Effect of Supplemental Vitamin E and A on Reproductive Performance and Serological Profiles of Ewes Managed in Drylot

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Summary

Forty Hampshire and 40 Suffolk ewes were allotted to one of four groups (VitA, VitE, VitA&E, Control) in a 2 x 2 factorial treatment arrangement to evaluate the effect of supplemental vitamin E (0 or 300 IU) and vitamin A (0 or 250,000 IU) on reproductive performance. Laparoscopy and ultrasonography were used to measure ovulation rate, embryonic loss, and fetal loss. Serum profiles of a-tocopherol (vitamin E) and retinol (vitamin A) also were monitored. There were no differences (P>.05) among treatment groups in any reproductive trait. Suffolk ewes exhibited a higher (P<.02) ovulation rate than Hampshire ewes, and yearling ewes incurred higher (P<.001) embryonic loss than other age groups, resulting in a lower (P<.001) litter size. Serum levels of a-tocopherol were higher (P<.05) for Hampshire than for Suffolk ewes and were lower (P<.001) in yearling ewes versus ewes two years of age and older. Serum levels of a-tocopherol declined (P<.01) throughout the study in VitA and Control ewes but remained unchanged in VitE and VitA&E ewes. Serum level of retinol remained unchanged in VitA ewes, whereas the level increased (P<.01) initially in VitE, VitA&E, and Control ewes before declining toward initial levels. Correlations were detected between ovulation rate and the change of pre-mating a-tocopherol serum level (r=-.29; P<.02), the change in pre-mating retinol serum level (r=-.50; P<.02) and the interval from vitamin A injection (r=-.60; P<.05). These data indicate significant influences of breed, age, and treatment on a-tocopherol and retinol serum levels in ewes and suggest that the timing of vitamin A administration may influence ovulation rate; however, vitamin supplementation, administered at random stages of the estrous cycle, was unable to alter flock reproductive performance.

Introduction

Vitamins E and A are essential nutrients for sheep reproduction. The NRC daily requirements for 60 to 90 kg. ewes during the flushing period are 26 to 30 IU of vitamin E and 2,820 to 4,230 IU of vitamin A. Most nutritionists assume that reproductive performance will not be limited when animals are fed diets that meet the NRC levels. However, little is known about the effects of vitamin E and A supplementation on specific reproductive events in sheep. Because fertilization in sheep is an all-or-none phenomenon (i.e., either all ovulated eggs are fertilized or none are fertilized), the three major variables that contribute to litter size are ovulation rate, embryonic survival, and fetal survival.

Research with other species indicates that vitamin supplementation may increase embryonic survival and the number of young born. In a preliminary study, we evaluated the effect of supplemental vitamins E and A on reproductive performance of ewes maintained under pasture or drylot breeding systems. Diet (pasture versus harvested hay) caused dramatic differences in a-tocopherol serum level as ewes progressed from the mating period into early and mid-gestation.

The objectives of this study were to monitor the serological levels of a-tocopherol and retinol in ewes maintained in drylot and to evaluate the effects of vitamin E and A administration on ovulation rate, embryonic loss, fetal loss, and litter size.

Materials and Methods

Animals and Diet

Forty Hampshire and 40 Suffolk mixed age (1 to 5 years) ewes had been maintained as one flock on a grass/legume pasture during the summer months. On August 27 (five days before the experiment began), ewes were moved to drylot and were started on a flushing diet consisting of 454 g. corn/head/day, *ad libitum* oat hay, and free choice trace mineralized salt (containing 90 ppm selenium, but no vitamins A or E). A teaser ram was introduced at this time to stimulate and enhance ewe reproductive activity. Body weight (BW) and body condition score (BCS; on a scale of 1 [emaciated] to 5 [obese]) were recorded one day before commencement of the experiment (Hampshires: BW of 87.0 ± 1.6 kg. and BCS of 3.1 ± .1; Suffolks: BW of 85.7 ± 1.5 kg. and BCS of 2.9 ± .1) and at monthly intervals thereafter over the next four months.

On September 15, a 35-day mating period began. The flock was divided by breed to permit single-sire purebred matings. Experienced rams (one Hampshire and one Suffolk) that had passed a thorough breeding soundness exam were fitted with a marking harness before being joined with the ewes. Raddle marks were recorded twice daily during the mating period.

After the end of the mating period, ewes were combined into one flock. Oat hay was gradually replaced with grass hay (due to exhaustion of supply of oat hay) during a one-week period, and corn feeding continued in order to

maintain body condition of the ewes. Table 1 shows the nutrient analyses for the feedstuffs provided during the experiment.

Treatment Groups

On September 1 (14 days before the beginning of the mating period), ewes within each breed were allotted to groups in a 2 x 2 factorial treatment arrangement, blocking for age and balancing for BW and BCS. The resultant treatment groups were designated as VitA, VitE, VitA&E, and Control.

The commercial vitamin preparations used in this study were Vital-E™-300 (Schering-Plough, Kenilworth, NJ) containing 300 IU/ml. vitamin E (as d-a-tocopherol) in a nonaqueous solution and vitamin AD_3 AgriLabs® (Agri Laboratories, Ltd., St. Joseph, MO.) consisting of 500,000 IU vitamin A (as vitamin A propionate), 75,000 IU vitamin D_3, and 5 IU vitamin E (included as an antioxidant) per ml. in an emulsifiable solution. No commercial products were available that contained solely vitamin A.

The treatment dosages for the experiment were based on the concentration of the vitamin preparations, label recommendations, and NRC daily vitamin requirements of ewes. Ewes received 0 or 300 IU of vitamin E and 0 or 250,000 IU of vitamin A (given every 14 and 28 days, respectively) in order to provide a typical therapeutic dose while avoiding potential toxicosis. All treatments were administered intramuscularly in the gluteus muscle.

Serum Collection and Analysis

A total of nine blood samples was collected from each ewe via jugular venipuncture at 14-day intervals over a period of 16 weeks. Samples were collected prior to vitamin administration into non-heparinized, 15-ml. vacutainer tubes. Samples were centrifuged (30 min., 3,000 rpm., 6°C), and serum was stored at -20°C until analysis.

Serum samples were analyzed for a-tocopherol and retinol by high performance liquid chromatography. Duplicate samples were analyzed, and any sample with concentrations that differed by more than 10% was reanalyzed. The duplicate concentrations were averaged to obtain the final sample concentration.

The serum samples from all ewes were analyzed for a-tocopherol. Because of extreme cost, however, only a subset of samples was analyzed for serum retinol. The subset consisted of 24 mature ewes selected from those believed to have maximal opportunity for reproductive loss (i.e., those with an ovulation rate of 3 or 2). These ewes (denoted subset ewes) were also balanced within treatment group for breed, BW, and BCS.

Ovulation Rate Assessment

Laparoscopic ovarian examinations were performed on every ewe between days 4 and 10 of gestation (d 0 = day of first observed raddle mark) to determine the number of corpora lutea (CL). Each CL was assumed to represent the ovulation of one oocyte. Any ewe that returned to estrus during the mating period was re-examined at the same stage of gestation after the subsequent breeding.

Fetal Number Determination

Real-time ultrasound scanning began one day after the end of the mating period and continued at weekly intervals for nine weeks to encompass days 35 to 98 and 0 to 63 of gestation for ewes that conceived on the first and last day of the mating period, respectively. (Hence, RTU was performed weekly on **all** ewes between days 35 and 63 of gestation, irrespective of the actual day of mating.) Ultrasonography was performed by a commercial technician using a VETSCAN 2® (BFC Technology, Ltd., Livingston, West Lothian, Scotland) sector scanner equipped with a 3.5 MHz transducer. Ewes were held off feed for 18 hours (but were allowed access to water) before being scanned twice on each day. The technician was asked to provide a count of the number of fetuses present in utero or, if that was not possible, to provide an assessment of fluid accumulation and/or presence of cotyledons as an indicator of pregnancy. If the result of the first scan did not match the result of the second scan, a third scan was performed to obtain two scans in agreement.

Litter Size

At lambing time, ewes were segregated into small groups and were closely monitored to facilitate accurate recording of the number of lambs born for a final assessment of litter size (LS).

Statistical Analysis

The records of each ewe were reviewed before performing analyses. Ovulation rate (OR) was calculated as the sum of the CL present on the left and right ovaries. The CL count that corresponded with the breeding date that yielded a gestation length of approximately 147 days was used as the OR for ewes that lambed. For ewes that had a confirmed pregnancy by RTU but did not lamb, the CL assessment consistent with the first potential detection of pregnancy by RTU (≈ 26 to 33 days) was used as OR. The last observed CL assessment was utilized for the OR of ewes not lambing or not having a pregnancy confirmed by RTU. Ovulation rate was not determined on one ewe due to the absence of a breeding mark. Although this ewe was subsequently found to be pregnant, she was removed from the data set to avoid inaccurate estimation of OR and/or embryonic loss.

Embryonic loss (EL) was calculated for each ewe using the formula EL = [(OR - first fetal count) / OR]. Fetal loss (FL) was calculated for each ewe using the formula FL = [(first fetal count - number born) / first fetal count]. The pre-mating a-tocopherol and retinol serum levels were defined as those immediately preceding a fertile mating. The change of a-tocopherol and retinol serum levels were defined as the difference between the

serum level immediately preceding a fertile mating and the serum level 14 days prior to that. Three records of BW and BCS encompassed the mating period, and the pre-mating changes in BW and BCS were defined as the difference between the second and first measurements for ewes mated prior to the second record, or as the difference between the third and second measurements for ewes mated after the second record.

Data were analyzed utilizing analysis of variance, correlation, regression, and t-test procedures. The main effects of breed, age, and treatment were examined for their effects on OR, EL, FL, LS, and serum level of a-tocopherol and retinol. Where appropriate, some traits (BW and BCS corresponding to mating; the change in pre-mating a-tocopherol, retinol, BW, and BCS; a-tocopherol and retinol level) were utilized as covariates in the analyses.

Results

Reproductive Traits

Data analysis revealed no effect of vitamin administration on OR, although OR tended ($P<.10$) to be lower in the VitA group (Table 2). Suffolk ewes exhibited a higher ($P<.02$) OR than Hampshires, and yearling ewes tended ($P<.10$) to have a lower OR than ewes of other ages.

No correlation existed between OR and the pre-mating a-tocopherol serum level or between OR and any of the changes in BW or BCS. Interestingly, a negative correlation ($r=-.29$; $P<.02$) was found between OR and the change in a-tocopherol serum level preceding mating. Further analyses revealed that this correlation approached significance in Suffolk ewes ($r=-.27$; $P<.09$) and was significant in Hampshire ewes ($r=-.39$; $P<.02$). Within treatment group analysis (ignoring effect of breed) indicated that only the VitE treatment group experienced a correlation ($r=-.56$; $P<.01$) between OR and the change in serum a-tocopherol level.

Embryonic loss was affected by age ($P<.001$) with yearling ewes exhibiting greater EL than ewes two years of age and older. Neither breed nor vitamin treatment affected EL, but EL tended ($P<.08$) to be influenced by OR, as greater EL was observed as OR increased (12.5, 22.7, 27.4% EL for OR of 1, 2, and 3, respectively).

Because only five ewes (6.3%) had FL, data were too few to analyze statistically. Two of the five ewes incurred complete loss of all fetuses, and the other three ewes sustained partial litter loss. One mature Hampshire ewe lost both fetuses in the seven-day period ending on day 31 of gestation, and one yearling Hampshire ewe lost her single fetus in the seven-day period ending on day 49. Another mature Hampshire ewe lost one of three fetuses in the seven-day period ending on day 36 of gestation, while one mature Suffolk ewe lost one of two fetuses in the week ending on day 60 and one yearling Suffolk ewe lost one of three fetuses sometime after day 80 of gestation but before lambing.

The LS at lambing was not affected by breed or vitamin administration but was affected by age. Yearling ewes produced fewer ($P<.001$) lambs than either the two-year-old or three-year-old and older ewes (Table 2).

The mean OR and LS for the subset ewes (the ewes for which serum retinol was assayed) was $2.4 \pm .1$ and $1.9 \pm .2$, respectively. However, further analyses of data from the subset ewes were not performed because the ewes had been selected based on their high reproductive potential.

Serum Level

Analysis of a-tocopherol serum data of all experimental ewes revealed no difference ($P>.05$) among the four treatment groups at the beginning of the experiment (Figure 1). Analyses comparing the first a-tocopherol sample with each of the eight remaining samples revealed no difference in a-tocopherol level over time for VitE and VitA&E treatment group ewes. Conversely, a-tocopherol levels declined ($P<.01$) between sample one and each of the eight subsequent samples for VitA and Control treatment group ewes.

Unexpectedly, analysis revealed an effect of breed ($P<.05$) on serum a-tocopherol at all but the ninth serum sample (Table 3) where, although not significant, the a-tocopherol serum level of Hampshire ewes remained numerically higher than that of Suffolk ewes. Age also affected ($P<.001$) a-tocopherol concentration at the first sample (yearlings: $1.22 \pm .06$ ppm; two-year-olds: $1.61 \pm .07$ ppm; three-year-olds and older: $1.52 \pm .06$ ppm) but not at subsequent samples.

Analysis of data from only the subset ewes revealed that a-tocopherol levels were affected by treatment ($P<.05$) and breed ($P<.05$). However, because of the replacement of oat hay with grass hay after the mating period and the known influence of diet on a-tocopherol serum level, two separate regression analyses were performed--using the first five samples and the second five samples, respectively--to enable analysis within a dietary regimen. Serum a-tocopherol levels declined over the first five samples in all treatment groups except VitE ($P<.47$), but a-tocopherol levels over the last five samples were essentially unchanged in all treatments.

In contrast, serum retinol levels of the subset ewes increased over the first five samples in all treatment groups except VitA which remained unchanged. Across the last five samples, serum retinol values tended to decline in all treatment groups, although not significantly so. Further analysis revealed a correlation ($r=-.50$; $P<.02$) between the pre-mating serum retinol level and OR. In addition, a correlation ($r=-.60$; $P<.05$) was discovered between OR and the injection interval (mean of 14.8 ± 2.8 days) from vitamin A administration to the time of mating. Ewes receiving vitamin A closer to (but prior to) mating had an increased OR.

Discussion

Reproductive Trait

The failure of vitamin E and A injections to alter the reproductive events of the ewes utilized in this

experiment is clear; however, this may be related to the frequency and timing of injections or to the relatively low dose given (especially for vitamin E). In research conducted elsewhere, sows that experienced an increase in the number of young born were given vitamin A injections at critical stages of their reproductive cycle (at weaning, mating, and seven days after mating) rather than on a calendar day as was done in the present experiment. The correlation between OR and the injection interval of vitamin A to mating suggests that ovulation rates are higher when the injection is given closer to (but prior to) ovulation. If the effectiveness of supplemental vitamin A injections is dependent on specific timing of administration to the reproductive cycle of sheep, application of this technology to entire flocks would require intensive management and/or synchronization of estrus.

The correlation between OR and the change in pre-mating a-tocopherol serum level superficially implies that supplemental vitamin E administration would be deleterious to OR because it elevates a-tocopherol serum levels; however, the OR of vitamin E-treated ewes was not different from non-E-treated ewes. Further, careful review of the data reveals stronger correlations for groups of ewes that are expected to have a higher a-tocopherol serum level (i.e., Hampshire ewes and vitamin E-treated ewes), so the biological significance of this statistical correlation is questionable.

The effect of vitamin A administration on retinol serum level is puzzling. The serum retinol level of the VitA treatment group ewes did not increase during the study as it did in the other three groups. This unexpected response may be due to an inhibitory effect of the vitamin A injection on mobilization of vitamin A from the liver, which reduces the serum concentration. Use of smaller doses of vitamin A may be necessary to avoid this phenomenon. Although a negative correlation existed between OR and the pre-mating serum retinol level, implying that higher ovulation rates are experienced at lower pre-mating retinol levels, the biological interpretation of this correlation is not clear.

The reproductive data collected in conjunction with serum a-tocopherol and retinol levels are intriguing, but interpretation of these data is difficult. Nevertheless, the one conclusive finding is that age is a highly influential factor in determining the reproductive performance of sheep, as yearlings are more prone to embryonic loss. This finding suggests that producers should consider segregation of yearling ewes during early and mid-gestation.

Nutrition/Serum Level

The primary dietary nutrients were present in levels adequate to meet the daily requirements of the ewes in this experiment. Interestingly, replacement of the oat hay with grass hay (which had twice the content of a-tocopherol and four times the content of b-carotene as oat hay) after the mating period appeared to prevent further declines of a-tocopherol in the VitA and Control ewes, whereas the previously rising retinol serum level in the VitA&E, VitE, and Control ewes declined.

The a-tocopherol level of ewes housed in drylot was stabilized by administering 300 IU of vitamin E at 14-day intervals, whether it was given alone or in conjunction with vitamin A. The a-tocopherol level of non-vitamin E supplemented ewes housed in drylot, however, declined rapidly and approached levels considered to be deficient. Similarly, the serum retinol level of ewes housed in drylot can be maintained by injecting 250,000 IU of vitamin A (as vitamin A propionate) every 28 days. Interestingly, serum retinol levels did not decline in vitamin E-treated ewes.

Implications

This study documented that ewe reproductive traits are difficult to influence with an injection of vitamin E and/or A administered at random times of the estrous cycle. Repeated parenteral administration of vitamin E is effective in maintaining serum a-tocopherol level, but diet, breed, and age also impact these levels. Serum retinol levels can be influenced by parenteral injection of vitamin A and by diet, but not to the same degree that vitamin E treatment influences serum a-tocopherol levels. Ewe age deserves careful consideration when planning flock management, as yearling ewes managed contemporarily with the mature flock seem more susceptible to embryonic loss. These findings will be useful to help further explore the frequency, timing, and amount of vitamin supplementation that may alter reproductive performance of the ewe.

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Table 1. Diet analysis on a dry mattor basis[a].

Nutrient	Oat hay	Grass hay	Corn
Crude protein (%)	13.0	17.8	10.8
Metabolizable energy (kcal/kg.)	476	426	588
Vitamin E (U/kg.)[b]	13.3	38.5	3.4
β-carotene (mg/kg.)	2.9	12.1	1.9
Selenium (ppm)[c]	< .11	< .11	. 12

[a]AII samples were analyzed at a commercial laboratory using industry-approved methods.
[b]Epressed as total tocopherol.
[c]Assay detection limit of .11 ppm.

Table 2. Summary of ewe reproductive components.

	n	Ovulation rate	Embryonic loss (%)	Fetal loss (%)	Lambing rate
Breed					
Hampshire	40	2.0 ± .1[b]	19.2 ± 5.6	5.8 ± 3.6	1.5 ± .1
Suffolk	39	2.3 ± .1[a]	24.3 ± 5.7	2.1 ± 1.5	1.6 ± .2
Age Group					
yearling	28	1.9 ± .1	42.8 ± 7.6[d]	4.8 ± 3.7	.9 ± .1[d]
2	20	2.3 ± .1	4.2 ± 2.9[c]	1.7 ± 1.6	2.2 ± .1[c]
≥ 3	31	2.3 ± .1	14.0 ± 5.7[c]	4.8 ± 3.6	1.8 ± .2[c]
Treatment					
VitA	19	1.9 ± .1	17.5 ± 8.6	5.3 ± 5.3	1.4 ± .2
VitE	20	2.2 ± .1	21.7 ± 7.6	2.5 ±2.5	1.7 ± .2
VitA&E	20	2.3 ± .1	23.3 ± 8.7	6.7 ±5.2	1.6 ± .2
Control	20	2.3 ± .1	24.2 ± 7.6	1.7 ±1.6	1.7 ± .2

[a,b] Means within a column having unlike superscripts are different (P<.02).
[c,d] Means within a column having unlike superscripts are different (P<.001).

Table 3. Serum α-tocopherol level by breed[a].

Breed	1	2	3	4	5	6	7	8	9
Hampshire	1.58±.05a	1.44±.05a	1.36±.05a	1.29±.05a	1.18±.06a	1.18±.05a	1.22±.06a	1.24±.06a	1.23±.07
Suffolk	1.29±.05a	1.24±.06b	1.14±.06b	1.07±.06b	1.03±.06b	1.02±.06b	1.07±.07b	1.10±.07b	1.15±.09

[a]Means within a column having different superscripts are signficantly different (P<.05).

Figure 1. Serum α-tocopherol profile of Hampshire and Suffok ewes mated in drylot.