The Veterinary Journal 197 (2013) 205–210

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Uterine artery blood flow characteristics assessed during oestrus and the early luteal phase of pregnant and non-pregnant bitches

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ARTICLE INFO

Article history:
Accepted 15 February 2013

Keywords:
Bitch
Blood flow
Fertility
Perfusion characteristics
Uterine artery

ABSTRACT

The aim of this study was to measure uterine artery blood velocity daily using Doppler ultrasonography in 10 young and 10 older clinically normal bitches throughout oestrus.

Typical arterial waveforms identified in young bitches were characterised by a systolic peak and subsequent flow throughout diastole, whereas in older bitches, flow was sometimes absent in diastole. For 3 days immediately prior to ovulation, at the time of declining plasma oestrogen and increasing progesterone concentrations, resistance index (RI) increased, principally associated with decreased diastolic velocity; in some bitches there was absent late diastolic flow during this time. In older bitches, the waveform appearance was more variable, with absent late and early diastolic flow observed in some cases. Mean RI was higher throughout oestrus for older bitches compared with young bitches, although both groups had a similar 3-day duration increase before ovulation. Nine of the young bitches and five of the older bitches became pregnant; litter size was smaller for the older bitches. Non-pregnant bitches: (1) were significantly older; (2) had fewer waveforms with continuous diastolic flow 2 days before ovulation; (3) had lower end diastolic velocity, higher RI and fewer waveforms with continuous diastolic flow 2 days after ovulation, and (4) had lower plasma progesterone concentrations 5 days after ovulation. These are the first detailed observations of uterine artery blood velocity and waveform appearance throughout oestrus in bitches, and this is the first description of a link between impaired diastolic flow and reduced fertility. Assessment of uterine artery velocity could be useful to promote understanding of physiological mechanisms and could also become an important tool to assess potential infertility.

Introduction

Doppler ultrasonography has been used for the evaluation of uterine artery blood flow in a number of species, including women and mares (Goswamy and Steptoe, 1988; Bollwein et al., 1998, 2000, 2002). Common assessments of the uterine artery include measurement of peak systolic velocity (PSV) and end diastolic blood velocity (EDV), as well as calculation of blood flow volume or average velocity. Although useful, these calculations might be flawed, especially when vessel size is very small and when the Doppler angle and machine gain are not consistent (Dickey, 1997). However, calculation of resistance index (RI) is not subject to these errors and as a simple ratio of the PSV and EDV, it forms a useful estimation of downstream impedance to blood flow and therefore the degree of uterine perfusion.

Fluctuations in uterine artery blood flow have been related to stage of the oestrous cycle in cows and mares (Bollwein et al., 1998, 2000, 2003). A crucial association between the time-averaged maximum blood velocity and the uterine nitric oxide synthase (NOS) system has been established; it is likely an oestrogen-mediated stimulation of NOS (Honnens et al., 2011). Important variations in uterine artery RI have been documented, associated with age and parity in women and mares (Dickey, 1997; Bollwein et al., 1998; Ousey et al., 2012) and as part of an increased uterine perfusion following mating (Bollwein et al., 2003). Doppler ultrasound examination of the uterine artery is common in human pregnancy since there is an association between blood flow parameters and adverse fetal outcome (Dickey, 1997; Iacovella et al., 2012). Interesting observations have been made between decreased uterine perfusion during the menstrual cycle and human infertility (Goswamy et al., 1988; Kurjak et al., 1991; Steer et al., 1994), and although recent work suggests limited association between these parameters (Kim et al., 2010), the initial studies of Goswamy et al. (1988) did not just make flow-associated measurements, rather, they devised descriptive criteria for classification of the waveforms, and suggested that these were valuable estimates of blood flow. Despite those early observations, analysis of waveform characteristics is rarely performed because it is not

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http://dx.doi.org/10.1016/j.tvjl.2013.02.015

amenable to evaluation by ultrasound machine software, and as a result it is often omitted from clinical investigation of infertility (Dickey, 1997). Despite this, there has recently been increasing interest in the relationship between ultrasound-measured endometrial blood flow and infertility (Ng et al., 2007).

In the bitch, measurement of the uterine artery blood flow with Doppler ultrasound has been reported in non-cycling and pregnant animals (Alvarez-Clau and Liste, 2005), and recently interesting physiological changes have been documented after the deposition of semen into the uterus (England et al., 2012b) and in cases of endometrial disease (England et al., 2012a). However, to the authors' knowledge, there are no published studies of the variation of uterine artery blood flow in bitches during oestrus, and investigations of any possible associations with age, parity, or fertility have not previously been reported.

We hypothesise that in the bitch there are fluctuations of uterine artery blood flow and waveform characteristics during oestrus, and that these could differ according to age and might be associated with reduced likelihood of establishing a pregnancy.

Materials and methods

Ethical approval

All procedures were conducted as part of normal veterinary clinical practice with owner permission and the ethical approval of the School of Veterinary Medicine and Science, University of Nottingham.

Animals

Twenty Labrador retriever bitches that were presented for monitoring of oestrus to determine the optimal time for natural mating were used for the study. The bitches were mated to one of 12 different Labrador retriever dogs that had all been fertile within the previous 3 months. Semen samples were collected from all male dogs between 5 and 7 days after the last mating. Ejaculates were separated using glass funnels into pre-sperm, sperm-rich and prostatic fractions. The sperm-rich fractions were evaluated for volume, motility, concentration and morphology (Pacey et al., 2000) to enable calculation of the total number of normal live spermatozoa per ejaculate.

Bitches were divided into two groups of 10 designated as 'young' (aged 28–38 months) and 'older' (aged 50–68 months). Five of the young bitches and all of the older bitches had previously been pregnant; the remaining five young bitches had not previously been mated. All pregnancies had been approximately 1 year previously and none of the bitches had been mated at the preceding oestrous cycle. Each bitch had a normal clinical reproductive tract examination, including normal uterine ultrasonography at the onset of proestrus.

Bitches were examined daily for collection of vaginal epithelial cells and cytological examination was performed after staining with a Romanowsky stain (Diff Quik) to establish the anuclear cell index (Moxon et al., 2010), and for measurement of plasma concentrations of progesterone by ELISA (Ridgeway Science). Approximately 90 min before vaginal cytology was performed, the bitch was placed into left lateral recumbency and the uterine arteries were located using trans-abdominal B-mode ultrasonography with a 10 MHz mechanical sector transducer (Pie-Data UK, Ltd.). The transducer was placed on the ventral abdomen in the transverse imaging plane with an incident angle of approximately 45°. The uterine artery (positioned lateral to the uterine vein) was detected adjacent to the mid uterine body and was confirmed using the colour Doppler setting. Using pulsed-Doppler, a small sample gate was placed over the artery and minor positional adjustments were made to facilitate recording of uterine artery waveforms. The waveforms were examined subjectively and classified as defined by Goswamy and Steptoe (1988) and modified by Dickey et al. (1994): Type C was a systolic peak followed by the diastolic wave continuous with systole and extending throughout the remainder of the cardiac cycle to the next systole; Type A was a systolic peak with absence of the early diastolic wave, but with flow present in mid and late diastole; Type B was a systolic peak followed by the diastolic wave continuous with systole but not extending to the next systole (i.e. there was no flow in late diastole), and Type D was a systolic peak with absence of the early and late diastolic wave, but with flow present in mid diastole. Other waveform profiles reported in women (Goswamy and Steptoe, 1988; Dickey, 1997) were not identified in preliminary studies (data not shown) and so no further classification categories were assigned. Measurements were made of PSV and EDV; in cases of Type B and Type A waveforms, the latest diastolic velocity before the decline to baseline was measured. RI was calculated using the formula:

$$RI = (PSV - EDV)/PSV$$

Measurements were made from three waveforms each from the left and right uterine artery.

Mating occurred 1 day after plasma progesterone concentrations exceeded 5 ng/mL (designated as the day of ovulation, day 0) and a second mating was performed 48 h later. The owners of the dogs provided pregnancy and whelping data.

Statistical methods

Means were calculated and the differences between time periods within treatments (young or older bitches) were analysed using a two-way analysis of variance and Tukey's post hoc test, while differences between treatments (young and older bitches) for each time period were analysed using the Mann Whitney *U* test. Data were then re-classified according to the pregnancy outcome (pregnant or non-pregnant) and were compared between the pregnant and non-pregnant groups for days −2 and +2 in relation to ovulation, and day +5 for plasma progesterone, using either the Mann Whitney *U* test or chi-squared test with Yates correction. Analysis was performed with InStat3 (GraphPad) and data were considered statistically significant when $P < 0.05$.

Results

Each of the bitches had an apparently normal oestrous cycle, ovulated and was mated. Semen quality was within the following ranges: % normal motility, 70–85%; total live normal spermatozoa, 510–880 $\times$ 10^6 per ejaculate.

It was possible to identify the uterine arteries in all bitches and at least three suitable waveforms were recorded at each examination. Each waveform had a characteristic appearance with high systolic flow and an obvious diastolic wave. All four of the waveform classifications were identified at various times during the study (Fig. 1). Since statistical analysis did not demonstrate significant differences between values for PSV, EDV and RI of the left and right arteries, all six daily waveform measurements for each bitch were pooled for subsequent evaluation.

For young bitches, there was a significant difference in RI across the days of the study. RI values were lowest and not significantly different to each other on days −5 to −3 and days +1 to +5, and were significantly higher on days −2 to 0, respectively (Fig. 2a). The change in RI was predominantly associated with a decrease in EDV, which was significantly lower on days −2 to 0 compared to all other days. There were no significant differences in PSV across any of the days, although values were numerically higher on days −2 to 0. Higher RI values corresponded with the graphically-identified trough in the peak of anuclear vaginal epithelial cells at the time of the initial rise of plasma progesterone (Fig. 2a). Examination of the waveform classification for the young bitches showed that eight bitches had Type C waveforms throughout the study, while two bitches had a Type C waveform on days −5 to −2, and days 0 to +5 inclusive, with Type B waveform on day −1. Nine of the young bitches became pregnant with a mean (±SD) litter size of 6.2 ± 1.8 pups.

For the older bitches, values for RI were significantly higher than the young bitches for each day of the oestrous cycle (Fig. 2b). Higher RI values were associated with lower EDV values; PSV was not different between the two groups. The older bitches had a similar trend in RI throughout the cycle as the young bitches; there was a significant increase in RI on days −2 to 0, associated with significantly lower values for EDV on these days. The subjective relationship between RI and the peak of anuclear vaginal epithelial cells and first detection of a rise in plasma progesterone concentration was similar for the older and young bitches (Fig. 2b). RI returned to lower values on day +1, similar to the young bitches (Fig. 2b). Examination of the waveform characteristics for the older bitches showed that two of the bitches had a Type C waveform on each day of the study. Of the remaining eight bitches, four had Type C waveforms on days −5 to −3 and days +3 to +5 inclusive and Type B waveforms on days −2 to +2, while three bitches had Type C waveforms on days −5 to −3, and days