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Effects of estradiol on uterine perfusion in anesthetized cyclic mares affected with uterine vascular elastosis

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ABSTRACT

Uterine vascular elastosis in mares is characterized by degeneration of uterine vasculature through thickening of the elastin layers. Factors commonly associated with this degeneration include age, parity, and chronic uterine endometritis. Affected mares have also been shown to exhibit decreases in uterine blood flow and perfusion of the uterus. Due to the increased thickness of the elastin layers, we hypothesize that vasodilatation of the uterine vasculature is also impaired. To test the functionality of these vessels, we evaluated the vasodilatory effects of estradiol on the uterine vascular bed in mares with normal vasculature and mares with severe elastosis. Both groups were tested in estrus and diestrus. Fluorescent microspheres were used to determine basal blood perfusion, followed by the intravenous administration of 1.0 μg/kg of 17β-estradiol. After 90 min, perfusion was measured once again to determine the vascular response to estradiol. Control mares in estrus displayed a significant increase in total uterine blood flow after the administration of estradiol when compared to baseline levels. No other group had a significant increase in total blood flow and perfusion after estradiol administration. The administration of estradiol in control mares induced regional increases in perfusion in the uterine horns and uterine body during estrus and only in the uterine horns during diestrus. Mares affected by elastosis exhibited no regional differences in perfusion levels post-estradiol administration. The difference in the vasodilatory response induced by estradiol between reproductively healthy mares and mares affected with elastosis indicates that the functionality of the affected vessels is compromised.

1. Introduction

Uterine vascular elastosis is a degeneration of the elastin layer in uterine vessels that is associated with mares affected by chronic endometritis. This degeneration was first reported in endometrial biopsies from aged infertile mares (Oikawa et al., 1993) and consists of enlargement, duplication and splitting of the elastic membrane and perivascular deposits of elastin. Such changes are displayed in contrast to the otherwise thin, single and continuous layer found in nulliparous, presumptively fertile mares (Nambo et al., 1995; Esteller-Vico et al., 2012).

Several factors have been associated with these lesions: among them, mare age, parity, endometrial biopsy grade, infertility and number of foals (Nambo et al., 1995; Esteller-Vico et al., 2012). Our previous research indicated that elastosis was strongly associated with the number of foals produced by each mare (Esteller-Vico et al., 2012) and that elastosis had a negative effect on uterine blood perfusion throughout the estrous cycle (Esteller-Vico et al.,

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2015). In mares with normal vasculature, perfusion levels were significantly higher during estrus compared to diestrus. In mares affected with elastosis, we found no change in perfusion throughout the estrous cycle and significantly lower perfusion levels compared to control mares.

Higher levels of perfusion in reproductively healthy mares during estrus are likely related to the increase of circulating estradiol produced by the pre-ovulatory follicle. Estradiol has been shown in sows, cows and ewes to be a vasodilator of the uterine vascular bed (Greiss and Anderson, 1970; Ford, 1982). The effect of several estrogens on uterine blood flow (UBF) has been compared in ewes (Resnik et al., 1974), and 1.0 μg/kg of 17-β estradiol caused a significant increase in UBF 90–120 min after intravenous administration (Killam et al., 1973). It was later determined that this dose of estradiol had no systemic effects on cardiovascular parameters (Magness and Rosenfeld, 1989), which made it an ideal vasodilator to specifically test uterine vasculature. The effects of estradiol were further confirmed in intact cyclic animals (Gibson et al., 2004) and also by reversing the vasodilatory effect of estradiol using an estrogen receptor antagonist in ovariectomized, intact and pregnant ewes (Magness et al., 2005). In the mare, treatment with estradiol benzoate 5 mg orally increased UBF around the peri-ovulatory period but not during diestrus (Bollwein et al., 2004).

We hypothesized that the lower levels of perfusion and lack of difference between estrus and diestrus in mares affected by elastosis was caused by a decreased response to circulating estradiol and increased resistance of the uterine vascular bed. Therefore, the objectives of this study were to evaluate the response of uterine perfusion to estradiol on reproductively normal mares during estrus and diestrus as well as to compare the response in mares with or without uterine vascular elastosis.

2. Materials and methods

2.1. Experimental design

All experiments were conducted in accordance with and approval of the Committee on Animal Use and Care Protocol at the University of California, Davis (protocol number 15212). Twelve mares were classified according to the degree of vascular degeneration ($n = 6$ mares in each group) based upon Gruninger's classification: no degeneration, mild, moderate or severe (Gruninger et al., 1998). Nulliparous, presumptively fertile mares with normal vasculature or mild vascular changes were used in the control group and multiparous mares with severe vascular degeneration were used in the elastosis group. Mares were also classified according to their stage of cycle. To determine the stage of cycle, mares were followed by transrectal ultrasonography to visualize the presence of ovulatory follicles or *corpus luteum* (CL). Circulating progesterone concentrations were used to confirm the stage of cycle. Mares with serum progesterone concentrations <0.5 ng/mL were considered in estrus and mares with circulating progesterone concentrations >1.0 ng/mL were considered in diestrus.

2.2. Blood perfusion measurement

Mares were prepared as previously described (Esteller-Vico et al., 2015). Briefly, mares were fasted overnight and sedated with 1 mg/kg of xylazine hydrochloride IV. Anesthesia was induced with 2 mg/kg of ketamine hydrochloride and maintained with 1.57% isofluorane corresponding to 1.2 X MAC for horses (Brosnan et al., 2008). Once anesthetized and on the surgery table, standard instrumentation to monitor cardiovascular parameters (systolic arterial pressure, mean arterial pressure, diastolic arterial pressure and heart rate) were placed on mares. Additionally, an intracardiac catheter in the left ventricle and four arterial catheters (carotid artery, facial artery, right and left dorsal metatarsal arteries) were placed for microsphere injections and for arterial blood sampling. All arterial catheters were connected to automated withdrawal pumps with 50-mL glass syringes (containing 2 mL of Heparin sodium, Baxter International). The intracardiac catheter was connected to an injection pump with a glass syringe loaded with 40×10^6 fluorescent microspheres (15 μm in diameter) suspended into 40 mL aqueous solution (0.01% Tween 80 and 0.01% Thimerosal). To evaluate the uterine vascular response to estradiol two consecutive measurements using fluorescent microspheres (Dye-Trak-F, Triton technology, San Diego, CA) were performed to determine a baseline and post estradiol UBF levels. Each measurement used microspheres containing a different fluorescent dye. Microsphere injections were performed as previously described (Glenny et al., 1993; Brosnan et al., 2008; Esteller-Vico et al., 2015). The four arterial samples from different locations were used to determine that the concentration of microspheres per mL of blood was the same, ensuring adequate mixing of microspheres in the blood during perfusion measurements. Following baseline determination, mares received a single dose of 17β-estradiol (Steraloids, Ltd., Croydon, England) IV *via* a jugular catheter of 1.0 μg/kg in ethanol and further diluted 1:10 in saline. The second injection of microspheres was performed 90 min after the administration of estradiol. The microsphere injection and blood sampling were repeated in the same manner as the baseline determination using microspheres with a different fluorescent dye. After completion of the experiment, mares were euthanized with an overdose of pentobarbital sodium and phenytoin sodium (Beuthanasia-D Special Euthanasia Solution, Schering–Plough Animal Health Corporation, Kenilworth, NJ, USA) while under general anesthesia. Post mortem, the uteri were retrieved through an incision lateral to the abdominal midline. As described previously (Esteller-Vico et al., 2015), uteri were weighted and sectioned into small pieces (6–8 g). The sample weights and locations within the uterus were recorded. Sections were then placed in a sample-processing unit (SPU) (Raab et al., 1999) with digestion solution (KOH 4 M). Following an incubation of 48 h at 60 °C, contents of SPU were filtered, rinsed and centrifuged to recover the microspheres at the bottom of the filter (7 μm pore size). To collect the fluorescent dyes, the microspheres' outer polystyrene layer was dissolved by adding 2 mL of 2-ethoxyethyl acetate, releasing the dyes that were then collected in a sample tube by centrifugation.