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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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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 differences between treatment groups over time (SAS version 9.2; SAS Institute, Cary, NC, USA). Dietary treatment, day, and the day x treatment interaction were included in the model as fixed effects. Data collected before parturition was analyzed separately from the 7 days after parturition. Data for days before parturition were normalized to day of parturition (day 0). Means of both indices and arterial diameter between the day before parturition and the days immediately after parturition were compared by ANOVA followed by a post hoc Tukey test. Comparisons of gestation length between age, parity, EFD, sex of resulting foal, as well as all foaling parameters, between treated and control mares were evaluated by Students *t*-test. Values are reported as mean ± SEM. A probability of $P \leq .05$ indicated a significant difference and a probability between $P > .05$ and $P \leq .1$ indicated a trend toward significance.

3. Results

3.1. Gestation and Foaling Parameters

All mares completed the study without complication. No feed refusals were observed, and mares were allowed back on pasture after all (>99%) feed was consumed. The mean age for control and Arg mares was 11.4 ± 1.3 and 11.5 ± 1.7 years, respectively. The EFD for all mares ranged from January 14 to May 15, 2010, with majority (n = 10) of mares having EFD before March 1. No deleterious physical effects of feeding Arg were observed in treated mares.

Parturition proceeded without complication in all mares, and all foals were born clinically healthy with no observable differences in size or physiological abnormalities. The mares fed with Arg beginning 21 days before EFD had a shorter gestation length (337.9 ± 1.7 days) than control mares (345 ± 2.1 days; $P \leq .05$). No significant differences were found in any other foaling parameters observed. Combined means for time from rupture of the chorionallantois to foal delivery was 16.5 ± 1.5 minutes; time from rupture of chorionallantois to placenta shed was 34.8 ± 12.2 minutes; time from foal delivery to first nursing 95.1 ± 9.5 minutes; placental weight was 6.98 ± .31 kg. No acute effects were observed on initiation of parturition in Arg-supplemented mares, with rupture of the chorionallantois ranging from 20:59 to 06:20 hours. Time of chorionallantois rupture in control mares ranged from 20:02 to 04:12 hours.

When examining gestation length in mares by EFD, for EFD prior to March 1 (n = 10), Arg-supplemented mares had a shorter (336.8 ± 2.4 days) gestation length as compared with control (345.8 ± 2.8 days; $P \leq .05$). No significant differences were observed in gestation length in Arg-supplemented or control mares with an EFD after March 1 (n = 6; 337.3 ± 2.3 days and 342.5 ± 4.5 days, respectively). When separated for gender, gestation length for fillies born from Arg-supplemented mares was shorter (n = 4; 336.4 ± 2.2 days) as compared with control mares (n = 5; 342.8 ± 2.2 days; $P \leq .05$). Similarly, there was a trend for colts born from Arg-supplemented mares to have a shorter gestation length (n = 3; 340.3 ± 2.7 days) when compared to control (n = 4; 347.3 ± 3.3 days; $P = .09$). When the data were combined for both groups, fillies tended to have a shorter gestation length as compared with colts ($P = .06$). When examining the previous year's foaling records, the mean gestation length was 342.9 ± 2.0 days for mares identified as control (n = 7) and 342.3 ± 2.3 days for Arg-supplemented mares (n = 6); the difference found was not significant. Finally, no differences could be detected in gestation length as a result of any potential sire (n = 11) affects, and breed, parity, or age of mares.

3.2. Uterine Artery Characteristics

Differences in diameter of the GUA compared with NGUA were found ($P \leq .05$); therefore, each was evaluated separately. A day effect was observed in both GUA and NGUA after parturition with decreasing uterine artery diameter ($P \leq .001$). Supplementation with Arg had no effect on the GUA diameter in the days leading up to parturition (Fig. 1A); however, a treatment effect was observed in the GUA after parturition, with Arg-supplemented mares having smaller uterine artery diameter ($P \leq 05$). In the NGUA, differences were found between groups before parturition, with treated mares having smaller uterine artery diameter ($P \leq .01$); and a trend toward significance in the days after parturition ($P = .1$; Fig. 1B). Across treatments, a decrease in diameter of the GUA was found when comparing the day before parturition (1.21 ± .05 cm) with day 7 postparturition (0.67 ± .06 cm; $P \leq .01$); a similar decrease was found in the NGUA (1.07 ± .07 cm and 0.59 ± .02 cm, respectively; $P \leq .01$). Interestingly, control mares NGUA had a larger diameter

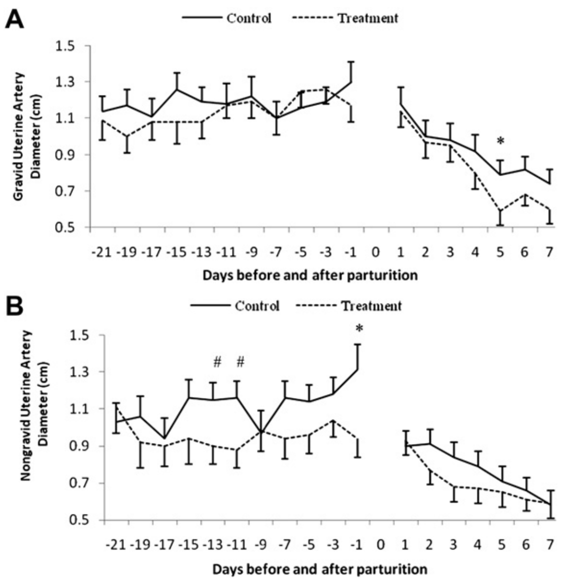

Fig. 1. Mean (±SEM) diameter of the uterine arteries of L-arginine treated and control mares in the 21 days before and 7 days after parturition, with day 0 as day of parturition. (**A**) Gravid uterine artery for days −21 to −1 (T: NS, D: NS, T × D: NS) and days 1 to 7 (T: $P \leq .001$, D: $P \leq .01$, T × D: NS). (**B**) Non-gravid uterine artery for days −21 to −1 (T: $P \leq .001$, D: NS, T × D: NS) and days 1 to 7 (T: $P = .10$, D: $P \leq .001$, T × D: NS). T, treatment; D, day; NS, not significant; an asterisk (*) indicates significance ($P \leq .05$); and pound sign (#) indicates a trend toward significance ($P > .05$ to $P \leq .10$).

the day before parturition ($P \leq .05$), but no differences were evident the day after parturition.

3.3. Uterine Artery Blood Flow Characteristics

Differences in blood flow measurements were found between the GUA (Fig. 2) and NGUA (Fig. 3), before and the days after parturition ($P \leq .05$); therefore, each were evaluated separately. In the days leading up to parturition, a day effect was observed in both the RI and PI as index values decreased in the GUA ($P \leq .01$). This was not observed in the NGUA. Before parturition, no overall treatment effect was found in the GUA; however, control mares had greater PI values on days 7 and 3 (Fig. 2A; $P \leq .05$), whereas treatment mares had a greater RI value on day 17 (Fig. 2B; $P \leq .05$). An overall treatment effect was observed for PI ($P < .0001$) and RI ($P < .0001$) in the NGUA leading up to parturition. Control mares had greater NGUA PI values on days 21, 19, and 13 ($P \leq .05$), and a trend for significance on day 3 (Fig. 3A; $P = .07$). Additionally, control mares had greater RI values in the NGUA on days 21, 19, and 13 ($P \leq .05$), and a trend for greater RI values on days 17 and 1 (Fig. 3B; $P \leq .1$).

When compared with the measurements to the day before parturition, blood flow significantly decreased across both groups in both uterine arteries the day after parturition. Across treatments, uterine blood flow significantly ($P \leq .05$) decreased from the day before to the day after parturition. Postpartum, a day ($P \leq .01$) and treatment ($P \leq .05$) effect was observed in both GUA indices, and treatment by day effect was observed in the GUA PI ($P \leq .05$). Arg-supplemented mares had smaller PI values on days 3, 4, and 6 ($P \leq .05$), and a smaller RI value on day 6 ($P \leq .05$) and days 1 and 4 ($P \leq .1$). Only a day effect was observed in both indices of the NGUA postpartum ($P \leq .05$), with no significant differences noted between groups.

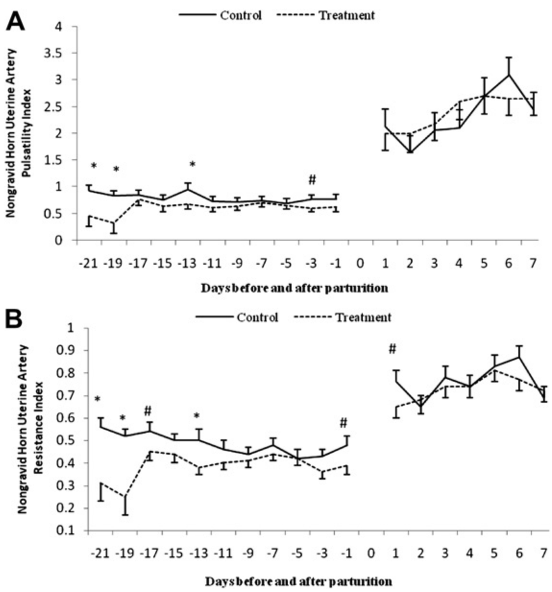

Fig. 3. Mean (±SEM) PI and RI values in the uterine arteries leading to the non-gravid horn of pregnancy of L-arginine treated and control mares in the 21 days before and 7 days after parturition, with day 0 as day of parturition. (**A**) Non-gravid PI for days −21 to −1 (T: $P \leq .0001$, D: NS, T × D: NS) and days 1 to 7 (T: NS, D: $P \leq .01$ T × D: NS). (**B**) Non-gravid RI for days −21 to −1 (T: $P \leq .0001$, D: ND, T × D: $P \leq .05$) and days 1 to 7 (T: NS, D: $P \leq .01$, T × D: NS). T, treatment; D, day, NS, not significant; an asterisk (*) indicates significance ($P \leq .05$); and pound sign (#) indicates a trend toward significance ($P > .05$ to $P \leq .10$).

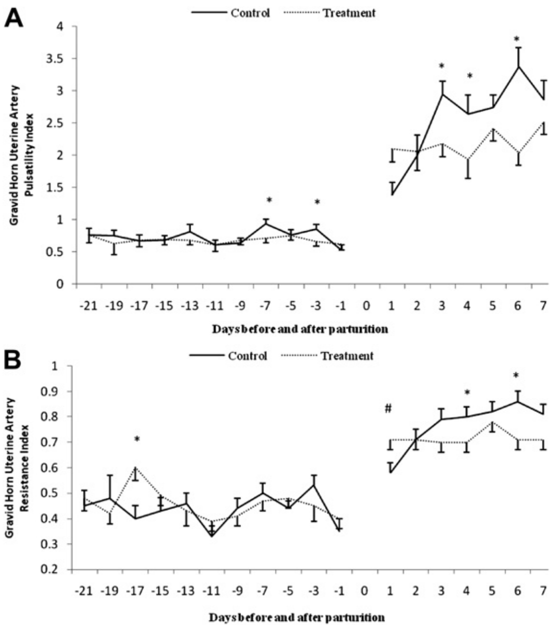

Fig. 2. Mean (±SEM) PI and RI values in the uterine arteries leading to the gravid horn of pregnancy of L-arginine treated and control mares in the 21 days before and 7 days after parturition, with day 0 as day of parturition. (**A**) Gravid PI for days −21 to −1 (T: NS, D: $P \leq .01$, T × D: NS) and days 1 to 7 (T: $P \leq .001$, D: $P \leq .0001$, T × D: $P \leq .05$). (**B**) Gravid RI for days −21 to −1 (T: NS, D: $P \leq .01$, T × D: $P = .10$) and days 1 to 7 (T: $P \leq .05$, D: $P \leq .05$, T × D: NS).

4. Discussion

The results of this study have demonstrated that uterine arterial blood flow can be measured during late pregnancy in mares and in the immediate postpartum period. Our results indicated that supplemental feeding of Arg beginning 21 days before EFD shortened gestation length. An increase in uterine artery blood flow through the NGUA was observed in treated mares before parturition, with no differences in the GUA. The day immediately after parturition for both groups, indices significantly increased and uterine artery diameter decreased in both the GUA and NGUA as mares transitioned back to the nonpregnant state. Additionally, a treatment effect was observed, with Arg-supplemented mares having greater blood flow (lower indices) in the GUA. As previously mentioned, supplemental Arg in the diets of pregnant animals has no apparent deleterious effects on pregnancy, fetus, or resulting neonate [4]. This also seems to be true with short-term feeding of Arg during late pregnancy in mares.

The most surprising finding in the current study was the significantly shorter gestation length in Arg-supplemented mares as compared with unsupplemented controls. Many factors influence gestation length in mares, including season, breed, gender of fetus, parity, and age of mare.

Although we did not find any differences in breed, age, or parity of the mares in this study, we did find differences in season and foal gender. When examining season for mares with an EFD before March 1, Arg-supplemented mares had a significantly shorter gestation length. Although Arg-supplemented mares with an EFD after March 1 had an overall shorter gestation length, significance was not achieved and is most likely because of the smaller sample size ($n = 6$).

For gender differences, our results are in agreement with previous work [16], with mares carrying fillies having a significantly shorter gestation length in comparison with colts, regardless of the treatment. However, when foal gender and gestation length were examined by treatment, Arg-supplemented mares had a significantly shorter gestation length in both fillies and colts. Furthermore, to evaluate any potential mare differences, records from the previous foaling season were evaluated for gestation length. Of the 13 mares that foaled in the past year, no differences in gestation length were found when mares were separated based on this study, control or Arg-supplemented. Therefore, our data support Arg as the contributing factor to reduce gestation length.

These findings are in contrast to the study of Mateo et al. [5], who did not find any differences with gestation length in gilts fed Arg from day 30 to term. There are differences in characteristics of pregnancy between pigs and horses, most noticeably a litter-bearing versus a typical singleton-bearing species, respectively. In the mare the sequence of prepartum endocrine events are generally similar to those seen in other farm animals; however, there are differences [17].

Across mammalian species, a fetal rise in glucocorticoids and a fall in maternal progesterone levels are the endocrine changes most consistently observed leading up to parturition. However, the prepartum rise in fetal cortisol concentrations occurs much closer to term in the mare than in other species [18]. Fowden et al. [17] stated that the basal fetal cortisol levels remain low until 4 to 5 days before term and rise exponentially only in the final 24 to 36 hours before birth. This rise in cortisol is essential for fetal maturation, and foals born before this increase typically do not survive [19]. Conversely in swine, a rise in cortisol happens earlier and is observed beginning the final 2 weeks of gestation [20]. It is currently unknown how NO might influence fetal foal, or fetal pig, corticoid synthesis. Riquelme et al. [21] established that NO is important in regulating adrenal blood flow and corticomedullary function in the llama fetus. In their study, treatment with an NO synthase inhibitor (L-NAME) decreased fetal adrenal blood flow and prevented increases of fetal plasma cortisol under hypoxic conditions. Although inhibition of NO has been shown to affect fetal adrenal function, to our knowledge increased NO production has not. However, increased NO production through Arg-supplementation could possibly enhance fetal adrenal function or efficiency and deserves further investigation.

Although we did not observe any outward differences in fetal outcomes or placental weights, greater uterine blood flow potentially could have hastened the end of fetal development, thus reducing gestation length. The increased blood flow in the uterine arteries in Arg-treated mares could possibly be attributed to the known vasodilative properties of NO [22,23]. NO is produced by the conversion of Arg to L-citrulline by nitric oxide synthase (NOS) enzymes and is a potent vasodilator that has been shown to regulate blood flow to reproductive tissues in ewes [24] and sows [25]. It is interesting that increased blood flow was more evident in the NGUA prepartum, and may possibly indicate that during late pregnancy in mares blood flow through the GUA is at maximal flow and is not manipulated with a NO-donor such as Arg; or conversely, the indices are so low that significant changes in blood flow are not detectable with the Doppler ultrasound used in this trial. These results are supported by the study of Neri et al. [26] who only observed increases in blood flow (lower PI) in the nonplacental side uterine artery in Arg-treated women.

Another contributing factor to shorter gestation length, and the differences found between porcine and equine pregnancy, may be because of mare prostaglandin metabolites slowly increasing over approximately the last 30 days until term [17], whereas in pigs no rises in prostaglandins are detected until term [27]. This is interesting because NO has been positively correlated in the mare endometrium with both prostaglandin E synthase (PGES) and prostaglandin F synthase (PGFS), enzymes responsible for conversion of important reproductive prostaglandins (PGE_2 and $PGF_{2\alpha}$, respectively) [28]. In the mare, PGE_2 promotes cervical ripening and relaxation, whereas $PGF_{2\alpha}$ stimulates uterine contractions [17,29]. NO, like PGE_2, is also known to be a critical component in cervical ripening, and interestingly has been shown to inhibit spontaneous uterine contractions [30]. Therefore, it is possible that Arg treatment late in gestation may enhance prostaglandin synthesis and cervical ripening, thus playing a role in hastening the onset of labor and this deserves further study.

The second most interesting data from this study are the increase in uterine arterial blood flow in the NGUA prepartum, and the increase in uterine arterial blood flow in the GUA postpartum. Color Doppler ultrasonography is becoming a commonly used diagnostic tool to evaluate blood flow to reproductive tissues in livestock animals. Doppler indices (RI and PI) as ratios are useful in quantitative assessment of blood flow velocity measurements for reproductively important tortuous vessels such as the uterine arteries, as stated by Ginther [12]. The studies of Mortensen et al. [11] and Bollwein et al. [10] both observed differences in uterine arterial blood flow, leading to the gravid or non-gravid uterine horns during early equine pregnancy. Results from both studies showed greater blood flow through the uterine artery leading to the horn (gravid) of established pregnancy. Our results indicated that this also seems to hold true during late pregnancy in mares, with greater blood flow to the GUA a compared with the NGUA, regardless of group. Related studies on other species has shown that during pregnancy uterine blood flow increases from 10- to 100-fold above nonpregnant levels [31]. As expected, uterine arterial blood flow decreased in the days after parturition, as the foal and placenta were shed. These decreases have previously been documented in sheep and dogs [32] and more recently in postpartum dairy cows [33]. This seems to be the first study in mares documenting differences pre- and postpartum in uterine arterial blood flow and diameter.

Another observation was the significant treatment effect, with Arg-supplemented mares having greater uterine arterial blood flow on days after parturition in the GUA. It is surprising as to why this was not observed in the NGUA after foaling, when before foaling there were significant differences. Osol and Madala [31] stated that the available evidence points to local rather than systemic factors playing a primary role in gestational uterine vascular remodeling and increases in blood flow. Additionally, the authors stated that a reduction in downstream resistance would be an effective stimulus for increasing the velocity of blood flow in upstream arteries. In women, enhanced myometrial vascularity (EMV) is common in the early postpartum period, is located at the former placental site, and is associated with lower PIs and a greater maximal flow velocity in the uterine arteries [34]. Therefore, it is plausible from our results that as the uterus recovers from the trauma of parturition and undergoes involution, greater vascular development in the previously gravid horn benefits more from an NO donor such as Arg as compared with the non-gravid horn. It remains to be elucidated as to how a greater blood flow to the uterus might benefit the mare; however, greater blood flow to the reproductive tract has been associated with greater fertility in mares [15] and cows [14].

Finally, uterine artery diameter did not differ before parturition in the GUA, but was observed to be smaller in the NGUA in Arg-supplemented mares. Conversely, after parturition Arg-treated mares had smaller GUA diameter with increased flow. This is interesting, as outward hypertrophic growth of uterine arteries has been correlated with increased blood flow [31]. However, based on these results, Arg-supplemented mares had smaller uterine artery diameter with increased blood flow. It is possible that these differences may reflect physiological differences between the two groups, rather than a treatment effect. Yet, it is also possible that Arg-supplementation, and presumably through NO action, may have altered the vascular remodeling further downstream than our point of examination of the uterine arteries. As stated earlier, greater vasculature downstream increases blood flow upstream, and based on our results this seemed to not influence uterine artery diameter. Further work should be attempted to try and delineate these differences.

5. Conclusions

Supplementing pregnant mares with Arg 21 days before EFD seemed to be safe and reduced gestation length. Additionally, supplemented mares were observed to have greater uterine arterial blood flow before, and on the days immediately after parturition. The sharp reduction in uterine arterial blood flow and diameter after parturition could be predicted as the mares transitioned from the pregnant to the nonpregnant state. Because most equine breed registries recognize an annual January 1 birth date, and because of the long equine gestation length (range: 329-353 days in this study), each day a mare remains open after parturition potentially reduces the value of the proceeding year's foal, or risks her being left open and losing a year's production. Therefore, any reduction in gestation length, or ensuring a mare foals near her EFD, is extremely valuable to horse breeders. Previous attempts at reducing gestation length through pharmacologic manipulation potentially leads to dystocia or still-born foals [19,35]. Therefore, if results from the current study can be confirmed, this would seem to be an important finding in safely reducing gestation length in mares by diet manipulation.

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