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Real-time characterization of the uterine blood flow in mares before and after artificial insemination

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ABSTRACT

The present experiment was divided into two studies to investigate the effect of age and endometrial degeneration on uterine blood flow of mares throughout the immediate post-breeding period. In study 1, uterine blood flow was characterized in mares ($n = 7$ mares/group) with minimal, moderate or severe endometrial degenerative changes (GI, GII and GIII, respectively). In study 2, the effect of age was investigated using young (≤6 years) and old (≥15 years) mares ($n = 7$ mares/group). Uterine vascular perfusion and mesometrial pulsatility index (PI) were evaluated every hour from H0 (moment immediately before AI) to H12. In study 1, a pronounced and transitory increase on uterine vascular perfusion was detected ($P < 0.001$) between H1 and H3 in the three endometrial groups. In addition, GIII mares had greater ($P < 0.05$) mesometrial PI than GI mares during the post-breeding period, denoting reduced uterine blood flow. In study 2, a transitory increase on uterine vascular perfusion was also observed in both age groups during the first hour after mating. However, mesometrial PI of young and old mares was similar ($P > 0.05$) and constant ($P > 0.7$) through the first 12 h after AI. Results demonstrated, for the first time, the immediate changes on uterine vascular perfusion and mesometrial PI in response to semen infusion. Moreover, reduced blood flow of the uterus during the post-breeding period was strongly associated with endometrial degenerative changes in mares, regardless of age.

1. Introduction

Doppler ultrasonography is a noninvasive pulsewave technique that has been used for the real time evaluation of blood flow in the reproductive tract of large animals (Ginther, 2007). For providing precisely values of blood velocities from mesometrial attachment arteries, spectrum mode is an objectively method for the uterine blood flow evaluation (Ferreira et al., 2008). Furthermore, power flow exam of vessels located in the endometrium and myometrium provides immediately qualitative information of the vascularity of the uterine tissue (Ginther and Utt, 2004).

In mares, a transient breeding-induced inflammation is a physiological acute mechanism (Troedsson, 2006) characterized by increased myometrial activity (Katila et al., 2000) and local innate immune reactions (Kotilainen et al., 1994; Troedsson, 1999). Although adequate blood flow is essential for the normal function of the reproductive

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system (Ginther, 2007), there are few in vivo studies describing the uterine hemodynamics of non-pregnant mares (Ferreira et al., 2008; Silva et al., 2005). Changes on blood flow velocity of uterine arteries after mating (Bollwein et al., 2003) suggest that an increased endometrial blood flow occurs during semen transportation and uterine clearance. However, at the present, evaluation of Doppler indices of mesometrial arteries and vascular perfusion of the uterine tissue during both pre- and post-breeding periods in mares has not been reported.

Pathologic changes in uterine vessels of aged and multiparous mares have been recently associated with delayed uterine clearance (Esteller-Vico et al., 2012) and subfertility (Lehmann et al., 2011). In addition, age-related endometrial degenerative changes might affect placental development (Abd-Elnaeim et al., 2006) and its associated blood flow in pregnant mares (Ousey et al., 2012; Ferreira et al., 2015).

Therefore, we have hypothesized that mares of advanced age or with diffuse endometrial fibrosis would have reduced uterine blood flow after mating when compared to young mares and mares with minimal endometrial changes. Accordingly, the main purpose of this study was to evaluate the effect of degree of degenerative endometrial changes and age on the uterine blood flow throughout the immediate post-breeding period. The specify objectives were to characterize the uterine vascular perfusion and the pulsatility index (PI) from arteries at the mesometrial attachment during the first 12 h after artificial insemination.

2. Materials and methods

2.1. Animals and experimental groups

Cycling mixed breed mares 3 to 25 years of age, weighing 250–380 kg were used in the present experiment. The mares were kept under natural light in an open shelter and outdoor paddock at the Reproduction Center of the Department of Animal Reproduction and Veterinary Radiology at the São Paulo State University (UNESP), Campus of Botucatu. Mares were handled in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (protocol number 13/2012-CEUA). All mares were maintained on grass hay, pelleted feed and trace-mineralized salt with free access to water. Score for body condition for all mares throughout the experiment was ≥7 (Henneke et al., 1983). None of the mares had foaled during the last 5 years preceding the experiment.

2.1.1. Study 1: Effect of endometrial degenerative changes on the uterine blood flow of mares during the post-mating phase

Twenty-one mares were used to study the influence of endometrial degenerative changes in the uterine vascular perfusion and mesometrial PI during the first 12 h after artificial insemination (AI). Initially, endometrial samples from all mares were obtained using transcervical uterine biopsy technique and alligator-jaw instrument. Samples were fixed in 10% buffered formalin, processed for histopathological sectioning and staining with hematoxylin–eosin. Endometrial degenerative changes were graded into four categories (Table 1) as previously established (Kenney and Doig, 1986). Therefore, the mares were arranged in three groups ($n = 7$ mares/group) based on the degree of endometrial histopathological changes: GI (category I), GII (categories IIA and IIB) and GIII (category III).

Table 1
Uterine classification in mares according to the intensity of degenerative endometrial changes.

Category	Histopathological changes
I	Unaltered endometria Mild, focal inflammation or fibrosis
IIA	To moderate inflammation Multifocal fibrosis with 1–3 layers of fibroblasts surrounding glands or <2 fibrotic nests per 5 mm linear field
IIB	Moderate inflammation Multifocal or diffuse fibrosis with 4 or more layers of fibroblasts surrounding glands or 2–4 fibrotic nests per 5 mm linear field
III	Severe inflammation Diffuse fibrosis with 5 or more fibrotic nests per 5 mm linear field

Adapted from Kenney and Doig (1986).

2.1.2. Study 2: Effect of age on the uterine blood flow of mares during the first 12 h after artificial insemination

Fourteen mares were used to evaluate the temporal relationship between age and uterine blood flow throughout the post-mating phase. The mares were assigned to two age groups: young (≤6 years, ranged from 5 to 6 years, $n = 7$ mares) versus old (≥15 years, ranged from 15 to 21 years, $n = 7$ mares). Age of mares was estimated from dental characteristics as described by the American Association of Equine Practitioners Manual (2002). Mares of age ranging from 7 to 13 years old were not used in order to increase the difference between the two age groups.

2.2. Artificial insemination

Reproductive status of each mare was based on records obtained by daily transrectal ultrasound scanning of the ovaries and uterus. A single insemination was performed for each mare when a pre-ovulatory follicle with ≥35 mm of diameter and endometrial edema was detected.

All mares were artificially inseminated between 5 and 6 a.m. using cooled semen from one fertile stallion. After collection, semen was diluted with skim milk extender (BotuSÊMEN, Inc., Botupharma, SP, Brazil) to obtain an insemination dose of 1–1.5 billion progressively motile sperm extended to a final volume of 20–30 mL. The insemination dose was maintained cooled at 15 °C using insulated box (BotuBOX®; Botupharma) until the time of insemination, which was done within 10–12 h after semen collection and processing. The semen dose was placed into the body of the uterus using a standard disposable AI catheter.

2.3. Doppler ultrasonography

Mares were scanned per rectum using a pulse wave color Doppler ultrasound unit (Sonoace Pico; Medison do Brasil Ltda) equipped with a linear-array multifrequency