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Influence of L-arginine supplementation on reproductive blood flow and embryo recovery rates in mares

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ABSTRACT

Supplementation with L-arginine can increase uterine arterial blood flow and vascular perfusion of the preovulatory follicle in mares. Increased vascular perfusion of the preovulatory follicle has been correlated with successful pregnancy in mares. The objective of this study was to determine if supplemental L-arginine would increase ovarian arterial blood flow, vascular perfusion of the preovulatory follicle, and embryo recovery rates in mares. Mares were blocked by age and breed and assigned at random within block to L-arginine supplementation or control groups. Mares were fed L-arginine beginning 17 days before and through the duration of the study. Transrectal Doppler ultrasonography was used to measure ovarian arterial blood flow and vascular perfusion of the preovulatory follicle daily when it reached 35 mm and subsequent CL on Days 2, 4, and 6. Mares, on achieving a follicle of 35 mm or more were bred via artificial insemination and an embryo collection was attempted 7 days after ovulation. Treatment did not affect interovulatory interval (arginine-treated, 18.1 ± 2.6 days; control, 20.7 ± 2.3 days) or embryo recovery rate (arginine-treated, 54%; control, 48%). Mares treated with L-arginine had a larger follicle for the 10 days preceding ovulation than control mares (30.4 ± 1.2 and 26.3 ± 1.3 mm, respectively; $P < 0.05$) and vascular perfusion of the dominant follicle tended ($P = 0.10$) to be greater for the 4 days before ovulation. No differences were observed between groups in diameter or vascular perfusion of the CL. Resistance indices, normalized to ovulation, were not significantly different between groups during the follicular or luteal phase. Oral L-arginine supplementation increased the size and tended to increase perfusion of the follicle 1, but had no effect on luteal perfusion or embryo recovery rates in mares.

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1. Introduction

Proper vascularization plays an important role in the selection, growth and maturation of follicles [1], and the increased blood flow to the dominant follicle is associated with increased pregnancy rates in mares [2]. One way to improve blood flow to the reproductive tract is through administration of L-arginine [3]. This amino acid acts as a substrate for biosynthesis of nitric oxide (NO), polyamines, proline, glutamate, creatine, and agmatine [4]. Nitric oxide is a vasodilator that inhibits vasoconstrictor inputs [5], acts downstream of vascular endothelial growth factor signaling to promote angiogenesis [6], and is thought to modulate preovulatory ovarian blood flow [7,8]. Supplementation of women, who have a history of poor response to ovarian hyperstimulation, with L-arginine, increased ovarian blood flow, oocytes retrieved, and embryos transferred [9]. In mares, oral supplementation with L-arginine increased uterine arterial blood flow and hastened the involution period in postpartum mares [10]. The objective

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of this experiment was to determine if oral supplementation with L-arginine would increase ovarian blood flow and embryo recovery in mares.

2. Materials and methods

2.1. Animals

Animal use was approved by the University of Florida Institute of Food and Agricultural Sciences Animal Research Committee. Mares were blocked into a control or treatment group based on age and breed. The control consisted of five Thoroughbreds (mean age: 14.8 ± 1.4 years) and three American Quarter Horse (mean age, 6.7 ± 4.7 years) mares aged 2 to 17 years (control mean age, 11.8 ± 2.3 years). The treatment group contained three Thoroughbred (mean age, 14.3 ± 1.7 years) and four American Quarter Horse (mean age, 9.5 ± 3.4 years) mares aged 2 to 19 years (treatment mean age, 11.6 ± 2.1 years). Mares had apparently normal reproductive activity as determined using ultrasound examination of the cervix, uterus, and ovaries. All mares had uterine biopsies obtained before the study and were examined by a Diplomate of the American College of Theriogenology using the Kenney system [11]. All mares had either a biopsy score of II or IIA and there was no difference between groups in biopsy score as determined by chi-square test.

The study was conducted between May and September (Latitude 18′ 22″N; Longitude 10′ 3″W). The first estrus cycle was induced with an injection of PGF2α (Lutalyse; Pfizer, New York, NY, USA) on Day 7 after ovulation, as were subsequent estrous cycles. Data were collected from up to six cycles; however if uterine bacterial culture was positive, mares were treated and not bred. Mares were housed on pasture and supplemented with a commercial mixed concentrate ration formulated for gestating and lactating mares (minimum guarantees: 16% crude protein, 3.5% crude fat, 0.9% Ca, 0.55% P; Ocala Breeder's Feed and Supply, Ocala, FL, USA) and fed individually in paddocks once daily. All mares had free access to water and trace mineral salt. Treatment mares were fed 100 g of L-arginine (Ajinomoto, Raleigh, NC, USA) once daily in the morning beginning from 17 days before the start of the study and remained on supplementation once daily until the completion of study. Ovulation was determined using transrectal ultrasonography.

2.2. Doppler ultrasonography

Ovaries and ovarian arteries were examined via transrectal ultrasonography using Doppler ultrasonography with a 5 to 10 MHz broadband linear array (Micromaxx; Sonosite, Bothell, WA, USA) every other day during estrus until the largest follicle reached a diameter of 35 mm and then daily until ovulation. Profiles of the eight largest follicles (designated F1 through F8, respectively) were categorized using a nontracking method (without regard to day-to-day follicle identity). A retrospective analysis identified the dominant ovulatory follicle (F1) and the largest subordinate follicle (F2). Corpus luteum measurements were taken on Days 2, 4, and 6 postovulation. Diameters (average of height and width) of the largest follicle and CL were measured and recorded. Vascular perfusion to the follicle and CL was determined with color power Doppler ultrasonography. Resistance index (RI) was measured for both ovarian and uterine arteries as described by Smith, et al. [12]. All examinations were recorded digitally for future analysis. Images from the digital video disc recordings were used to determine the percent perfusion of the retrospectively identified dominant follicle and CL as previously described by Kelley, et al. [13] and Smith, et al. [12].

2.3. Embryo collections

Mares were bred with semen from one of two fertile stallions. Semen was collected using artificial vagina and evaluated for total progressive motility and sperm concentration. The concentration was estimated using an Equine Densimeter (Animal Reproduction Systems, Chino, CA, USA). Each mare received a total dose of 500×10^6 fresh, motile spermatozoa extended to a volume of 15 mL using E-Z Mixin original formula (Animal Reproduction Systems). Mares were bred every other day until ovulation via artificial insemination, beginning from the time they displayed estrus to a teaser stallion and had a follicle with a diameter of 35 mm or greater. All ovulations were spontaneous (not induced). Nonsurgical embryo collection was performed on Day 7 after ovulation. The uterus was infused four times with a 500-mL flush medium (Biolife Advantage; Agtech, Manhattan, KS, USA) utilizing a silicone 34 French catheter (Reproduction Resources, Walworth, WI, USA) and the effluent was collected through Y-junction tubing (Reproduction Resources) into a 75-μm filter (Reproduction Resources). Mares were administered PGF2α (Lutalyse; Pfizer), 10 mg intramuscularly, at the end of the flushing procedure. The contents of the filter were rinsed into collection dishes, which were examined using a stereomicroscope at magnification ×20. Embryos were evaluated based on a one (excellent) through four (nonviable) scale as previously described [14].

2.4. Statistical analysis

Mares were divided by age into young (<16 years; arginine, n = 4; control, n = 4) and old (≥16 years; arginine, n = 3; control, n = 4) for analysis to examine the effect of age. Follicular and RI data (during the follicular phase) were normalized to ovulation (Day 0). Corpus luteum and RI data (during the luteal phase) were normalized to ovulation beginning an estrous cycle (Day 0). Continuous data were analyzed using the SAS MIXED procedure (version 9.2; SAS Institute Inc., Cary, NC, USA). A random statement was used to account for variability of animals within treatment and cycle by animals within treatment. A repeated statement for day was used, with the subject being cycle by animals within treatment, using compound symmetry as the model best fitting the covariance matrix. A Tukey's multiple range test was used to detect differences among groups within days when there was a significant interaction between group and day. The embryo recovery rates were compared using a Chi-square test. Data are presented as least square means ± standard error of the mean. A significant

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