Animal Reproduction Science 138 (2013) 233–240

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Oral L-arginine supplementation impacts several reproductive parameters during the postpartum period in mares

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ARTICLE INFO

Article history:
Received 9 August 2012
Received in revised form 26 February 2013
Accepted 1 March 2013
Available online 14 March 2013

Keywords:
Mare
Postpartum
Ovary
Blood Flow
Uterus

ABSTRACT

L-Arginine is an amino acid which can alter pituitary function and increase blood flow to the reproductive tract. The objective was to determine the effect of supplementing 100 g of L-arginine on plasma arginine concentrations, follicular dynamics and ovarian and uterine artery blood flow during the estrus that occurs subsequent to foaling. In Experiment 1, mares were fed 100 g L-arginine for 1 day during the last 3 weeks of pregnancy and plasma samples taken for every hour for the first 4 h and every other hour until 12 h. L-Arginine supplementation elevated plasma arginine concentrations from 1 to 8 h post feeding; arginine peaked at 6 h (arginine: 515 ± 33 μmol/L; control: 80 ± 33 μmol/L). In Experiment 2, mares received either 100 g L-arginine or control diets beginning 21 d before the expected foaling date and continued for 30 d postpartum. The reproductive tract was evaluated by transrectal Doppler ultrasonography from Day 1 postpartum through Day 30. There were no differences in ovarian follicular dynamics, ovarian or uterine resistance indices between groups. Vascular perfusion of the F1 follicular wall was greater in L-arginine supplemented mares (37.3 ± 2.6%) than controls (25.4 ± 2.7%; $P < 0.05$). L-Arginine supplemented mares had a smaller uterine body and horns and accumulated less uterine fluid than controls ($P < 0.05$). The combination of reducing uterine fluid accumulation, while not altering follicular development, raises the possible use of L-arginine supplementation as a breeding management tool during the postpartum period to increase reproductive success.

1. Introduction

L-Arginine is one of ten essential amino acids in horses (National Research Council, 2007) and a biologically-active regulator of several physiological systems including the reproductive, cardiovascular, pulmonary, renal, and immune systems (Wu et al., 2009). L-Arginine can behave both as a receptor ligand (Joshi et al., 2007) and as a substrate for biosynthesis of nitric oxide (NO), polyamines, proline, glutamate, creatine, and agmatine (Wu and Morris, 1998). L-Arginine activates nitric oxide synthase (Joshi et al., 2007; Morrissey and Klahr, 1997) which catalyzes the conversion of L-arginine to NO and L-citrulline. Nitric oxide is a vasodilator that inhibits vasoconstrictor signals (Thiriet, 2008) and acts downstream of VEGF signaling to promote angiogenesis (Murohara et al., 1998). In gilts, oral supplementation of L-arginine starting on Day 30 of gestation increases litter size and birth weights (Mateo et al., 2007). Supplementing L-arginine (1% of diet) in pre- and postpartum mares increased uterine blood flow (Mortensen et al., 2011). Given that blood flow surrounding the dominant follicle is associated with increased pregnancy rates in mares (Silva et al., 2006); fertility may be improved in mares by feeding L-arginine.

In the present study, the impact of L-arginine supplementation on the reproductive characteristics of the

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http://dx.doi.org/10.1016/j.anireprosci.2013.03.001

postpartum mare are further examined. Mares are distinctive among domestic livestock in the ability to return to estrus and potentially conceive shortly after giving birth. This first postpartum estrous period has been coined 'foal heat', with mares having ovulations from 8 to 13 d after giving birth parturition (McCue and Hughes, 1990). Scant literature exists on ovarian follicular development and follicle selection during the first postpartum estrous cycle in mares. Shortly after parturition, the diameter of largest follicle ranges from 13 to 16 mm (Ginther et al., 1994) and reaches a diameter of 36.4 mm by Day 8 (Gündüz et al., 2008). The objectives of this study were to observe baseline follicular dynamics and ovarian blood flow during the first postpartum estrous cycle in mares and evaluate the influence of supplemental L-arginine on ovarian follicular dynamics, ovarian and uterine blood flow and uterine involution.

2. Materials and methods

2.1. Animals and experimental design

Studies were approved by the Institute of Food and Agricultural Sciences Animal Care (IFAS) and Use Committee at the University of Florida and were conducted at the IFAS Equine Science Center (Latitude 29°18′12″N; longitude 82°10′3″W). In Experiment 1, six multiparous Quarter horse mares were randomly assign to either a control or L-arginine group ($n = 3$) during the last 3 weeks of pregnancy to evaluate plasma availability of arginine in response to a meal supplemented with L-arginine. Both groups were fed 2.4 kg of 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). Mares were fed individually in stalls. L-Arginine supplemented mares received 100 g of L-arginine (Ajinomoto AminoScience LLC, Raleigh, NC, USA) that was mixed into the grain ration. Mares in both groups were fed Coastal Bermuda grass (3.5 kg) hay 4 and 8 h after receiving grain. Control mares were not fed an isonitrogenous (diets equal in nitrogen) vehicle to ensure that the comparison would be between L-arginine and a standard pregnant mare diet. Blood samples were obtained via jugular catheters into heparinized tubes, prior to feeding (0 h) and at 1, 2, 3, 4, 6, 8, 10 and 12 h post feeding. Samples were immediately centrifuged at 11,000 *g* for 15 min and plasma was stored at -80 C until analysis.

In Experiment 2, 16 mares were blocked by age (range 5–19 yr), breed [Thoroughbred ($n = 8$) and Quarter Horse ($n = 8$)], and expected foaling date and assigned randomly to receive L-arginine or no supplementation ($n = 8$/group). The mean (±SEM) age of mares was 11.5 ± 1.7 yr for L-arginine and 11.4 ± 1.3 yr for controls. The basal diet consisted of ad libitum Coastal Bermuda grass hay and 3.8 ± 0.3 kg of a commercial mixed concentrate ration formulated for gestating and lactating mares (minimum guarantees: 16% CP, 3.5% crude fat, 0.9% Ca, 0.55% P; Ocala Breeder's Feed and Supply, Ocala, FL, USA). Mares were fed individually in stalls at 0700 and 1500 h. L-Arginine-supplemented mares received 100 g of L-arginine (Ajinomoto AminoScience LLC, Raleigh, NC, USA) that was mixed into the grain ration immediately before each morning feeding. Treatments began 21 d before expected foaling dates and continued for 30 d postpartum.

2.2. Amino Acid Analysis

Plasma samples from Experiment 1 were deproteinized using 35% (w/v) sulfosalicylic acid. The acid soluble fraction was separated by centrifugation (4 °C, 11,000 *g* for 20 min). The supernatant was filtered (0.2 μm, Fisher Scientific, Pittsburgh, PA, USA) and then mixed 1:1 with 0.02 N HCl (Boucher et al., 1997). Plasma samples were then analyzed for amino acid composition using an amino acid analyzer (L-8900, Hitachi-High Technologies, Pleasanton, CA) as previously described by Ma et al. (2010).

2.3. Ultrasonongraphy

In Experiment 2, ovaries and ovarian arteries were examined via trans-rectal ultrasonography daily from the day after foaling until the first postpartum ovulation. The uterus and uterine arteries were examined daily from foaling until 30 d post-foaling. Ultrasonographic exams (900–1100 h) were performed using a Micromaxx Sonosite digital color Dopplar ultrasonic equipment with a 5–10 MHz broadband 52 mm linear array (Bothell, WA, USA). Exams were recorded (Sony DVDIRECT®, San Diego, CA, USA) and subsequent videos were reviewed for analysis. The length of the first estrus subsequent to foaling was defined as the number of days from foaling to the first post partum ovulation.

Follicles were grouped by diameter (6 to 10, 11 to 15, 16 to 20 and >20 mm) each day without regard to day-to-day identity as previously described by Kelley et al. (2011). Data were normalized to the 10 d preceding ovulation with the day of ovulation considered Day 0. Following ovulation, retrospective analysis determined the largest (ovulatory) follicle (F1), largest subordinate follicle (F2) and the second largest subordinate follicle (F3). The diameter of the F1 and F2 at the time of follicular deviation was ascertained as described elsewhere (Kelley et al., 2011). Deviation was defined as occurring on the day (exam) prior to the examination with the greatest change in differences in diameter between the two largest follicles.

For uterine measurements the screen depth was increased to visualize the entire uterine section and an outer cross sectional measurement for the uterine body was taken at the point of maximum height using electronic calipers. The diameter of each uterine horn was calculated by averaging the height and width measurements and was taken at approximately the midpoint of each horn. Maximum intrauterine fluid accumulation was measured within the uterine body via ultrasonography using electronic calipers.

Spectral Doppler measurements of both ovarian arteries and uterine arteries were evaluated as described by Ginther (2007) and calculated by an algorithm package in the Micromaxx® ultrasonography. Retrospective analysis identified the ovarian arteries as either ipsilateral or contralateral to the ovulatory follicle. The sample cursor