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L-Arginine supplementation 0.5% of diet during the last 90 days of gestation and 14 days postpartum reduced uterine fluid accumulation in the broodmare

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ABSTRACT

L-Arginine is an essential amino acid in many species that has been shown to influence reproduction. However, in horses a dose of 1% L-arginine of total dietary intake impaired absorption of other amino acids, whereas a dose of 0.5% did not. The objectives of this experiment were to evaluate postpartum parameters on mares supplemented with 0.5% L-arginine through the last 90 d of gestation and 14 d postpartum. Sixteen light-horse mares were randomly divided in two groups: 8 mares supplemented with 0.5% L-arginine and 8 mares fed an isonitrogenous equivalent. Gestation length, days to uterine clearance and days to first ovulation were compared. Uterine body depth, diameter of uterine horns, and length of largest pocket of uterine fluid were recorded daily via transrectal ultrasound. Measurements of foal weight, height, and cannon bone circumference were recorded for 9 weeks. Arginine treatment had no effect on gestation length ($P = 0.58$). Supplemented mares cleared fluid quicker postpartum (6.8 ± 0.53 d; $P = 0.026$) compared to control (9.0 ± 0.38 d). Mares supplemented with L-arginine had smaller diameter of fluid present in the postpartum uterus ($P \leq 0.05$). Days to first postpartum ovulation were not affected by treatment nor any influence on uterine involution. Finally, treatment had no effect on any foal's measured parameters. L-Arginine supplementation fed at 0.5% of daily intake during the last 90 d of gestation and early postpartum in mares decreased uterine fluid accumulation, yet did not appear to have any effect on any other parameters measured.

1. Introduction

L-Arginine (L-Arg) is an essential amino acid in mammals, even when it can be endogenously synthesized, and requirements in most species exceeds its biosynthetic production (Castillo et al., 1994). L-Arg is also involved in diverse physiological functions as a mediator in endocrine and immune systems, and serves an essential role in the cardiovascular system by being a nitric oxide precursor. Furthermore, L-Arg is required for synthesis of proteins, urea, polyamines, creatine, and agmatine (Wu and Morris, 1998).

Numerous studies have evaluated the effects of arginine supplementation on animal models to include effects on hypertension (Sanders, 1996; Siani et al., 2000) preeclampsia (Kim et al., 2006), and growth hormone secretion (Fisker et al., 1999). In studying L-Arg and influences on reproduction, Mateo et al. (2007) reported L-Arg supplementation in pregnant gilts elicited an increase in litter survival with increased live litter birth weight. The author's

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hypothesized that supplemented gilts could have experienced enhanced placental angiogenesis and increased placental blood flow that promoted better circulation and improved environment for the fetuses. It has also been suggested that arginine is an insulin stimulator in human fetuses and newborns, therefore causing an anabolic response (King et al., 1971). L-Arg deficiencies have also been associated with fetal growth restrictions (Vosatka et al., 1998).

Mares supplemented with 1% of their diet with L-Arg had shorter gestation lengths, improved uterine arterial blood flow and decreased uterine fluid accumulation postpartum (Kelley et al., 2013, 2014a; Mortensen et al., 2011). However, recent evidence suggested that L-Arg supplemented at this amount reduced the absorption of other amino acids. Kelley et al. (2014b) reported, mares supplemented with 1% L-Arg had a decrease in plasma lysine, methionine, histadine, and proline. This was not observed in mares fed a single dose of 0.5% dose L-Arg.

The purpose of this experiment was to evaluate reproductive parameters of postpartum mares supplemented with 0.5% L-Arg of the total daily dietary intake through their last 90 d of gestation and first 14 d post parturition. Our hypothesis was mares supplemented with 0.5% L-Arg of the total for the 90 d leading up to their expected foaling date, would have a significantly shorter gestation length, greater reproductive performance postpartum and foals born from supplemented mares would report faster growth rates.

2. Materials and methods

2.1. Experimental design

The study was conducted from November 2012 to April 2013 at the Institute of Food and Agricultural Sciences (IFAS) Equine Science Center in Ocala, FL. The experiment was approved by the Animal Care and Use Committee at the University of Florida. A total of 16 light-horse mares and of their foals were used for this experiment and housed as previously described by Mortensen et al. (2011). Mares were blocked by parity, randomly assigned into either an L-Arg or control group and were weighed weekly for dietary estimation as described by Kelley et al. (2014b). The basal diet consisted of ad libitum access to Coastal Bermuda grass hay and 0.5–1% of their bodyweight of a grain-mix concentrate (minimum guarantees: 16% crude protein, 3.5% crude fat, 0.9% Ca, 0.55% P; Gest-O-Lac Ocala's Breeder's feed and Supply, Ocala, FL) during gestation. Amino acid analyses of diets used in this study are similar to those described in Kelley et al. (2014b). Briefly, estimated arginine content of forage was 0.32 g per 100 g and concentrate was 0.85 g per 100 g. After parturition, the offered grain increased to 1.25% of their body weight from the same concentrate. The treated group was supplemented with L-Arg (Ajinomoto AminoScience LLC, Raleigh, NC, USA) at 0.5% of the total estimated daily intake. The control group was fed an isonitrogenous equivalent urea supplement. Mares were fed individually and the supplement was mixed with the morning feed. Supplementation started 90 d before the expected foaling date and continued for 14 d postpartum.

2.2. Mare reproductive performance

Transrectal ultrasonographic measurements began within 24 h of foaling as previously described by Mortensen et al. (2011). Briefly, a digital color Doppler ultrasound (Micromaxx®, Sonosite, Bothell, WA) with a 10–5 MHz broadband and 52 mm linear probe was used and the same technician performed all the exams. Uterine body depth, diameter of the gravid and non-gravid horns, diameter (height × width) of the largest pocket of uterine fluid was recorded to estimate uterine involution. Spectral-Doppler blood flow measurements for uterine arteries were obtained operating an algorithm package of the Micromaxx ultrasound. Uterine arteries were classified as gravid or non-gravid according to the diameter of the ipsilateral uterine horn on the first post-partum measurement. The arterial spectrum obtained with Doppler, with two uniform cardiac cycles, was used to generate the resistance index (RI) [(peak systolic velocity (PSV) − end diastolic velocity (EDV))/PSV] (Ginther, 2007). Blood flow data was performed in triplicate and the average was considered for statistical analysis. Retrospectively identified dominant follicle diameters and presence of a corpus luteum was recorded to identify first postpartum ovulation. All exams were digitally recorded (Sony DVDIRECT®, San Diego, CA, USA) for future analyses.

2.3. Foal measurements

Within 24 h of foaling, all 16 foals were weighed using an electronic livestock scale and height and cannon bone circumference was recorded using a tape measure. Height was measured from the ground to the top of the foal's withers on a uniform concrete surface. Cannon bone size was calculated by measuring the circumference of the medial front right large metacarpal bone. Individual measurements of the foals continued weekly for the 9 weeks following parturition.

2.4. Statistical analysis

Variables were analyzed for normal distribution using Shapiro–Wilk test; in case of normal distribution, the Barlett test was used to verify the homogeneity of variances. If any of the assumptions were not assured, data was transformed using a Box–Cox transformation using the Lmsupport package on R, and subsequently assumptions were verified again. These statistical analyses were performed using R version 3.0.1 (R Foundation). Measurements collected at a single time point, such as gestation length, days to ovulation and days to uterine clearance were compared by one-way ANOVA. For all comparisons a P-value ≤ 0.05 was considered significant and a P-value < 0.1 and > 0.05 was considered a trend. Graphed values are given as mean ± SEM.

Data collected at multiple time points was analyzed using the MIXED procedure in SAS (V.9.2, SAS Inst., Inc., Cary, NC). Fixed effects of treatment, day, and the interaction of treatment by day were assessed by the restricted maximum likelihood (REML) estimation method, with repeated measures over time (days). The mares