

Effect of using supplementation mineral or organic selenium with vitamin E as antioxidants in the flushing diet on the fertility of ewes

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ABSTRACT

In this study, 44 Afshari ewes were investigated that were 1–4 years old with a mean weight of 54–51 kg, with a physical score of approximately three in four experimental groups. Ewes were randomly assigned into four experimental groups including Group A Control (base diet recipient), Group B (Flushing), Group C (Flushing + Vitamin E Supplement + Organic Selenium), and Group D (Flushing + Vitamin E Supplement + Mineral Selenium). Blood sampling was carried out at different intervals namely beginning of the course, 24 h prior to CIDR, 24 h after CIDR (estrus), and 21 days after mating (at the time of embryonic implantation). Blood serum hormones (estrogen, progesterone, and insulin) were measured. The results of this study showed improvement in reproductive efficiency, especially the percentage of lambing, the fertility rate in Afshari breed. The use of organic selenium supplement with vitamin E and mineral selenium with vitamin E in the flushing diet before mating increased the reproductive performance of Afshari breed sheep by increasing the number of reproductive hormones (estrogen, progesterone, and insulin). C and A treatment had the highest and lowest lambs, respectively with 14 and 10 progenies. The highest birth weight of lambs was related to C treatment with an average of 4.49 kg. The use of two Flushing factors and vitamin E and organic selenium supplementation significantly increased the estrus and fertility, and significantly reduce the non-pregnant ewes by controlling oxidative conditions and increasing sex hormones.

Keywords: Afshari sheep, Organic selenium, Reproduction, Vitamin E

Afshari sheep have tail fat and colored wool, which are meat-dairy ewes and distributed in Iran in Zanzan province, and also in other provinces of the country (West Azerbaijan and East). In normal conditions, the rate of ovulation in sheep is 1 to 3, but all the eggs do not fertilize. Also, about 40% of deaths occur within 28 days after artificial insemination and 14% between 28 and 45 days after artificial insemination (Bilby *et al.* 2006). The use of mineral and vitamin supplements in the flushing diet can have favorable effects on their reproduction. Many nutrients such as cobalt, selenium, manganese, beta-carotene, and vitamins play an important role in sexual behavior by affecting ovarian steroids (Hirunpanich *et al.* 2006). Although little information is available on the effects of selenium and vitamin E on reproduction in sheep, it has been shown that selenium, *in vitro*, promotes the proliferation of ovarian granulosa cells and the synthesis of estradiol β -17 in animals and ultimately increases oocytes and the number of living embryos (Aranda-Avila *et al.* 2010).

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MATERIALS AND METHODS

Forty four single birth 1–4 years old Afshari ewes with a mean weight of 54–51 kg, and body scores of approximately 3 were randomly divided into control and 4 experimental groups. Control group (A) was maintained on basal diet and other treatment (T) groups fed flushing diet (B) or flushing diet supplemented with 0.048 g of vitamin E along with 150 mg of organic selenium (C) or 13.5 mg of mineral selenium (D). Dietary requirement of the sheep was set during the testing period, based on the requirements of an average weight of 50 kg and the beginning of the reproductive season with the first 5 weeks of breeding. The amount of dietary energy in the flushing treatments was approximately 1.5 times the base ration (Table 1). The tested livestock received 450 g of feed per ewe in one meal after returning from total mixed ration (TMR).

Prior to distribution in the groups, all ewes were ascertained for their health and reproductive performance. The ewes in different groups received the respective diet for 5 weeks consisted of 2 weeks before mating and 3 weeks after mating. Body weight of all ewes were recorded at the beginning and end of experimental period. The estrus

Table 1. Test diets

Edible food (dry matter percentage)	Treatment			
	A	B	C	D
Wheat bran	–	11.00	11.00	11.00
Soybean meal	–	8.00	8.00	8.00
Mineral supplement	–	1.00	1.00	1.00
Protein supplement	–	5.00	5.00	5.00
Barley seeds	–	73.00	73.00	73.00
Salt	–	5.00	5.00	5.00
Molasses	–	6.00	6.00	6.00
<i>The amount of vitamin E and selenium supplementation per sheep (in g)</i>				
Mineral selenium	–	–	–	135.00
Organic selenium	–	–	135.00	–
Vitamin E	–	–	48.00	48.00
<i>Chemical compounds</i>				
TDN (%)	–	78.00	78.00	78.00
Crude protein (%)	–	2.13	2.13	2.13
Digestible energy (mega calories per kg)	–	41.30	41.30	41.30
Metabolism energy (mega calories per kg)	–	1.30	1.30	1.30

ewes synchronized with CIDR and 400 PMSG hormone units were injected 36 h after CIDR during mating using Afshari pure breed in each treatment.

Statistical analysis: The experiment was conducted in a completely randomized design. Chi-square analysis was used for non-returning to estrus, generation rate, multiplicity, and flock breeding. The statistical model of the design was

$$y_{ij} = \mu + T_i + e_{ij}$$

where, μ was population mean, T_i was the treatment effect ($i = A, B, C, D$), y_{ij} was observation effect and e_{ij} was error component. GLM and Mixed procedures were used to analyze the traits of generation rate, flock incubation, birth weight of estrogen, prostaglandin, and insulin levels using following model

$$y_{ijkl} = \mu + \text{Treat}_i + \text{Time}_j + (\text{Treat} \times \text{Time})_{ij} + \text{Type}_k + \text{Animal}_l + e_{ijkl}$$

where, μ was population mean, y_{ijk} was the observed data for the measured parameters, Treat_i was the effect of the i^{th} treatment, type k was the effect of k^{th} generation, $(\text{Treat} \times \text{time})_{ij}$ was the interaction of time and treatment, Animal_l

Table 2. Comparison of mean least squares between treatments and estrogen concentration (pg/ml) during estrus and day 21 of generation

Experi- mental Diets	24 h before CIDR	24 h after CIDR	Day 21 of generation	Total Estrogen of the Course
A	8.35±0.45 ^b	29.01±1.45 ^c	8.40±0.43 ^b	16.02±0.23 ^b
B	8.02±0.49 ^b	32.88±1.59 ^b	8.27±0.47 ^b	16.39±0.61 ^b
C	10.80±0.45 ^a	36.99±1.45 ^a	8.39±0.43 ^b	19.43±0.56 ^a
D	11.45±0.45 ^a	37.60±1.45 ^a	9.98±0.43 ^a	19.35±0.56 ^b

*Non-similar Latin letters in each column indicate a significant difference in the level ($P < 0.01$).

is the animal, and e_{ijkl} is the residual effect or the error. The results were analyzed by SAS software (2003) after the normalization test.

RESULTS AND DISCUSSION

Reproductive biochemical hormones associated with reproduction in Afshari ewe

Estrogen (E2): Tables 1–3 show significant differences in serum estrogen concentrations during estrus between treatments. Serum estrogen was 36.39 and 37.60 pg/ml in TC and TD, respectively. Highest estrogen concentration observed after 24 h of CIDR TC and TD in which eventually had the highest number of offspring among the treatments. Although estrogen levels during estrus (24 h after CIDR) in treatments C and D were higher than treatment B (Flushing Barley) and A (control). This indicates that using mineral or organic selenium antioxidants with vitamin E significantly ($P < 0.01$) increased estrogen levels in the estrous phase and it had cascading effect on the incidence of fecundity and progeny (Table 2).

Progesterone (P4): Table 3 shows a comparison of mean serum progesterone concentrations. Progesterone concentration at the beginning of the period did not differ significantly between Flushing treatments. However, the results showed a significant difference in serum progesterone concentrations 21 days after mating. 24 h before CIDR progesterone concentration increased because of CIDR. Progesterone concentrations in TC and TD were significantly different with the control group ($P < 0.01$) but did not differ significantly with TB. The concentration of progesterone decreased 24 h after CIDR using (estrus) to remove CIDR in all groups. At this stage, the progesterone

Table 3. Comparison of mean least squares between treatments and progesterone concentration (ng/ml) in whole period

Experimental diet	24 h before CIDR	24 h after CIDR	Day 21 of generation	Total progesterone of the course	24 hours before CIDR
A	1.12±0.07 ^a	1.42±0.09 ^b	0.54±0.08 ^b	3.31±0.13 ^c	1.65±0.64 ^c
B	1.24±0.07 ^a	1.41±0.09 ^{ab}	0.85±0.09 ^a	4.24±0.15 ^b	1.89±0.67 ^b
C	1.29±0.06 ^a	1.29±0.06 ^a	0.59±0.08 ^{ab}	5.10±0.13 ^a	2.12±0.61 ^a
D	1.30±0.06 ^a	1.30±0.06 ^a	0.65±0.08 ^{ab}	5.31±0.13 ^a	2.19±0.61 ^a

*Non-similar Latin letters in each column indicate a significant difference in the level ($P < 0.01$).

Table 4. Comparison of mean squares between treatments and insulin concentration (ng/ml) in four blood sampling times

Experimental diet	24 h before CIDR	24 h after CIDR	Day 21 of generation	Total insulin of the course	24 h before CIDR
A	0.39±0.01 ^a	0.41±0.01 ^b	0.51±0.02 ^b	0.39±0.02 ^b	0.44±0.01 ^b
B	0.37±0.01 ^a	0.45±0.01 ^b	0.54±0.03 ^b	0.45±0.01 ^a	0.47±0.01 ^a
C	0.39±0.01 ^a	0.55±0.01 ^a	0.62±0.02 ^a	0.52±0.02 ^a	0.52±0.12 ^a
D	0.40±0.01 ^a	0.53±0.01 ^{ab}	0.70±0.02 ^a	0.54±0.02 ^a	0.54±0.12 ^a

*Non-similar Latin letters in each column indicate a significant difference in the level ($P < 0.01$).

content was the highest in TB, but no significant difference was observed with TC and TD.

The concentration of progesterone was high at 21 days after mating. This appears to be due to the introduction of ewes into generation and embryo formation. Also, the level of this hormone was significantly different between TB ($P < 0.01$) at 21 days after mating. Meanwhile, TD (5.7 ng/ml) and TC (5.9 ng/ml) had the highest levels of progesterone which was significant ($P < 0.01$) with barley flushing treatment and control group. Also, the level of this hormone showed a significant difference in the TB with the CG ($P < 0.01$). In fact, CG (31 ng/ml) had the lowest level of progesterone in the first month of generation and embryo implantation time.

The results of this study were compared with the results of Naqvi *et al.* (2012). In Suffolk sheep, the use of mineral selenium with vitamin E prior to ovulation induced an increase in progesterone levels during embryo formation compared to the control group. Also, Lopez-Sebastian *et al.* (1997) showed that increasing selenium in the ration during mating and generation increases serum progesterone on days 50, 90, and 106 of generation compared with the CG and thus preserves the fetus. However, Ambrose *et al.* (2006) concluded that the use of organic selenium supplementation in ewe's diet reduces progesterone production compared to the control group, resulting in backwardness and abortion.

Insulin: Table 4 indicate significant differences in serum insulin concentration at all times, except for the beginning of the experiment between treatments. Treatment A with 0.41 (ng/ml) insulin 24 h before CIDR showed the lowest amount of insulin hormone among treatments. Treatment C with 0.55 (ng/ml) (organic selenium with vitamin E) had the highest amount of insulin hormone among treatments. There was a significant difference between treatments C with A and B treatments ($P < 0.01$). Also, 24 h after CIDR (estrus), D treatment with 0.70 (ng/ml) (mineral selenium with vitamin E) had the highest (0.75) insulin hormone among treatments, which had a significant difference with A and B treatments ($P < 0.01$). In the 21st day after mating, treatment D had the highest insulin concentrations in the treatments, which had a significant difference only with control treatment ($P < 0.01$).

Insulin is a well-known factor for follicular function in several species of livestock, and the roles that it carries are increasing the activity of steroid genesis in granulosa cells, cell mitosis and changes in morphological differentiation

in follicles and increasing the concentration of progesterone in follicular fluid. The addition of growth hormone and insulin on bovine granulosa cells increased progesterone production (Scaramuzzi *et al.* 1993). Increasing insulin concentration leads to the growth and development of more follicles, and subsequently, the amount of ovulation is increased and more progeny will be born (Scaramuzzi *et al.* 2006).

Fertility parameters

Fertility parameters, viz. percent fertility (Percentage of delivered ewes to the total mated ewes), percentage of lambing (number of born lambs to the number of delivered ewes), propagation percentage in the herd (Number of born lambs to the number of mated ewes) and twin birth (number of ewes with twin birth to the total delivered ewes) are presented in Table 5.

The research results indicate a significant increase in the degree of twin birth, lambing, and fertility (Tables 3–5), and the reproduction rate of the herd was more than 130% among TB, TC and TD, which confirms ideal breeding conditions.

According to the findings of this study, the addition of mineral or organic selenium with vitamin E in the diet during the reproductive season increased the number of infants and improved fertility (Tables 3–5).

Nutrition directly will affect processes such as the development of oocytes, ovulation, embryo survival, and generation establishment through the provision of nutrients. It also indirectly affects fertility through effects on hormones and blood metabolites (Robinson 1990). The use of nutrients such as cobalt, selenium, manganese, beta-carotene, and vitamins play a major role in reproduction by influencing ovarian steroids. It is proven that Vitamin E is effective in oviposition and survival of the embryo (Hess *et al.* 2005). It has been shown in some references that selenium, increases the chance of fertilization by increasing

Table 5. Descriptive statistics of progeny and fertility percentages in experimental treatments

Treatment	Number of progenies	Estrous	Fertility	Twin birth	Lambing
A	10	100	90	0.00	90.000
B	12	100	100	9.00	109.000
C	14	100	100	3.27	3.127
D	12	100	100	9.00	109.000

the uterine movement during fertilization, as a result, reduces the number of Ghasr sheep. In addition, the results of Scalz *et al.* (1974) showed that the use of sodium selenate (mineral selenium) orally and 17 days before mating caused a 12% decrease in Ghasr sheep.

Analyzing the results of variance analysis of logistic regression and correlation between hormones, it can be stated that any factor or factors that can increase estrogen concentration at the onset of the reproductive cycle and estrus time in oviposition, increase the number of lambs, fertility, and twin birth. The results showed that mineral selenium increased the success rate in the first estrus and also had the highest fertility, duality, lameness, and reproduction in the herd of Afshari sheep. According to Robinson (1990), increasing estrogen in the follicular fluid improves ovarian characteristics and oocyte rate, fertility, cleavage, and generation. The results of this study indicate improvement of reproductive efficiency especially lambing percentage and fertility rate in Afshari sheep. The use of organic selenium supplement with Vitamin E and mineral selenium supplement with Vitamin E in the flashing diet (fertilization) improved reproductive performance in Afshari sheep through TMR method before mating with the growth of a series of reproductive-related hormones (estrogen, progesterone, and insulin).

It seems that the use of antioxidant supplements in ruminants, especially sheep, has positive effects before mating, after, and during generation. Therefore, the evaluation of oxidative stress and the antioxidant status during mating and embryonic replacement time can play a significant role in the production of sheep.

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