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Original Research

Supplemental L-Arginine Shortens Gestation Length and Increases Mare Uterine Blood Flow before and after Parturition

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ABSTRACT

Supplementing diets with L-Arginine (Arg) improves female reproductive performance and reproductive blood flow in other species. The objectives of this study were to investigate uterine artery blood flow changes before and after parturition, and evaluate blood flow in Arg supplemented and control mares by Doppler ultrasonography. Sixteen light-horse mares began Doppler ultrasonography evaluation, 21 days before expected foaling date (EFD) and continued until day 7 postparturition. The mares under treatment (n = 8) were supplemented with 100 g Arg, once daily, beginning with 21 days before EFD. Blood flow measurements were calculated as pulsatility index (PI) and resistance index (RI) for both uterine arteries, either ipsilateral or contralateral; to uterine horn of established pregnancy; defined gravid uterine artery (GUA) and non-gravid uterine artery (NGUA), respectively. The mares under treatment had a shorter gestation length (337 ± 1.7 days) as compared to control (345 ± 2.1 days; $P \leq .05$). No differences in gestation length were observed between groups when examined by age, parity, EFD, or sex of foal. Both GUA and NGUA uterine artery diameter decreased from the day before parturition to day 7 after parturition ($P \leq .001$). During this time period, both PI and RI increased ($P \leq .01$); indicating less blood flow. A treatment effect was observed with Arginine-treated mares having greater blood flow prepartum in the NGUA ($P \leq .001$) and postpartum in the GUA ($P \leq .05$), for both indices. The data demonstrated that supplementing mares with Arg shortened gestation length and increased uterine arterial blood flow before and after parturition.

1. Introduction

L-Arginine (Arg) is one of the 10 essential amino acids for horses [1]. Arginine supplementation has been shown to improve reproductive, cardiovascular, pulmonary, renal, gastrointestinal, liver, and immune functions in various species [2]. In addition, Arg metabolism results in production of nitric oxide (NO), polyamines, proline, glutamate, creatine, and agmatine [3]. Previous work has established the safety of supplementing Arg in diets of pregnant sheep, pigs, and rats [4]. Supplementing diets with Arg has been shown to enhance reproductive performance of pigs [5] and reduce embryonic mortality in rats [6]. In the study of Mateo et al. [5], it was shown that Arg supplemented in the diets of gilts from day 30 of gestation until term resulted in a greater number of live piglets born. The author's hypothesized that one mode of action for these increases could have been greater uteroplacental blood flow and greater placental angiogenesis. In another study, Takasaki et al. [7] reported that women with a thin endometrium who were supplemented orally with Arg (24 g/d) had greater blood flow to the uterine radial arteries and improved endometrial thickening. In horses, the Arg-NO pathway has been shown to mediate endothelium-dependent relaxation [8]. Potentially,

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increased availability of NO and its metabolites may improve blood flow in mares, as seen in other species [9].

Bollwein et al. [10] and Mortensen et al. [11] have documented changes in uterine blood flow during early pregnancy in mares. The biological changes leading up to parturition in the mare have been documented; however, uterine blood flow characteristics during late pregnancy and immediately after parturition have not been documented. Therefore, our goals were twofold: document the uterine blood flow changes leading up to, and the immediate days after parturition; evaluate any potential uterine blood flow increases in response to supplemental feeding of Arg beginning in late pregnancy to mares fed a standard diet.

2. Materials and Methods

2.1. Animals

A total of 16 pregnant light-horse mares were used for this trial. Animal use was approved by the Institute of Food and Agricultural Sciences Animal Care and Use Committee at the University of Florida. Mares were maintained on pasture and individually fed in box stalls daily at 07:00 and 15:00 hours. Mares showing outward physical signs of nearing parturition (e.g., waxing of teats) were moved to a 1-acre dry lot for overnight monitoring until foaling. Once impending parturition was spontaneously initiated with rupture of the chorioallantois, mares were moved into an adjacent 6×3 m^2 foaling stall. The following foaling parameters were recorded: time from rupture of the chorioallantois to termination of stage II (foal delivery); time from foal delivery to shedding of the placental tissues; placental weight; and time from foal delivery to first nursing. Mares and foals were allowed back on pasture 24 hours after foaling.

2.2. Experimental Design

The mares were blocked by age (range: 5-19 years), breed (Thoroughbred [$n = 8$] and American Quarter Horse [$n = 8$]), and expected foaling date (EFD); they were then randomly assigned to one of the two dietary treatments: Arg supplementation ($n = 8$) or nonsupplemented control ($n = 8$). Mares were fed, before and throughout the study, according to the National Research Council's recommendations for pregnant and lactating broodmares [1]. The basal diet consisted of ad libitum access to Coastal bermudagrass hay and 3.8 ± 0.3 kg of a grain-mix concentrate formulated for gestating and lactating mares (16% CP, 3.5% crude fat, 0.9% Ca, 0.55% P; Ocala Breeder's Feed and Supply, Ocala, FL, USA). To acclimatize mares to the study diet for observations during the immediate pre- and postpartum period, beginning 21 days before EFD, Arg-supplemented mares received 100 g of high purity (>98.5%) Arg (Ajinomoto AminoScience LLC, Raleigh, NC, USA), which was hand-mixed into the 07:00 hour concentrate feeding. This amount of Arg represented approximately 1% of the total diet (dry matter basis), and was based on previous reports of the efficacy and safety of 1% Arg supplemented to pregnant pigs [4,5]. There are currently no dietary recommendations for Arg in the National Research Council's recommendations on nutrient requirements for horses [1].

2.3. Color Doppler Ultrasonography

Transrectal examinations of blood flow to the reproductive tract of all mares began 21 days before the EFD between 08:00 and 11:00 hours by an operator blind to treatment. Pregnant mares were evaluated every other day until parturition. Twenty-four hours after parturition, ultrasound examinations were continued daily until 7 days after foaling. A digital color Doppler ultrasound with a 10-5 MHz broadband, 52-mm linear probe (Micromaxx, Sonosite, Bothell, WA, USA) was used for all examinations. All examinations were digitally recorded (Sony DVDIRECT, San Diego, CA, USA) and subsequent videos were reviewed for analysis.

Spectral-Doppler measurements of both uterine arteries were evaluated and calculated by the algorithm package in the Micromaxx ultrasound. The sample cursor gate was set at 5 mm and at a magnification depth of 7.7 cm. The measurements were: resistance index (RI) = ([peak systolic velocity {PSV} − end diastolic velocity {EDV}]/PSV); and pulsatility index (PI) = ([PSV − EDV]/time-averaged maximum velocity [TAMV]) [12]. Uterine arteries were identified as described by Bollwein et al. [13], with measurements of both uterine arteries taken near the branching of the external iliac artery or deep circumflex artery or both. Similar to the methods described by both Siddiqui et al. [14] and Silva et al. [15] the angle cursor in relation to the direction of blood flow in the uterine arteries (insonation angle) was unknown; thus the RI and PI provide relative, rather than actual, velocity measurements. Ginther [12] stated that the indices (RI and PI) are ratios of velocity measurements and therefore are independent of the Doppler angle. A reciprocal relationship exists between these indices and blood flow, whereby an increase in either PI or RI indicates a decrease in blood flow through that examined vessel. The setting for the range of flow-velocity was adjusted to clearly visualize the spectral graph, and a Doppler spectrum with at least two uniform cardiac cycles was generated, with one of the cycles used for spectral measurements. This was done a second time, and the mean of the two measurements was used for statistical analysis. Finally, diameter of both uterine arteries was recorded during each examination for the duration of the trial.

After parturition, the mares underwent ultrasonic evaluation as described earlier and the uterine arteries were determined to be either ipsilateral or contralateral to the previously gravid horn. A retrospective analysis was then conducted on the blood flow measurements before parturition and defined as the gravid uterine artery (GUA) or non-gravid uterine artery (NGUA).

2.4. Statistical Analyses

Differences in blood flow velocities (RI and PI) were compared within and between the GUA and NGUA using the SAS MIXED procedure to determine the main effects of treatment and day and their interactions with a repeated measures statement; and LSMEANS to evaluate