

## Impacts of supplemental arginine on reproductive performance in sheep

A. R. Crane<sup>1,2</sup>, R. R. Redden<sup>1</sup>, M. L. Van Emon<sup>1,2</sup>, T. L. Neville<sup>1</sup>, J. S. Caton<sup>1</sup>, C. S. Schauer<sup>2</sup>

<sup>1</sup>Department of Animal Sciences, North Dakota State University, Fargo, ND

<sup>2</sup>Hettinger Research Extension Center, North Dakota State University, Hettinger, ND

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*The objective of this study was to determine the effects oral and injectable supplementation of arginine two weeks post breeding on ewe reproductive performance and lamb growth. Prenatal lamb loss accounts for a large portion of economic loss in the sheep industry. Sheep producers could benefit from a supplementation protocol that recovered these losses.*

### INTRODUCTION

Reproductive performance is the largest determinant of income in livestock production. In the U.S. sheep industry, embryonic and fetal death can account for 25-50% of the total number of ovulations (Knights, et al., 2003; Dixon et al., 2007). The majority of embryonic loss occurs before d 18 of gestation (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). However, the loss of individual embryos can occur without a complete loss of pregnancy, such as in the case of multiple pregnancies (Rhind et al., 1980; Schrick and Inskeep, 1993). In sheep, it has been reported that 30% of fertilized ova are not represented by live births, resulting in frequent, but unrecognized economic loss (Knights et al., 2003; Bolet, 1986; Dixon et al., 2007). Furthermore, a small percentage of embryos are inherently non-viable (Wilmot et al., 1986), which suggests early embryonic loss is likely preventable in the ewe. Strategies to enhance prenatal growth and survival could clearly have a major economic impact in the sheep industry. Past research by NDSU has shown that supplemental arginine (**Arg**) can recover embryonic and/or fetal loss in fall lambing ewes synchronized to estrus with exogenous hormones (Saevre et al., 2011; Luther et al., 2009).

The amino acid L-arginine is a precursor for nitric oxide and important in the synthesis of polyamines and proteins, all of which are essential to proper development of the embryo and placenta. Past NDSU research has observed increased pregnancy rate in ewes treated with injectable L-arginine when compared to control ewes by 45% (Saevre et al., 2011; Luther et al., 2009). However, this previous research has always utilized an injectable Arg source, which is not readily adaptable to producer use. In order for producer acceptance of Arg supplementation to occur, a feed option must be found. It is reasonable to hypothesize from the previous studies that supplementation of rumen-protected arginine would have beneficial impacts on prenatal growth and survival for ruminant livestock.

The objective of this study was to determine the effects of injectable (Exp. 1) and oral (Exp. 2)

Arg supplementation provided two wk post breeding on reproductive performance of naturally stimulated fall lambing ewes.

## PROCEDURES

All procedure were approved by the Animal Care and Use Committee of North Dakota State University. This study was conducted at the Hettinger Research Extension Center in Hettinger, ND.

**Animals and Diets.** Rambouillet ewes of a similar BW ( $142.6 \pm 15.01$  lbs.) were randomly assigned to one of six treatment groups: control (**CON**; n= 25), IV-alanine (**IVALA**; n=20), IV-arginine (**IVARG**; n=23), rumen-protected arginine (**RPARG**; n=20), soybean meal (**SBM**; n=23), fishmeal (**FM**; n=24). Ewes were exposed to 15 fertile ram lambs for 2 weeks before the trial start. During this time, ewes were fed one pound of corn/hd/d. Ewes were exposed to fishmeal at 12% of corn intake for 4 days during the 2 weeks pre-breeding to adapt them to the taste and smell of fishmeal. Ewes were fed 6 lb per day (as fed) a ration consisting of 25% alfalfa haylage and 75% grass hay. Ewes were exposed to mature rams one day before the start of the trial. Any ewes that were bred during the two weeks before the start of the project were removed from the trial. Ewes that received breeding marks 10 - 17 d post ram introduction were allocated to treatments. Thereafter, ewes were moved to a different pen and exposure to fertile rams for an additional 14 days. From d 0 (estrus) to d 14 (post estrus) ewes received their assigned treatment. In Exp. 1, all ewes received 1 lb of corn daily and injected with similar volumes of their treatment to provide  $30 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$  Arg. Intravenous injections of arginine, alanine, and saline were administered daily to IVARG, IVALA, and CON ewes. In Exp. 2, all ewes received 1 lb/d of their respective treatments to provide  $30 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$  Arg to the Arg supplemented treatments. Treatments were: RPARG (0.15 g/kg BW rumen protected product mixed with ground corn), SBM (25:75 soybean meal: corn), and FM (37.5:62.5 fishmeal: corn). The CON treatment from Exp. 1 served as the control treatment for Exp. 2. Blood samples were collected from 12 ewes per treatment group prior to administration of treatment every other day during 14-day trial treatment period. Blood samples were assayed for concentrations of progesterone. At lambing, birth weight, birth type, and sex were collected. Weaning weights were collected when the average age of lambs was 60 d.

**Statistical Analysis.** Pregnancy, prolificacy, and lambing rates were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Arginine treatment served as the fixed effect. The fixed effect of day was utilized in the REPEATED measures analysis for progesterone concentrations. The model included the fixed effects of dietary treatment, day, and treatment x day. Significance was determined at  $P \leq 0.05$ . To partition day effects and treatment x day interactions, LS Means were utilized ( $P \leq 0.05$ ).

## RESULTS

In Exp. 1, no differences were detected for pregnancy, prolificacy, and lambing rates among treatments ( $P = 0.95, 0.35,$  and  $0.70$ , respectively; Table 1). Similarly, in Exp. 2, no differences were detected for pregnancy, prolificacy, and lambing rates among treatments ( $P = 0.94, 0.61,$  and  $0.80$ , respectively; Table 2). Additionally, there were no differences detected for

progesterone concentrations for treatment or treatment by day interactions among treatments in Exp. 1 or 2 ( $P = 0.58$  and  $0.34$ , respectively; Figures 1 and 2, respectively). There was a day effect for both Exp. 1 and 2, but this observation was expected ( $P < 0.0001$ ) due to the estrous cycle. Similar to gestational performance, there were no differences detected for birth weights in Exp. 1 and 2 among treatments ( $P = 0.57$ ;  $P = 0.73$ , respectively; Tables 1 and 2). In Exp. 1, male lamb birth weights were significantly higher than female lambs ( $P = 0.014$ ; data not shown). However, birth weights for Exp. 2 were similar for all treatments. Prolificacy had no effect on lamb birth weight in Exp. 1 ( $P = 0.07$ ), but for Exp. 2 single born lambs were significantly heavier than twin lambs ( $P < 0.0001$ ; data not shown). There were no differences detected for weaning weights in Exp. 1 and 2 among treatments ( $P = 0.53$ ;  $P = 0.57$ , respectively; Tables 1 and 2). In Exp. 1, no differences were detected in weaning weights among the birth type ( $P = 0.17$ ), however, in Exp. 2, single born lambs were significantly heavier than twin born lambs ( $P = 0.04$ ; data not shown). In Exp. 1, male lamb weaning weights were significantly higher than female birth weights ( $P = 0.05$ ; data not shown), but in Exp. 2 no differences were seen between male and female lamb weaning weight ( $P = 0.92$ ).

## DISCUSSION

In the present study, pregnancy, prolificacy, and lambing rates were not influenced through injectable or oral treatments. In contrast, research from this laboratory reported greater pregnancy rates in ewes supplemented with injectable Arg from d 0 through 14 post breeding and also ewes supplemented d 9 through 14 post breeding (Luther et al., 2009; Saevre et al., 2011). Moreover, pregnancy rates were much lower in the previous studies than in our study. Pregnancy rates were as follows: ARG (55%) vs. CON (60%), ARG (55%) vs. CON (30%), and IVARG (88%) vs. CON (88%) vs. RPARG (86%) (Luther et al., 2009; Saevre et al., 2011). We hypothesize that the differences in pregnancy rates between these projects could be due to a difference in reproductive synchronization models utilized as a comparison. Ewes in the previous two studies were synchronized artificially with a CIDR and an injection of PG-600, whereas the ewes in the present study were naturally synchronized using ram exposure.

Arginine is important for many biological functions, including the synthesis of nitric oxide (Gouge et al., 1998; Manser et al., 2004). Other studies have hypothesized 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 (Luther, et al., 2009). In the current study, however, increased pregnancy, prolificacy or lambing rates were not observed for arginine treated ewes.

As stated previously, arginine is important for the synthesis of nitric oxide, which is important for dilating blood vessels, therefore increasing tissue blood flow. Increases in ovarian blood flow or flow to the corpus luteum during early pregnancy could result in higher progesterone concentrations. This could result in a more ideal environment for early embryonic survival in arginine treated ewes. However, no differences in progesterone concentrations were observed between arginine treated ewes and the controls.

## IMPLICATIONS

Although previous results imply that embryonic survival in sheep can be enhanced when supplemented with arginine, we did not detect any improvements in reproductive performance or lamb growth in ewes supplemented with either injectable or rumen-protected forms of arginine. We hypothesize that supplemented arginine might enhance reproductive performance in compromised models, such as the previous studies (chemical synchronization, etc.). However, further research is needed to develop this hypothesis.

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**Table 1.** Effects of daily injection of treatments<sup>1</sup> two weeks post breeding on pregnancy, prolificacy and lambing rate in sheep

Item	Control	IV-Alanine	IV-Arginine	SEM	<i>P</i> -value <sup>2</sup>
Pregnancy <sup>3</sup>	88	91	88	7.1	0.95
Prolificacy <sup>4</sup>	1.32	1.21	1.43	0.11	0.35
Lambing Rate <sup>5</sup>	1.16	1.10	1.25	0.13	0.70
Birth Weight	11.8	12	11.5	0.53	0.57
Weaning Weight	54.3	48.7	50.6	3.0	0.53

<sup>1</sup>Control, 7 mL/kg BW saline (n=25); IV-Alanine, 0.110 mL/kg of BW (n=20), IV-Arginine, 0.093 mL/kg of BW, (n=23).

<sup>2</sup>*P*-value for F test of the mean.

<sup>3</sup>Pregnant treated ewes that lambbed to the first estrus.

<sup>4</sup>Lambing rate of ewes that lambbed.

<sup>5</sup>Lambing rate of ewes treated.

**Table 2.** Effects of daily injection of treatments<sup>1</sup> two weeks post breeding on pregnancy, prolificacy and lambing rate in sheep

Item	Control	RPARG	FM	SBM	SE	<i>P</i> -value <sup>2</sup>
Pregnancy <sup>3</sup>	88	86	89	83	8	0.94
Prolificacy <sup>4</sup>	132	117	117	126	10	0.61
Lambing Rate <sup>5</sup>	1.16	1.00	1.04	1.04	0.12	0.80
Birth Weight	11.8	11.6	11.6	11.4	0.57	0.73
Weaning Weight	55.0	56.7	51.3	52.7	3.0	0.57

<sup>1</sup>Control, 7 mL/kg BW saline (n=25); RPARG, 0.15 g/kg BW (n=20); FM, 25:75 ration (FM:corn) (n=24); SBM, 37.5:62.5 ration (SBM:corn) (n=23).

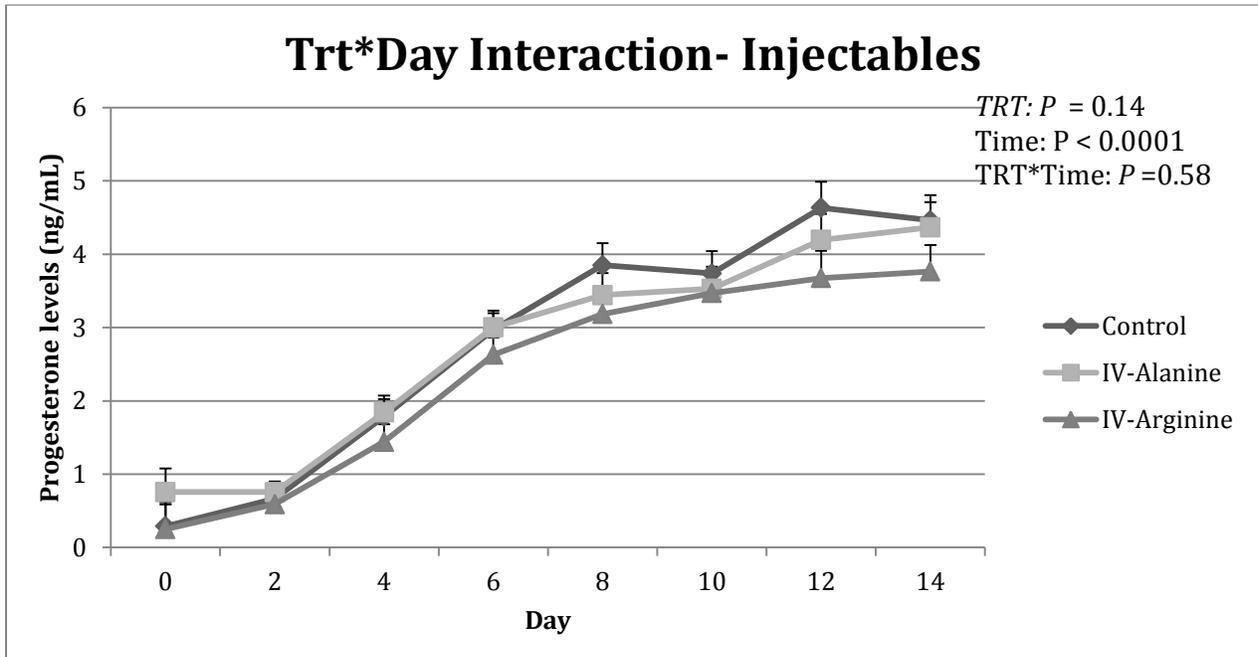
<sup>2</sup>*P*-value for F test of the mean.

<sup>3</sup>Pregnant treated ewes that lambbed to the first estrus.

<sup>4</sup>Lambing rate of ewes that lambbed.

<sup>5</sup>Lambing rate of ewes treated.

**Figure 1.** Progesterone concentrations throughout the treatment period in arginine and alanine injected ewes. Data are means  $\pm$  S.E.



**Figure 2.** Progesterone concentrations throughout the treatment period in rumen-protected arginine (RPARG), fishmeal (FM), and soybean meal (SBM) treated ewes. Data are means  $\pm$  S.E.

