*)

two modulations were seen above the baseline and one was seen below the baseline [7]. Images were collected and downloaded to a computer.

In order to evaluate the DV blood flow patterns, several Doppler velocities and indices were calculated. We measured the peak velocity during ventricular systole (S cm/s), during ventricular diastole (D cm/s) and during atrial contraction (aV cm/s) [5]. Moreover the Doppler indices were recorded. The S/D index (S/D) was the ratio between the peak systolic (S) and the end-diastolic maximum (D) frequency shift. The pulsatility index (PI) was calculated according to the formula: $PI = (S - aV)/M$ where aV represents the velocity during atrial contraction and M the mean maximum Doppler-shifted frequency over the cardiac cycle. The resistance index (RI) was calculated according to the formula: $RI = (S - aV)/S$. These velocities and indices were automatically calculated by the ultrasound unit. Each index and velocity value was the mean of three symmetric and consecutive waveforms.

A statistics software package (JMP ver. 7 for Windows) was used to measure by a multiple regression model the association between the ultrasonographic values (S/D , S , D , aV , PI , RI) and the independent variables: age of the bitch, pregnancy duration, and number of delivered puppies. A regression model was applied for the variables aV , PI , and RI (SPSS ver. 18 for MacOS).

The Pearson product moment correlations between the ultrasonographic variables (S/D , S , D , aV , PI , RI) were also calculated. Statistical significance was set to $p \leq 0.05$.

Results

Twenty-nine bitches, 1–7 years old (2.8 ± 1.6), and weighing from 1.5 to 56 kg (25 ± 18.8 kg) underwent ultrasound examination.

Out of 119 total puppies, 78 were born by vaginal parturition from fifteen bitches and 41 by Caesarean section from fourteen bitches. Litter sizes ranged from 1 to 15 (mean value of 4.25 ± 3.54). Neonatal mortality (within 48 h of birth) was 5 % (6/119). The remaining puppies were healthy.

Bitches underwent ultrasonographic examination once during pregnancy. The bitch with lowest gestational age was 36 days pregnant and the bitch with the higher gestational age was 66 days pregnant.

It was possible to identify and measure the DV waveform in all considered fetuses ($n = 55$).

Two different types of waveforms were found (diphasic and threephasic waveforms).

The 48/55 (87 %) waveforms were diphasic while the 7/55 (13 %) waveforms were threephasic. No monophasic waveforms were found.

Six of seven threephasic waveforms were observed in litters with neonatal mortality.

The Pearson's correlations coefficients were all significant among the ultrasonographic variables (S , D , aV , S/D , PI , RI) ($p < 0.05$) (Fig. 2). The aV value showed a high relation with PI and RI ($p < 0.0001$). The age of bitches and the number of newborn puppies were not statistically related to the flow values and indices. The gestational age was directly proportional to PI ($p < 0.02$).

Discussion

The morphological evaluation and velocimetry of the DV during pregnancy has been described in humans and sheep [4, 5, 7]. Our study describes the DV in canine fetus and its main flow characteristics recorded using non-invasive Doppler sonography.

Fig. 2 Correlation matrix reporting the Pearson correlation coefficients (*upper diagonal*) and the scatterplots of the variables, along with linear regression lines and confidence bands. All coefficients are significant with $p < 0.05$. The character body of the coefficients is proportional to the magnitude of the correlation. On the diagonal the variable symbols are reported: S/D ratio (SD); peak systolic frequency shift (S); end-diastolic maximum frequency shift (D); velocity during atrial contraction (aV); pulsatility index (PI); resistance index (RI)

The DV is a small trumpet-shaped vessel of a few millimeters length and width, originating from the umbilical vein and reaching the caudal vena cava near the confluence of the hepatic veins, gradually reducing its caliber (Fig. 1).

The DV was always identified using color Doppler in longitudinal or transverse section of the fetus abdomen near the diaphragm, before the confluence of the hepatic veins. The sparkling appearance of the DV (due to the sudden change of the blood flow that becomes disturbed with nonuniform and irregular distribution of low wall shear stress) allowed the operator to distinguish this vessel from the hepatic veins: the blood flow passing through the DV is accelerated and, because of its shape, creates a mosaic of colors called “color aliasing artifact” [5]. The pulse repetition frequency was set for high-speed blood flow; however, the maximum measurable velocity exceeded the Nyquist’s limit and the aliasing artifact was always present. This means that the blood flow through the DV is highly turbulent and accelerated without changing in directions.

Ductus venosus flowmetry was measured in the 100 % of fetuses considered. This percentage was slightly higher than that shown in lambs and humans [5–16]. The major difficulty in identifying the DV in the early pregnancy depended on small size of the fetuses, while in late pregnancy on active fetal movements and maternal intolerance to the manipulation of the abdomen.

We were able to visualize the flow in the DV from a minimum of 36 days of pregnancy until the day of delivery. However most of our measurements were performed on bitches over 49 days of pregnancy.

Concerning waveform morphology, we found 48 diphasic waves (Fig. 3) and 7 threephasic waves (Fig. 4).

No monophasic waveform was found. Six of seven threephasic waveforms belonged to litters in which perinatal mortality occurred.

In humans, the waveform is commonly used to assess fetal hemodynamics [10] and the presence of threephasic wave in the DV (reverse-a-wave which corresponds to a deflection during atrial contraction) is considered a strong predictor of neonatal mortality independently of gestational age [17].

In our study, the percentage of stillbirths is low and insufficient to draw conclusions about it.

The gestational age was not related to the flow values or indices except for the PI that increase during pregnancy ($p < 0.02$). These results are different from those described in human fetuses, where Doppler indices decreased continuously with increasing of gestational age [18, 19] and different from those described as fluctuating in fetal lambs during pregnancy [5].

The aV value was highly positively correlated to PI ($p < 0.0001$) and RI ($p < 0.001$). The aV value reflects the simultaneous hemodynamic status of the venous reservoir of blood awaiting return to the heart, the compliance of the right ventricle and the pressure and contractility of the right atrium. The aV value decreases in human fetuses and lambs as the stiffness of the right heart increases; i.e. during myocardium hypoxia [20, 21]. The PI and RI values represent the vascular resistances. In our study, in fact, as PI and RI increased the aV value decreased.

Our study cannot be directly correlated with those conducted on lambs and humans as it was not performed on

Fig. 3 Diphasic waveforms of normal ductus venosus in a canine fetus. Peak systolic velocity (S); peak diastolic velocity (D) and velocity during atrial contraction (aV)

Fig. 4 Threephasic waveforms of ductus venosus in a canine fetus. Peak systolic velocity (S); peak diastolic velocity (D). Reverse-a-wave (aV)

the same fetuses during pregnancy. This comparison may be more accurate monitoring the fetus vascular blood flows in the same bitches during pregnancy. Moreover, the bitch has a greater number of fetuses compared to man and sheep, and it may lead to different results arising from the difference of species.

Our work describes the possibility to assess and monitor velocimetry of the DV during pregnancy in dogs. Currently the well-being of the canine fetus has been evaluated by ultrasonography considering fetal movements [14], fetal heartbeat [14, 15] and fetal arterial blood flows [22]; while the ultrasonographic study of DV was limited to the monitoring of its closure after birth [6].

The study of the DV may therefore be regarded, as occurs in human medicine [10], as an interesting parameter to assess fetal health in dogs.

The identification of DV and the measurement of its blood flow in canine fetuses were assessed in each bitch we considered.

Our work describes the possibility to easily evaluate the DV in dogs during pregnancy and to measure its flow using Doppler sonography.

Gestational age was found to be positively correlated to the PI, while the age of bitches and the number of newborn puppies were not correlated to the flow values and indices we considered.

The PI and RI indexes were strongly positively correlated to each other and with aV.

In our opinion the study of DV should be regarded as a parameter of fetal well-being as occurs for fetal movements and heartbeat. Further studies are needed.

As reported in human literature, the possible correlation between threephasic waveform of DV and neonatal mortality in canine fetuses should be further investigated.

Conflict of interest G. Barella, S. Faverzani, M. Faustini, D. Groppetti, and A. Pecile declare that they have no conflict of interest.

Informed consent The study was conducted in accordance with the ethical standards dictated by applicable law. Informed consent was obtained from each owner to enrolment in the study and to the inclusion in this article of information that could potentially lead to their identification.

Human and animal studies The study described in this article does not contain studies with human or animal subjects performed by any of the authors.

References

1. Arantii JC (1563) De humano foetu libellus. Ed. Lugduni Bata-vorum: Ex Officina Felicis Lopes de Haro 1563:40–50
2. Burton CA, White RN (1999) The angiographic anatomy of the portal venous system in the neonatal dog. *Res Vet Sci* 66:211–217
3. White RN, Burton CA (2000) Anatomy of the patent ductus venosus in the dog. *Veterinary Record* 146:425–429
4. Tchirikov M, Eisermann K, Rybakowski C, Schröder HJ (1998) Doppler ultrasound evaluation of ductus venosus blood flow during acute hypoxemia in fetal lambs. *Ultrasound Obstet Gynecol* 11:426–431
5. Panarace M, Garnil C, Cané L, Rodriguez E, Medina M (2008) Echo-Doppler assessment of resistance and velocity of blood flow in the ductus venosus throughout gestation in fetal lambs. *The-riogenology* 70:648–654
6. Lamb CR, Burton CA (2004) Doppler ultrasonographic assess-ment of closure of the ductus venosus in neonatal Irish Wolf-hounds. *Veterinary Record* 155:699–701
7. Vade A, Lim-Dunham J, Iqbal N (2001) Imaging of the ductus venosus in neonates. *J Ultrasound Med* 20:681–687
8. Kiresud T, Eik-Nes ASH, Blaas HG, Hellevik LR (1992) For-amen ovale: an ultrasonographic study of its relation to the inferior vena cava, ductus venosus and hepatic veins. *Ultrasound Obstet Gynecol* 2:389–396
9. Kiresud T, Eik-Nes ASH, Hellevik LR, Blaas HG (1992) Ductus venosus. A longitudinal Doppler velocimetric study of the human fetus. *J Matern Fetal Invest* 11:2–5
10. Martins WP, Kiresud T (2013) How to record ductus venosus blood velocity in the second half of pregnancy. *Ultrasound Obstet Gynecol* 42:245
11. Concannon P, Hansel W, McEntee K (1977) Changes in LH, progesterone and sexual behaviour associated with preovulatory luteinisation in the bitch. *Biol Reprod* 17:604–613
12. Michel E, Spörri M, Ohlerth S, Reichler IM (2011) Prediction of parturition date in the bitch and queen. *Reprod Domest Anim* 46:926–939
13. Brugger N, Otdorff C, Walter B, Hoffmann B, Braun L (2011) Quantitative determination of progesterone (P4) in canine blood

- serum using an enzyme-lined fluorescence assay. *Reprod Domest Anim* 46:870–873
14. Zone MA, Wanke MM (2001) Diagnosis of canine fetal health by ultrasonography. *J Reprod Fertil Suppl* 57:215–219
 15. Yeager AE, Concannon PW (1990) Association between the preovulatory luteinizing hormone surge and the early ultrasonographic detection of pregnancy and fetal heartbeats in Beagle dogs. *Theriogenology* 34:655
 16. Kessler J, Rasmussen S, Hanson M, Kiresud T (2006) Longitudinal reference ranges for ductus venosus flow velocities and wave-form indices. *Ultrasound Obstet Gynecol* 28:890–898
 17. Turan OM, Turan S, Berg C, Gembruch U, Nicolaides KH, Harman CR et al (2011) Duration of persistent abnormal ductus venosus flow and its impact of perinatal outcome in fetal growth restriction. *Ultrasound Obstet Gynecol* 38:295–302
 18. DeVore GR, Horestein J (1993) Ductus venosus index: a method for evaluating right ventricular preload in the second-trimester fetus. *Ultrasound Obstet Gynecol* 3:338–342
 19. Rizzo G, Capponi A, Arduini D, Romanini C (1994) Ductus venosus velocity waveforms in appropriate and small for gestational age fetuses. *Early Hum Dev* 2:389–396
 20. Reuss ML, Rudolph AM, Dae MW (1983) Phasic blood flow patterns in the superior and inferior vena cava and umbilical vein of fetal sheep. *Am J Obstet Gynecol* 145:70–78
 21. Sakoda S, Mitsunami K, Kinoshita M (1990) Evaluation of hepatic venous flow patterns using a pulsed Doppler technique *J Cardiol* 193:20
 22. Blanco PG, Rodriguez R, Rube A, Arias DO, Tortora M, Diaz JD et al (2011) Doppler ultrasonographic assessment of maternal and fetal blood flow in abnormal canine pregnancy. *Animal Reprod Sci* 126:130–135