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Doppler ultrasonographic assessment of maternal and fetal blood flow in abnormal canine pregnancy

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ABSTRACT

The aim of this study was to describe the changes of uterine artery, umbilical artery and fetal abdominal aorta, renal and internal carotid arteries blood flow in abnormal canine pregnancy. Twenty-two, Brucella-negative pregnant bitches were retrospectively classified into abnormal (which had either interrupted their pregnancy between days 52 and 60 or had perinatal death >60% of the litter; $n = 11$) and normal (which had delivered healthy puppies at term; $n = 11$). In all the animals, color and pulsed-wave Doppler examinations of uterine artery were conducted every 10 days from Day 20 to 50 from estimated luteinizing hormone peak. Doppler ultrasonography was also conducted in the fetuses to assess umbilical artery, abdominal aorta, renal and internal carotid arteries from Day 40 to 60 of gestation. Throughout the study, resistance index (RI) of uterine, umbilical and fetal renal arteries decreased up to −15% compared to −36% ($P < 0.01$), −11% compared to −23% ($P < 0.05$) and 2% compared to −13% ($P < 0.05$), respectively in the abnormal and normal bitches. Fetal abdominal aorta and internal carotid did not differ between groups ($P > 0.05$). It is concluded that in dogs, uterine artery, umbilical artery and fetal renal artery RI differ between normal and abnormal gestation being useful for the prediction of adverse obstetric outcome.

1. Introduction

Doppler ultrasonography has been used in the assessment of maternal and fetal blood flow during pregnancy in many species (Blanco et al., 2008). This is a non-invasive technique to evaluate the flow velocity and resistance index (RI) within vessels. Resistance indices of uteroplacental and umbilical arteries, fetal aorta and fetal common carotid arteries progressively decrease throughout normal canine gestation (Nautrup, 1998; Di Salvo et al., 2006; Blanco et al., 2010; Miranda and Domingues, 2010) guaranteeing an appropriate perfusion of the placenta and fetal viscera.

In pregnant women, Doppler has become a routine technique for identifying fetal compromise associated with an abnormal uteroplacental or fetal circulation. Increasing RI of uterine or umbilical arteries indicates a greater risk of fetal or perinatal death (Strigini et al., 1995; Dickey, 1997; Papageorghiou et al., 2004). In pregnancies complicated with hypertension, the increase in placental resistance is generally associated with an intrauterine fetal growth retardation and fetal hypoxia (Acharya et al., 2004; Ghosh and Gudmundsson, 2009). Furthermore, pregnancies with abnormal umbilical artery waveforms are delivered at an earlier gestational age than those with normal waveforms. Infants with abnormal Doppler indices also have an increased frequency of neonatal complication (Acharya et al., 2005).

In humans, Doppler assessment of fetal blood flow also provides information about reflex responses of the fetal cardiovascular system. In this sense, the evaluation of fetal

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abdominal aorta adds information about the peripheral circulation to the lower limbs, spleen, skeletal muscles and mesentery (Akalin-Sel and Campbell, 1992). Moreover, oligohydramnios in fetuses with prolonged hypoxia is probably a consequence of decreased renal perfusion, evidenced by Doppler assessment of fetal renal artery (Mari et al., 1993). Additionally, the resistance of the fetal internal carotid artery is predictive for the development of an abnormal fetal heart rate trace (Groenenberg et al., 1993). Some reports have suggested that abnormal velocity waveforms at the level of the umbilical artery and fetal vessels may even preceed late decelerations of fetal heart rate (Arduini et al., 1992). Other reports stated that fetal heart rate does not seem to have any advantage over Doppler velocimetry (Chan et al., 1994).

In domestic animals, ultrasonographic evaluations of fetal biometry and fetal heart rate have only been used to assess fetuses condition (Bucca et al., 2005). Fetal size, growth rate, movements and fetal heart rate deceleration, are the primary parameters used in dogs (Zone and Wanke, 2001; Kutzler et al., 2003; Johnson, 2008b). Doppler measurements of uterine, umbilical and fetal blood flow in pathological pregnancies have not been reported in dogs. An experimental canine model of abnormal gestation suggested that RI of uterine artery could also be a predictor of compromised pregnancy and impending abortion in this species (Blanco et al., 2009). However, no information is available on spontaneous canine cases of pathological gestation. For these reasons, the aim of the present study was to describe the changes of uterine artery, umbilical artery, fetal abdominal aorta and fetal renal and internal carotid arteries blood flow in abnormal canine pregnancy.

2. Materials and methods

2.1. Animals

Purebred ($n = 22$), clinically healthy pregnant (primiparous or multiparous) bitches, 1–8 years of age, weighing 3–40 kg, were recruited in this study. Both groups included German Shepherd, Miniature Poodle, Yorkshire and Rottweiller bitches. All the cases were Brucellosis-negative and retrospectively classified into normal and abnormal. Abnormal group ($n = 11$) included animals in which pregnancy interruption occurred between days 52 and 60 (from estimated luteinizing hormone [LH] peak) or that had perinatal death greater than 60% within the first 48 h after parturition (Davidson, 2003; Münnich, 2008). The normal control group ($n = 11$) bitches delivered healthy puppies at term and showed perinatal death lower than 17% of the litter. None of the bitches in the study had dystocia or delivered via C-section. Litter sizes ranged from 2 to 11. This study was approved by the Faculty Institutional Care and Animal Use Committee (IACUC).

2.2. Vaginal cytology

The animals were checked daily for the presence of vulvar swelling and sanguineous vulvar discharge which was considered to indicate the onset of proestrus. Vaginal smears were subsequently obtained daily until vaginal cytology was indicative of estrus, which was estimated as the Day of LH peak. The smears were stained using Tinción 15 stain (Biopur, Rosario; Argentina), percentage of different cell types was calculated and interpreted as previously described (Olson et al., 1984).

2.3. Ultrasonographic evaluations

Pregnancy diagnosis, litter size, fetal death and pregnancy interruption were evaluated using two-dimensional ultrasonic examination (Toshiba Core Vision Pro, Japan) with a 5–8 MHz linear-array transducer (England, 1998; England and Russo, 2006). The female dogs were ultrasonographically evaluated every 10 days from Day 20 to 50 of gestation, defining Day 0 (LH peak) as the first day of typical estrous vaginal cytology (Olson et al., 1984). Color and pulsed-wave Doppler examinations of uterine arteries were conducted. The female dogs were positioned in lateral recumbency and the hair of the ventral skin was shaved. Acoustic gel was applied to the transducer and coupled directly to the skin. Two-dimensional ultrasonography was used to identify the uterine body in a transversal axis. Color flow mapping was subsequently used to localize uterine arteries at both sides of the cervix and pulsed-wave Doppler to obtain the waveforms (Alvarez Clau and Liste, 2005). To minimize variability, three uniform consecutive waveforms were recorded by a trained operator. Peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured. Resistance index [(PSV − EDV)/PSV] and pressure gradient (PG) were automatically calculated (Dickey, 1997).

In both groups, fetal Doppler and M Mode ultrasonography were performed from Day 40 to 60 of gestation in the most caudal fetus of the right uterus horn (Di Salvo et al., 2006; Scotti et al., 2008). Umbilical artery, fetal abdominal aorta, fetal renal artery and fetal internal carotid were assessed by color and pulsed wave Doppler (Di Salvo et al., 2006). To perform Doppler ultrasonography of the fetal renal artery, a longitudinal view of the fetal kidney was obtained. The renal artery was subsequently detected in the renal hilum of the fetus (Fong et al., 1999). The fetal internal carotid artery was detected at each side of the deep portion of diencephalo-telencephalic vesicle (Morales-Roselló, 2002; Beccaglia et al., 2008). Three uniform consecutive waveforms of each artery were recorded to measure PSV and EDV. Resistance index and PG were automatically calculated. Fetal heart rate was registered by M Mode echocardiography and three measurements were averaged (Verstegen et al., 1993). The last examination of all the patients was 4 ± 0.9 days (mean ± SEM) before abortion or delivery.

2.4. Statistical analysis

To verify the comparability of the groups, comparisons with regard to weight, age, parity and litter size were performed by Student's *t*-tests. Resistance indexes of the left and right uterine arteries were compared in both groups using the same test. Values of PSV, EDV, RI and PG of uterine artery, umbilical artery, fetal abdominal aorta, fetal renal artery and fetal internal carotid artery were transformed