Impacts of arginine on ovarian function and reproductive performance at the time of maternal recognition of pregnancy in ewes

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Reproductive performance is the largest determinant of income in the sheep flock. The objective of the current study was to determine if supplementation with the amino acid arginine surrounding the time of maternal recognition of pregnancy enhances ovarian function and reproductive performance in ewes. Recently arginine supplementation strategies have proven to be a suitable method to enhance reproductive loss in livestock creating a more profitable enterprise for the producer.

INTRODUCTION

As a precursor for nitric oxide, polyamines, and proteins, the amino acid arginine plays a vital role in metabolism and reproduction (Wu and Morris, 1998). Supplemental arginine has been reported to increase the number of live piglets born per sow (Mateo et al., 2007). Furthermore, pregnant rats supplemented with arginine throughout gestation exhibited an increase in embryonic survival and litter size (Zeng et al., 2008). Recently at NDSU we observed increased ovarian blood flow, serum progesteron and fetal number, despite similarities in ovulation rate, in ewes injected with L-arginine during the first 15 d post-breeding. Collectively, these studies suggest that reproductive efficiency can be enhanced via supplementation with arginine.

In sheep, embryonic and fetal deaths during pregnancy account for 25 to 50% of the total number of fertilized ova (Dixon et al., 2007). Most embryonic loss has been reported to occur before d 18 (Quinlivan, 1966). Only a small percentage of embryos are inherently non-viable in the ewe (Wilmut et al., 1986), which would suggest that the majority of early embryonic losses can be prevented.

Communication between the embryo and the maternal system must be established following conception to ensure normal development of the embryo. Maternal recognition of pregnancy in sheep occurs around d 13 following ovulation. During this critical period, the conceptus elongates from a blastocyst to a filamentous form, which produces interferon tau that is responsible for preventing the development of the endometrial luteolytic mechanism (Spencer and Bazer, 2002). The presence of interferon tau allows for maintenance of the CL, which is the primary structure responsible for progesterone production during early pregnancy in sheep.

The objective of this study was to determine the effects of arginine supplementation surrounding the time of maternal recognition of pregnancy on ovarian

hemodynamics, early reproductive loss and lamb birth weight in Rambouillet ewes.

PROCEDURES

Rambouillet ewes of a similar body weight and age were randomly assigned to one of two groups: control (**CON**; n = 47) and L-arginine (**ARG**; n = 47). All ewes received a CIDR device for 12 d. Following CIDR removal a single injection of PG -600 was given to help initiate follicular development and ensure ovulation. Thereafter, ewes were exposed to fertile rams. From d 9 to d 14 post-estrus ewes received L-arginine HCl (equivalent to 27 mg of L-arginine/kg of BW) or saline (CON) intravenously once daily. Daily blood samples were obtained (n = 10 ewes/ subgroup) immediately after treatment (0 h) to assess progesterone (P4) concentrations and at -0.5, 0, 0.5, 1, 2, 4, 6 and 8 h on d 10 to determine circulating concentration of arginine in response to treatment. Ovarian hemodynamics (d 12) was determined with color Doppler ultrasonography (n = 10 ewes/ subgroup) and reproductive losses (d 25, 45 and 65; n = 94) were determined using B-mode ultrasonography techniques.

RESULTS

On d 10 of pregnancy, serum concentrations of arginine (nmol/mL) were elevated in a subset of ewes (n=10 ewes/ treatment group) ewes injected with arginine vs. CON ewes at 0 ($P < 0.001$), 0.5 ($P < 0.001$), 1 ($P < 0.001$), 2 ($P < 0.001$) and 4 h ($P < 0.001$), but were similar ($P \geq 0.70$) at -0.5, 6, and 8 h (Figure 1). Metabolites of arginine, ornithine and citrulline were measured. On d 10, ornithine levels were elevated in ARG vs. CON ewes at 0.5 ($P < 0.03$), 1 ($P < 0.001$), 2 ($P < 0.001$), 4 ($P < 0.001$), 6 h ($P < 0.001$) and 8 h ($P < 0.02$). However there was no effect on circulating serum citrulline concentration ($P \geq 0.09$).

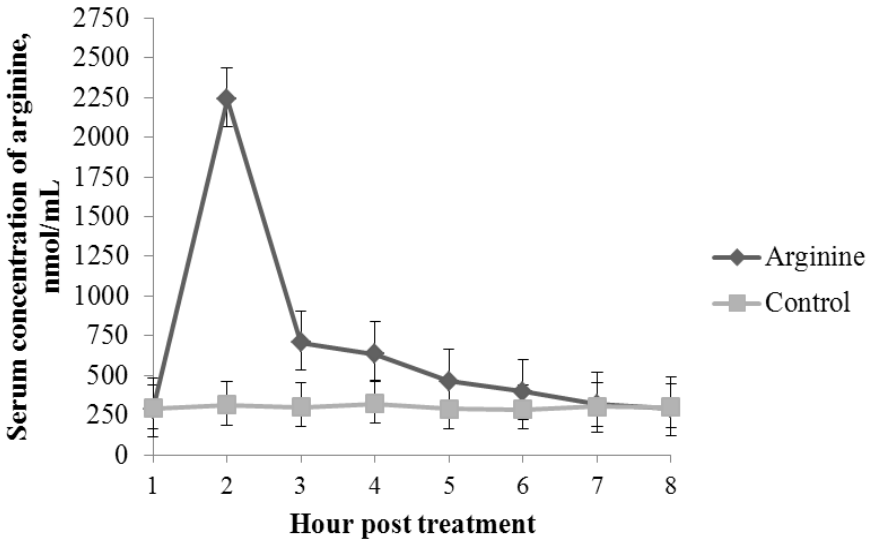

Figure 1. Effects of injectable L-arginine on serum arginine concentration (nmol/ mL) on Day 10 in Rambouillet ewes (**$P = 0.001$; *$P < 0.001$) from d 9 to 14 of the estrous cycle.

Carotid artery and ovarian hemodynamics were measured on d 12 with Doppler ultrasonography on a subset of ewes (n=10 ewes/treatment group). There were no differences in pulsatility index in those ewes treated with arginine vs. control in the ovarian hilus ($P \geq 0.49$). When measuring the vasculature surrounding the CL, there was no effect of arginine treatment compared with control ($P \geq 0.51$). Similar to the pulsatility index, resistance index was also not influenced with arginine treatment in the ovarian hilus or in the CL ($P \geq 0.49$ and $P \geq 0.51$; respectively). Despite similarities in CL in the subset of ewes blood sampled (ARG; 1.69 ± 0.12 and CON; 1.67 ± 0.16 CL/ewe; $P > 0.05$), CON ewes had greater serum progesterone concentration (ng/mL) compared with ARG on d 9 (6.11 ± 0.27 vs. 5.30 ± 0.15 respectively; $P < 0.02$) and 10 (6.50 ± 0.40 vs. 5.06 ± 0.21 respectively; $P < 0.005$) but similar for the remaining treatment period ($P \geq 0.06$) .

Treatment with arginine influenced pregnancy rate (ARG, 55%, n=47 and CON, 30%, n=47; $P \leq 0.02$), despite treatment similarities in pregnant ewes in CL number at d 25 in ARG ewes vs. CON (Table 1). As pregnancy progressed to d 45, a similar number of embryos were observed between ARG and CON ewes, with pregnancy rate remaining greater in ARG (ARG, 47% vs. CON, 26%; $P \leq 0.03$; Table 1). By d 65 of pregnancy, the ewes continued to maintain similar embryos in ARG ewes vs. CON (Table 1). Pregnancy rate was also greater

Table 1. Effects of L-arginine on number of corpora lutea and embryos

	Arginine[a]			Control[b]			
Item/ewe	Mean	SEM	n	Mean	SEM	n	*P*-value[c]
No. Corpora lutea	1.69	0.12	26	1.67	0.13	15	0.42
No. Embryos d 25	1.62	0.12	26	1.53	0.16	15	0.33
No. Embryos d 45	1.45	0.14	22	1.50	0.15	12	0.41
No. Embryos d 65	1.50	0.14	22	1.45	0.16	11	0.42

[a] Arginine, 27 mg/kg BW injectable arginine.
[b] Control, saline.
[c] *P*-value for F-test for treatment.

($P \leq 0.02$) at d 65 in ARG (47%) compared with CON (23%) ewes.

A total of 32 (n=47) and 16 (n=47) lambs were born from ewes exposed to rams on synchronized estrous in CON vs. ARG, respectively. Ewes treated with ARG gave birth to a similar number of lambs when compared with CON (1.78 ± 0.17 vs. 1.60 ± 0.27 respectively; $P < 0.58$). Average lamb birth weights (lb) did not differ between treatment groups (8.5 ± 0.44 vs. 9.0 ± 0.58 respectively; $P < 0.24$).

DISCUSSION

In the present study, pregnancy rate was greater in those ewes treated with injectable arginine when compared to control ewes. However, the overall pregnancy rates were lower than expected throughout the study. Ewes utilized were bred out of season and only those whom were mounted by a fertile ram following the synchronized estrous period were used for this study. This may be a justifiable reason for the overall low pregnancy rates across treatment groups.

In the current study, ewes treated with injectable L-arginine surrounding the time of maternal recognition of pregnancy (d 9 to 14) had enhanced pregnancy rates, increased circulating serum arginine and ornithine concentration, and elevated vascular resistance in peripheral blood flow. Arginine is important for many biological functions, including the synthesis of nitric oxide (Gouge et. al., 1998; Manser et. al., 2004). It may be reasonable to hypothesize that treatment with arginine at or slightly before the time of maternal recognition of pregnancy in the ewe may have enhanced the survival of the embryo during early embryogenesis through its role in polyamine and nitric oxide synthesis. Nitric oxide and polyamines may have directly enhanced embryonic cellular proliferation and differentiation to ensure proper embryonic survival.

Progesterone is important for histotropic nutrition of the early embryo and suppression of the luteolytic mechanism (Lamming et al., 1989). In the present study, ewes treated with arginine had lower concentrations of progesterone relative to control ewes on d 9 and 10 of gestation, but were similar for the remaining treatment period. The lower levels of progesterone in arginine treated ewes may be due to an increase in metabolic clearance rate of steroids within the liver. Several studies have shown that low levels of progesterone can lead to a greater incidence of embryonic loss in sheep, and ultimately result in decreased ewe productivity (Dixon et. al., 2007). However, this finding was not observed in the current study. In fact, ARG treated ewes actually had greater pregnancy rates when compared to CON ewes.

IMPLICATIONS

In summary, treatment with arginine surrounding the time of maternal recognition of pregnancy may have prevented pregnancy loss in some ewes. Despite similarities in total CL and embryo numbers, overall pregnancy rate was increased in ewes treated with arginine. The enhanced pregnancy rate may have been due to arginine supplementation creating a more ideal uterine environment for the maintenance of embryos. During the early stages of embryogenesis, the supplemental arginine could have rescued weaker embryos entering the early stages of regression through its role in nitric oxide and polyamine synthesis. Nitric oxide and polyamines may have directly enhanced embryonic cellular proliferation and differentiation to ensure and promote proper embryonic survival.

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