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Relationship between colour flow Doppler sonographic assessment of corpus luteum activity and progesterone concentrations in mares after embryo transfer

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ABSTRACT

Colour-flow Doppler sonography has been described as a means of assessing corpus luteum (CL) function rapidly, because area of luteal blood vessels correlates well with circulating progesterone (P_4) concentrations [P_4] in oestrous cycling mares. The aim of this study was to assess the relationships between CL size and vascularity, and circulating [P_4] during early pregnancy in mares, and to determine whether luteal blood flow was a useful aid for selecting an embryo transfer recipient. Equine embryos ($n = 48$) were recovered 8 days after ovulation and were transferred to available recipient mares as part of a commercial program with the degree of synchrony in timing of recipient ovulation ranging from 1 day before to 4 days after the donor. Immediately prior to embryo transfer (ET), maximum CL cross-section and blood vessel areas were assessed sonographically, and jugular blood was collected to measure plasma [P_4]. Sonographic measurements and jugular blood collection were repeated at day 4 after ET for all mares, and again at days 11, 18 and 25 after ET in mares that were pregnant. The number of grey-scale and colour pixels within the CL was subsequently quantified using ImageJ software. The CL blood flow correlated significantly but weakly with plasma [P_4] on the day of transfer and on day 4 after ET in all mares, and on days 11 and 25 after ET in pregnant mares ($r = 0.30–0.36$). The CL area and plasma [P_4] were also correlated on each day until day 11 after ET ($r = 0.49–0.60$). The CL colour pixel area decreased significantly after day 18, whereas CL area was already decreasing by day 4 after ET. The CL area, area of blood flow, or [P_4] was predictive of pregnancy. Findings in the present study suggest that both CL area and blood flow are correlated with circulating [P_4] at the time of transfer and in early pregnancy. Evaluation of the CL using B-mode or CF sonography, although practical, provides no improvement in the selection of recipients or prediction of pregnancy outcomes than methods employed currently.

1. Introduction

Maintaining and managing recipient mares is the greatest recurring expense in an embryo transfer (ET) program. However, despite attempts to select suitable recipients based on ovulation date, uterine and cervical tone (Carnevale et al., 2000) not all good quality embryos will develop into a viable pregnancy after transfer. While embryo quality is clearly an important contributor to pregnancy, and can to some extent be influenced by stallion choice and selection and management of the donor mare (Hendriks et al., 2015; Mortensen et al., 2009; Love et al.,

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2002), aspects of recipient mare management and selection could also be refined or adjusted in an effort to improve pregnancy rates. Currently, suitably oestrous synchronised mares are selected to receive an embryo based on ovulation date, uterine and cervical tone and absence of any gross abnormalities during a B-mode ultrasonic assessment of the reproductive tract.

Colour flow Doppler is an established technique for evaluating blood flow and/or patterns of vascular perfusion in human reproductive medicine (Kupesic, 1996; Fleischer, 2003) and has also been applied in studies of reproductive function in cattle (Herzog and Bollwein, 2007; Herzog et al., 2010; Lüttgenau et al., 2011; Miyamotoa et al., 2005; Utt et al., 2009), dogs (Bergeron et al., 2013; Polisca et al., 2013; Batista et al., 2013) and horses (Bollwein et al., 2001, 2002; Pozor and McDonnell, 2004; Acosta et al., 2004). Colour Doppler ultrasonography offers a reliable, non-invasive approach to evaluating vascular perfusion of the CL (Miyamotoa et al., 2005), a structure that has the greatest size-adjusted blood flow of any organ (Lüttgenau et al., 2011). Moreover, luteal blood flow is positively correlated with circulating [P_4] in the non-pregnant mare, where it is a more reliable predictor of CL function than CL size, particularly during luteal regression (Bollwein et al., 2001, 2002; Ginther et al., 2007).

In practice, more than one appropriately oestrous-synchronised recipient mare maybe available for a given embryo. In general, a mare is considered suitable if she had an ovulation no more than 1 day before and no more than 3 days after the donor mare and has a tonic cervix and uterus indicative of an elevated (>1 ng/ml) circulating [P_4] (Carnevale et al., 2000; McCue et al., 1999; Jacob et al., 2012). This may be supported by sonographic evidence of a CL of adequate size and homogenous echogenicity (Carnevale et al., 2000), although a mare CL with a central cavity or a less homogenous sonographic appearance can be as functional with regard to progesterone production (Townson et al., 1989). Ideally, the functionality of a CL should be evaluated by determination of an acceptable plasma [P_4] (greater than 2 ng/ml; Plotka et al., 1972; Remsen et al., 1982), however, in practice plasma [P_4] is rarely measured because of the added costs and the delay in ascertaining concentrations until after the time when transfers should occur. In this respect, assessment of CL blood flow would be an immediate and less expensive way of assessing CL function and thereby aid in the selecting a suitable recipient mare. The aim of the present study was, therefore, to evaluate relationships between plasma [P_4] and CL size and vascularity, and to determine whether there were any cut-off values that may affect the likelihood of establishing pregnancy in mares.

2. Material and methods

2.1. Study design

The study was conducted between April and September 2013 at Utrecht University's Equine Clinic in the Netherlands. As part of the commercial clinical service, 48 embryos recovered 8 days after ovulation were transferred into reproductively sound recipient mares (age: 3–15 years). Sonographic evaluation of the recipient mare's CL using both B-mode (grey scale) and colour flow Doppler was performed on the day of embryo transfer (ET + 0) and on day 4 after transfer (ET + 4) when the recipient mare was also examined for pregnancy. If the mare was pregnant, the CL measurements were repeated on days 11 (ET + 11), 18 (ET + 18) and 25 after transfer (ET + 25). Recipient mares that were not pregnant on day 4 after ET were re-examined 2 days later with no further CL measurements being performed if the mare was confirmed to be non-pregnant. At each time point the maximum cross-sectional diameter and area of colour pixels/abundance of blood vessels of the CL were recorded by one of three operators and a blood sample was collected from the jugular vein for subsequent assessment of plasma [P_4].

2.2. Animals

Warmblood mares (n = 48; 550–700 kg) with a median age of 6.5 years (range: 3–15 years) were available as recipients. Only mares receiving their first embryo of the season over which embryo transfers occurred were included in the study. All mares were free from signs of infectious disease, with grossly normal reproductive tracts and typical length oestrous cycles. The mares were maintained either on pastures with *ad libitum* access to grass and water or in stables with *ad libitum* access to hay and water. All animal procedures listed in the experimental methods were approved by Utrecht University's Animal Experimentation Committee (permission no: 2013.III.01.012).

2.3. Breeding management and oestrus synchronisation

Uterine and cervical tone were determined by rectal palpation and graded on a 1–3 point scale (1 = great tone typical of diestrus; 2 = intermediate or softening; 3 = flaccid as indicative of oestrus). Uterine oedema was graded: 1 = none, 2 = some, 3 = obvious, 4 = considerable. Follicular development and ovulation were monitored by means of trans-rectal ultrasonography. When recipient mares were in late oestrus (on the basis of a follicle ≥40 mm and uterine oedema >2), mares were examined daily until ovulation was detected by the disappearance of the pre-ovulatory follicle and replacement by a corpus haemorrhagicum. Mares had ovulations naturally or were induced to have an ovulation using human chorionic gonadotrophin (Chorulon®, 1500 iu per iv administration; Intervet, Boxmeer, the Netherlands). When required the luteal phase was shortened by administration of D-cloprostenol (37.5–75 μg i.m.; Genestranvet®, Eurovet, Bladel, the Netherlands). Preferred recipient mares were those that had ovulations 0–3 days after the donor mare and if none were available, mares having ovulations 1 day before or 4 days after the donor were also considered to have sufficiently synchronised times of ovulation to receive an embryo.

2.4. Embryo recovery

Embryos were collected from donor mares by uterine lavage on day 8 after ovulation, as previously described (Stout, 2006). Embryo collection was performed using