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Effects of vascular elastosis on uterine blood flow and perfusion in anesthetized mares

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ABSTRACT

In the uterus of the mare, data obtained using transrectal Doppler ultrasonography indicate that uterine blood flow (UBF) is dynamic and changes throughout the estrous cycle. Degenerative lesions in the uterus are associated with subfertility and infertility. Among these lesions, vascular elastosis has been reported in aged, multiparous, and infertile mares. Angiosis of the uterine vasculature could potentially compromise UBF. The objectives of this experiment are to determine levels of UBF and perfusion of reproductively healthy mares and compare them to levels of subfertile/infertile mares affected by uterine vascular elastosis. Twenty mares were classified on the basis of degree of vascular degeneration and stage of cycle. A fluorescent microsphere technique was used to measure reproductive organ perfusion, where microspheres were injected into the left ventricle of the heart and became trapped in capillary beds in proportion to blood flow and tissue perfusion. The reproductive tract was removed, sectioned, and the fluorescent intensity evaluated to measure blood flow and perfusion. Additionally, full-thickness samples of the uterine wall were examined postmortem to further assess the degree of vascular degeneration in all layers of uterine wall. The mean value of uterine perfusion for the control mares during estrus ($n = 5$) was higher ($P < 0.01$) than that during diestrus ($n = 5$); 17.6 and 11.9 mL/min/100g, respectively. For the subfertile/infertile mares, the mean value of tissue perfusion was not different ($P > 0.05$) during estrus ($n = 5$) and diestrus ($n = 5$); 5.9 and 7.2 mL/min/100g, respectively. Uterine perfusion in subfertile/infertile mares affected by elastosis was lower than that of control mares during both estrus ($P < 0.01$) and diestrus ($P < 0.01$). The differences in baseline levels of perfusion between the control and elastosis groups indicate that elastosis of the uterine vasculature is associated with decreased uterine perfusion during both phases of the estrous cycle. In the uterus, a compromise in UBF could have implications in endometrial glandular development, postbreeding endometritis, uterine clearance, development of the conceptus, and overall fertility.

1. Introduction

Blood flow and tissue perfusion are essential to many organ functions. In the reproductive tract of the mare, data obtained using transrectal Doppler ultrasound indicate that uterine blood flow (UBF) is a dynamic event [1] and subject to change under hormonal influence. Increases in blood flow were found to be associated with the presence in the uterine lumen of seminal plasma, raw semen [2], and embryonic vesicle, [3] and a progressive increase was associated with the week of gestation [4]. It has been hypothesized that increased blood flow is related to preparation of the endometrium, inflammation, uterine clearance, delivery of endocrine signals, and/or development of the embryo. It is clear that for all organs, blood flow is essential to the maintenance of normal physiology and

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function. Degenerative lesions in the uterus are associated with subfertility and infertility in the mare. Among these lesions, endometrial vascular elastosis has been reported in aged, multiparous, and presumptively infertile mares [5–7]. Uterine vasculature degeneration could potentially compromise UBF [8] and the overall fertility of the mare.

Decreased UBF has been associated with unexplained infertility in women [9,10], and restrictions in UBF caused decreased fetal growth in sheep [11]. In the mare, using color Doppler ultrasound, areas with reduced uterine perfusion were associated with endometrial cysts [12]. Additionally, it has been shown that occlusion of UBF caused impaired myometrial contractility in the rat [13]. In the mare, poor uterine contractility has been linked to delayed uterine clearance and chronic uterine infection [14]. During pregnancy, structural and morphologic changes in the microcotyledons suggested a compromise in hematological contact and fetal growth in aged mares [15].

The objectives of this study were to determine levels of UBF and perfusion throughout the cycle of reproductively healthy mares and compare these levels to UBF and perfusion in subfertile/infertile mares affected by uterine vascular elastosis.

2. Materials and methods

All experiments were conducted with the approval of the Committee on Animal Use and Care Protocol at the University of California, Davis (protocol number 15212).

2.1. Selection criteria

Thoroughbred mares were preselected for each group according to their degree of vascular degeneration on the basis of Gruninger's classification [8]. Nulliparous presumptively fertile mares with normal vasculature or mild vascular changes served as the control group, and multiparous mares, barren for at least 2 years, with moderate to severe vascular degeneration served as the elastosis group (Fig. 1) as determined by a uterine biopsy before the blood flow determinations. Once preselected for vascular status, each mare was randomly assigned to one of two subgroups: estrus or diestrus. Consequently, there were four groups: control-estrus, control-diestrus, elastosis-estrus, and elastosis-diestrus, with five mares in each group.

To assess the stage of cycle, mares were examined by transrectal palpation and ultrasound for at least one estrous cycle. For estrus, the selection criteria were based on the presence of an ovarian follicle of diameter greater than 35 mm, uterine edema, a relaxed cervix, and the absence of a visible CL. For diestrus, the criteria were based on the absence of large preovulatory follicles, absence of uterine edema, a closed cervix, and the presence of a CL. In addition to these requirements, stage of cycle was later confirmed by determining circulating levels of progesterone. Mares with serum progesterone greater than 1.0 ng/mL were considered in diestrus.

2.2. Blood perfusion measurement

Before each experiment, mares were fasted overnight, and water was available *ad libitum*. Mares were sedated with 1 mg/kg of xylazine hydrochloride, and anesthesia was induced with 2 mg/kg of ketamine hydrochloride. Mares were intubated with a 26-mm endotracheal tube for administration of inhalation anesthesia with 1.57% isoflurane corresponding to 1.2 X minimum alveolar concentration for horses as previously described [16]. The mares were transferred to a surgery table, placed in right lateral recumbency, and underwent cardiovascular stabilization for 20 to 30 minutes. During stabilization, four arterial catheters were placed (carotid artery, facial artery, right and left dorsal metatarsal arteries) to collect reference blood samples and standard instrumentation to monitor cardiovascular and respiratory parameters. The four reference samples served as blood flow calculations and to control for homogeneous mixing of microspheres within the circulating blood [16]. An additional 14-ga, 5¼ inch, intracardiac infusion catheter was placed transmurally into the left ventricle of the heart guided by an ultrasound. All arterial catheters were connected to automated withdrawal pumps with glass syringes (50 mL containing 2cc of heparin). The intracardiac catheter was connected to an injection pump loaded with 40×10^6 yellow fluorescent microspheres 15 μm in diameter (Dye-Trak-F, Triton technology, San Diego, CA, USA) that were previously vortexed for 15 seconds and sonicated for 2 minutes to ensure adequate mixing. Activation of all pumps followed the order described by Glenny et al. [17]. All withdrawal syringe pumps connected to arterial catheters were activated at the same time and blood samples were visually monitored *via* the glass syringes (approximately 15 seconds after starting). The fluorescent microspheres were injected through the intracardiac catheter into the left ventricle of the heart (total injection time 45 seconds). The total withdrawal time of arterial samples was 2 minutes and 30 seconds, allowing for at least one additional minute of blood withdrawal after the completion of the injection of microspheres. 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.

Postmortem, the uteri from mares were retrieved through an incision lateral to the abdominal midline and caudal to the last rib and extracted by excision through the broad ligament and caudal to the cervix. A full-thickness sample (1-cm long and 0.5-cm wide) was collected from the base of a uterine horn and placed in 4% formalin for histopathology.

Uterine specimens were embedded in paraffin using standard histologic procedures, and sections were stained for hematoxylin and eosin, and specific staining for elastic fibers with Verhoeff's Van Gieson stain [18]. The endometrial biopsy grade was determined using Kenney's modified criteria [19,20], and the degree of elastosis according to Gruninger's classification [8].

The uteri were weighted and excised into small sections (6 to 8 grams). The sample location within the uterus and weight were recorded for each sample, which was then placed in a sample-processing unit containing a built-in filter [21] with digestion solution (KOH 4M) for a minimum of 48 hours at 60 °C. Blood samples were transferred to Erlenmeyer flasks, and 50 mL of digestion solution was