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Original Research

Assessment of Uterine Vascular Perfusion During the Estrous Cycle of Mares in Connection to Circulating Leptin and Nitric Oxide Concentrations

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ABSTRACT

The objective of this study was to find difference in vascular perfusion of uterine horns or uterine body throughout the estrous cycle and their relation to circulating nitric oxide and leptin concentrations. Five cyclic mares were subjected to transrectal Doppler ultrasonography and blood sampling for 18 days. Area of color and power Doppler modes was measured in pixels. Day ($P = .0001$) of the estrous cycle and ovulation ($P = .0001$) influenced uterine blood flow. Uterine body blood flow directed away from the transducer (blue, $P = .0001$) increased from day −5 until day 0 (day of ovulation), and its power ($P = .0001$) blood flow increased from day −6 until day 0; then, both decreased until days 12 and 10, respectively. Conversely to the contralateral uterine horn, ipsilateral uterine horn blood flow directed away from the transducer (blue, $P = .0001$) increased from day −5 until day −1, and its power ($P = .0001$) blood flow increased from day −6 until day 0; then, both decreased until day 10. Nitric oxide concentrations ($P = .0001$) attained two major peaks; the first on day −3 and the other persisted from day 2 until day 5. Leptin concentrations increased ($P > .001$) with a maximum value on day 0 and then decreased until a minimum value on day 9. In conclusion, during the estrous cycle, ipsilateral uterine horn and uterine body blood vessels had similar blood flow. Both leptin and nitric oxide played a role during follicle growth, ovulation, and corpus luteum development and modulated uterine blood flow before and after ovulation.

1. Introduction

In mares, uterine hemodynamics has been subjected to several studies using Doppler ultrasonography [1]. Spectral Doppler ultrasonography has been performed to study uterine blood perfusion during early gestation [2,3] and in cyclic mares [4,5]. Color Doppler was used for studying changes in endometrial vascular perfusion comparable to changes in location of embryonic vesicle [6], as indicator for completed orientation of the embryo [7], and in subfertile mares [8].

Nitric oxide (NO) is created in vivo from the fundamental amino acid L-arginine by the vascular endothelium and has been shown to play a regulatory role in the reproductive system [9]. Nitric oxide plays a significant role in vascular smooth muscle relaxation to increase uterine blood flow during the luteal phase [10]. Nitric oxide, both ovarian cells derived and vascular endothelial cell derived, has an essential role in the physiology and biology of the ovary with respect to regulation of folliculogenesis and ovulation [11]. Nitric oxide synthase identified in the uterus plays a strong important role in the uterine function control [12]. In addition, the local production of NO is a major regulator of blood flow [13].

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The uterus has been demonstrated to have the capacity to deliver little amounts of leptin [14]. Leptin that is discharged from adipose tissue can effectively upregulate NO production [13]. Leptin is viewed as an angiogenic component [15]. The relaxing role of leptin on main blood vessels was partially controlled by the NO-dependent way, and phenylephrine-contracted blood vessel arterial rings were relaxed by leptin through endothelial NO production [16].

The aim of the present study was to evaluate uterine vascularization through monitoring blood perfusion to uterine body, ipsilateral and contralateral uterine horns using color and power Doppler modes throughout the estrous cycle of mares and discovering their relation to ovulation, circulating leptin and NO concentrations and to select the suitable Doppler mode for evaluating uterine vascular perfusion.

2. Materials and Methods

2.1. Animals and Ultrasound Scanning

Five crossbred brood mares of European × Egyptian horses (3–12 years old) were subjected to ultrasonographic examination for 18 successive days throughout two estrous cycles. The mares were given a week off rectal examinations between the two estrous cycles. The mares were kept in an indoor paddock with partition individually with a pubertal colt at the end of the stable to confirm estrus signs. The mares were kept under natural day light and temperature, and artificial lightening was used at night within the paddock. The mares were maintained on a commercial pelleted ration and hay with free access to water. Before directing this work, the mares were analyzed three times at week-by-week interim to affirm ovarian cyclicity and regulatory of estrus. This study was conducted from June 15 to July 28, 2014 at the Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University (30.0276°N, 31.2101°E). The experiment was conducted in accordance with an Institutional Animal Care and Use Committee.

A transrectal pulsed-wave Doppler ultrasound scanner equipped with a 5- to 12-MHz linear-array transrectal transducer (SonoVet R3; Medison, Samsung, South Korea) for the examination of uterus, by scanning all structures on both ovaries. Both uterine horns and uterine body were scanned with color and power Doppler modes. All scans were performed by the same operator during the experiment. Examinations were performed from 8 AM to 11 AM to avoid the high temperature and humidity.

2.2. Uterine Blood Flow Measurement

To quantify which color Doppler mode would be recommended for future evaluation of uterine vascular perfusion and which color (away or toward the transducer), the single color power Doppler mode, both color and power Doppler modes were used during this study. Vascular perfusion of the uterus (all layers combined) was estimated subjectively by one operator during the study period from the cross sections of a horn using single color power and color flow mode. Only the color signals that appeared to be within the limits of the uterine body or horns were considered. The uterine vascular perfusion was estimated subjectively using the percentage of uterine tissue with color signals of blood flow during real-time cross sectioning of the uterus. Multiple cross sections were viewed because of animal and uterine movements and the variation resulting from angles of insonation [17]. Spectral Doppler was not used because it was time consuming and required a sedated animal for longer time and special examination area that was not available. Each blood flow mode was activated, and the blood flow area within the uterine wall was quantified from the color images. All scans were done at a pulse-repetition frequency, and identical color gain settings were used. The color mode was used to determine the blood flow area within the uterine wall.

A real-time B-mode/color Doppler and power Doppler images were stored on the hard drive of the scanner, and images were exported at the end of the study using a removable hard disk to a computer for blood flow area analyses in the laboratory. The blood flow area was evaluated in a vertical plane at the maximum diameter of each uterine horn and body in every day examination. The distance between the transducer face and the uterus was minimized to reduce signal attenuation. The blood flow areas in the uterine wall were measured. The colored flow area was counted toward the transducer (red color), away from the transducer (blue color) and for power Doppler single color images per pixel using Adobe Photo Shop CC software (1990–2013, Adobe Systems). The days before ovulation (from day −6 to day −1, follicular phase) referred to the growth of the dominant ovulatory follicle, day 0 referred to the day of ovulation (the last day the ovulatory follicle monitored), and days from 1 to 13 referred to days after ovulation in which the corpus luteum is dominant.

2.3. Blood Sampling and Hormonal Assaying

Daily blood samples were collected via jugular vein punctures in plain vacuum tubes of all mares. Serum was harvested and stored at −20°C until hormone assaying. Leptin was assayed using commercially available Leptin ELISA kit (Sandwich) previously used for horses in our laboratory [18] using DRG diagnostics (DRG, Germany). Sensitivity of the assay was 1.0 ng/mL. Intra-assay and interassay coefficients of variation were 3.1% and 9.7%, respectively.

The procedure for determining plasma concentrations of total nitrates and nitrites was based on an assay for measuring NO metabolites (NOMs) in human plasma [19]. For measuring NOMs, 100 μL of serum samples were mixed with an equal volume of Griess reagent and incubated for 10 minutes at room temperature and absorbance was measured at 540 nm using a microtiter plate reader. Nitrite (NO_2) standards (0–50 μM) were used to determine NOMs concentration in each well as previously measured in our laboratory in mares [20]. The intra-assay and interassay coefficients of variation for the NOM assay were 5.3% and 6.9%, respectively. Sensitivity of the assay was 0.225 μmol/L of nitrites in the sample.