

Effect of Vitamin E –Selenium Administered During Estrus Synchronization on Antioxidant Activity, Hormonal Profile, and Lamb Rearing of Moghani Ewes

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Abstract

The study was conducted with 200 mature, non-lactating ewes aged 2–5 years and weighing about 70 kg, during the non-breeding season under natural photoperiod conditions. In the control group, only estrus synchronization was performed. The second group received two intramuscular injections (5 mL total) of E-Se (selenium as sodium selenite at 0.5 mg/mL and vitamin E as DL- α -tocopheryl at 50 IU) on the days of sponge insertion and sponge removal. Estrus synchronization involved placing intravaginal sponges for 14 days, followed by 400 IU eCG at sponge withdrawal. Two days after sponge withdrawal, ewes were mated with fertile rams. Three weeks after ram exposure, 20 ewes from each group were randomly selected for blood sampling. Hormone concentrations and antioxidant activity were measured. After parturition, reproductive traits were recorded for each group. The results showed that there was no significant effect of E-Se on the Lamb body weight at birth, body weight at 90 days, total weight gain and average daily gain. In E-Se treated group, number of lambs/ewes was significantly different ($P < 0.05$) compared with control. Progesterone concentration in E-Se groups was greater ($P < 0.05$) than the control. E-Se supplementation exerts a significant ($p < 0.05$) increasing effect on serum triiodothyronine (T3) concentration and the ratio of T3/T4. Total antioxidant capacity 3 weeks after ram exposure was significantly higher ($P < 0.05$) in E-Se than control. A significant ($p < 0.05$) increase in serum glutathione peroxidase concentration was observed in ewes supplemented with E-Se compared with the control group. Selenium concentration of serum was significantly higher in group receiving E-Se three weeks after mating ($P < 0.05$). However, there were no significant differences in serum malondialdehyde, superoxide dismutase, and hemoglobin concentrations between the E-Se and control groups. In summary, administering vitamin E-Se enhanced reproductive efficiency, antioxidant status, and serum progesterone levels in estrus-synchronized Moghani ewes during the non-breeding season.

Keywords: Antioxidant, Estrus Synchronization, Moghani Ewe, Progesterone, Vitamin E-Se.

Introduction

Livestock reproductive performance is influenced by four primary factors: genetic potential, environmental conditions,

management practices, and nutritional status. Among these, nutrition is arguably the most influential because of its direct impact on reproduction. Inadequate nutrition not only suppresses performance below genetic

potential, but also worsens adverse environmental effects [1]. Nutrition influences every stage of the reproductive process from gametogenesis and follicular development to ovulation and steroidogenesis in females, which helps explain why reproduction is so closely tied to food availability [1]. Administering nutritional supplements during the flushing period often increases both ovulation and lambing rates across many ewe breeds [2]. Micronutrients including minerals and vitamins are a critical component of livestock diets and can enhance reproductive performance. Specifically, elements like cobalt, selenium, manganese, β -carotene, and various vitamins support ovarian steroidogenesis and improve overall reproductive outcomes [3].

During the pre-estrus phase, a rise in metabolic rate and enhanced steroidogenic activity drive greater production of reactive oxygen species (ROS) [4]. Moreover, the application of intravaginal devices such as sponges or CIDR for estrus synchronization has been associated with increased oxidative stress [5,6]. Elevated ROS concentrations during the peri-mating period have the potential to impair ovulation, compromise oocyte and embryo quality, and diminish progesterone (P4) synthesis by the corpus luteum [7]. Consequently, around mating and particularly when estrus synchronization is used, maintaining or enhancing the antioxidant status of ewes may be beneficial. Vitamin E and selenium are two crucial antioxidants that help prevent cellular damage from free radicals. Vitamin E is a lipid-soluble antioxidant that protects cell membranes by reducing lipid peroxidation, while selenium is a key component of glutathione peroxidase enzymes that catalyze the reduction of harmful peroxides [8]. Selenium also supports amino acid metabolism, normal fetal growth and development, optimal thyroid function, and immune system activity [9]. Additionally, vitamin E can influence ovulation rate and the

survival of fetuses and embryos [10]. Deficiencies in vitamin E and selenium have been linked to impaired reproductive performance, increased incidence of retained fetal membranes, and higher perinatal mortality in sheep [11].

Numerous previous studies have demonstrated beneficial impacts of vitamin E-Se supplementation on the reproductive traits [12,13]. In Yankasa ewes, combined vitamin E and selenium injections significantly increased estrus response, pregnancy rate, lambing rate, and lamb growth performance [14]. Similarly, supplementation with these antioxidants in Awassi ewes improved conception rates, lambing outcomes, and physiological parameters critical for reproductive efficiency [15]. Some studies also show that Se supplementation can increase lamb survival, birth weight, and weaning weight [16]. This study aimed to assess the impact of repeated vitamin E-Se injections, administered alongside an estrus synchronization protocol, on reproductive efficiency, antioxidant status, serum hormone profiles, and lamb growth parameters in Moghani ewes.

Materials and Methods

Location, Animal Management, and Treatments

The current study was conducted in one of the smallholder flocks located in the village of Baqersali, Bilasuvar County, Ardabil Province, during the non-breeding season (March) under natural photoperiod conditions. All animal operations were carried out in compliance with protocols approved by the Research Ethics Committees of University of Mohaghegh Ardabili. Two hundred clinically non-pregnant, non-lactating and healthy Moghani ewes (2–5 years of age) with an average body weight 70 kg were divided into two groups (E-Se and control, n = 100). Ewes were balanced between groups for body weight, body condition score, and parity. The

body condition score of the ewes was between 3.0 and 3.5 on a five-point scale, indicating they were in good condition. They were managed under typical nomadic system, with supplementary feeding during early pregnancy (200 g barley and alfalfa per day for each ewe) and then grazing on pasture alone until lambing. The males were separated from the females in individual pens 500 m away from the females. All ewes were ear-tagged for identification and recording, and both experimental groups received identical management, including nutrition and hygiene. Estrus was synchronized in all ewes according to the following program: intravaginal sponges containing progestagens (60 mg medroxyprogesterone acetate, Esponjavet®, Hipra, Spain) were inserted and maintained for 14 days. Upon sponge removal, each ewe received an intramuscular injection of 400 IU eCG (Folligon®; Intervet, Holland). The ewes were naturally mated using fertile Moghani rams (one ram per 10 ewes), which were introduced the day after the injection of eCG. The experimental groups consisted of a control and an E-Se injection treatment. The E-Se group was received two intramuscular (5 mL) injections of E-Se (0.5 mg/mL of selenium as sodium selenite and 50 IU of vitamin E as DL- α -tocopheryl; Erfan Darou Research, Tehran, Iran) on the days of sponging and sponge removal. Throughout the experimental period, all animals were provided with unrestricted access to feed and water. The dosage and timing of vitamin E-Se administration were determined based on the manufacturer's guidelines for the prevention of E-Se deficiency in sheep, as well as relevant findings documented in previous studies.

Reproductive Performance and Rearing of Lambs

After parturition, reproductive parameters were assessed within each experimental group. Lambing rate (lambs born/ewes joined), and lamb viability rate were recorded.

Lambs were weighed at birth and every two weeks thereafter until weaning. Lambs were weaned and weighed at approximately 90 days of age using an electronic scale to record final body weight. This was performed at 0700 hours, prior to the daily feeding. For the calculation of average daily gain (ADG), total weight gain for the entire feeding period was divided by the number of days fed *i.e.* $([\text{final BW} - \text{initial BW}] / 90)$.

Blood Sampling and Analytical Methods

At the onset of the trial, blood samples were randomly collected from 20 ewes per group via jugular venipuncture using 18-gauge needles into non-heparinized tubes. The samples were left undisturbed at ambient temperature for 2 hours to allow clot formation, followed by centrifugation at $3,000 \times g$ for 20 minutes to isolate the serum. Serum samples were preserved at -20°C for hormonal and antioxidant assays. Moreover, whole blood samples were collected from the jugular vein into sterile EDTA tubes and subsequently stored at -20°C for the quantification of superoxide dismutase (SOD), glutathione peroxidase (GPx), and hemoglobin (Hb) levels. Blood samples were collected at two time points: prior to intravaginal sponge insertion and three weeks following ram exposure. The hormones estradiol-17 β , progesterone, T3, and T4 concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) method, using commercial kits (estrogen: Monobind No. 4925-300A; progesterone: Monobind No. 4825-300A; triiodothyronine (T3) and thyroxine (T4): Pishtaz TEB Diagnostics No. 14003), with measurements read on ELISA reader (Hiperion Microplate Reader, Germany). Intra-assay coefficients of variation for the T3, T4, estrogen and progesterone assays were 7.4%, 5.4%, 0.13% and 0.42%, respectively. The malondialdehyde (MDA) concentrations were determined by a colorimetric thiobarbituric acid (TBA) assay, following the

Esterbauer and Cheeseman [17] protocol. Thiobarbituric acid reactive substances (TBARS), produced as by-products of lipid peroxidation and detectable by TBA, were quantified in serum. Total antioxidant capacity (TAC), superoxide dismutase (SOD) activity, and glutathione peroxidase activity (GPx) were quantified using a commercial kit (Randox, Crumlin, UK). The absorbance was recorded by a spectrophotometer (T80 UV/VIS PG Instruments Ltd, UK) at the wavelengths recommended by the kit. Hemoglobin levels (Hb) were measured using the cyanmethaemoglobin method as described by Cannan (1958). Serum selenium levels were measured following sample preparation via microwave digestion using the ETOS 1 advanced system (Milestone, Italy), in accordance with the methodology outlined by Hefnawy et al. [18]. Selenium stabilization was achieved with a Pd+Mg(NO₃)₂ modifier as described by Zanão et al. [19], and Se was quantified by atomic absorption spectrophotometry (Unicam Atomic Absorption Spectrometer Model SOLAAR M5, UK).

Statistical Analysis

Reproductive data were evaluated using the Proc Genmod procedure within a completely randomized design in SAS version 9.2. Comparisons of lambing rates were performed through the Chi-Square test. Data related to lamb body weight, serum hormone concentrations, and antioxidant activity were analyzed using the General Linear Model (GLM) procedure in SAS. Statistical significance was established at P < 0.05. Baseline blood samples collected prior to intravaginal sponge insertion were used as covariates in analyses of hormonal profiles and antioxidant activity three weeks after ram exposure.

Results

Reproductive Traits and Production Performance of Lambs

The effects of E-Se on reproductive traits and lamb performance are presented in Table 1.

Table 1 Effects of vitamin E-Se injection in estrus-synchronized ewes on production performance of lambs up to 90 days of age

Performance of lambs	Experimental treatments		SEM	P-value
	Control	E-Se		
Number of mated ewes	100	100	-	-
Number of lambs born	82	111	-	-
Number of survived lambs	75	106	-	-
Lamb viability (%)	91.5	95.5	-	0.18
Number of lambs/ewes	0.82 ^b	1.11 ^a	0.06	0.02
Lamb live weight at birth (kg)	3.81	3.94	0.12	0.37
Lamb live weight at day 90 (kg)	17.3	18.6	0.94	0.25
Total weight gain (kg)	13.5	14.7	0.55	0.12
Average daily gain (g)	151.4	164.8	4.71	0.09

^{a,b} Means with different superscript letters in rows are significantly different, P < 0.05.

Lamb viability: survived lambs/lambs born alive × 100

The lambing rate in the group treated with vitamin E-Se was significantly higher (P <

0.05) than that observed in the control group (1.11 vs. 0.82, respectively). The percentage of

lamb viability in the group receiving vitamin E-Se did not differ significantly ($P > 0.05$) from that of the control group (95.5% vs. 91.5%, respectively). Findings indicated that administration of vitamin E-Se did not produce a statistically significant impact on lamb birth weight, weight at 90 days, overall weight gain (from birth to weaning), and average daily gain ($P > 0.05$).

Hormonal Profile

The effects of vitamin E-Se injection on hormonal profile in estrus-synchronized ewes are presented in Table 2. Administration of vitamin E-Se did not result in a significant alteration in estradiol-17 β levels compared to the control group ($P > 0.05$). Progesterone concentration in E-Se groups was greater ($P < 0.05$) than the control (5.48 vs. 4.15 ng/mL, respectively). The statistical analysis revealed that the mean serum T3 concentration was significantly ($P < 0.05$) higher in treatment group as compared to the control (1.81 vs. 1.34 nmol/l, respectively), but there were no significant treatment effects on serum T4 levels ($P > 0.05$; Table 2). The supplemented group exhibited a significantly reduced serum T4/T3 ratio ($P < 0.05$) compared to the control group (40.76 vs. 52.47, respectively; Table 2).

Antioxidant Activity and Selenium Concentration

Table 3 shows the effects of vitamin E-Se injection on antioxidant activity and selenium concentration in estrus-synchronized ewes. Although plasma malondialdehyde (MDA) levels and indicators of oxidative stress were not significantly influenced by the experimental treatments ($P > 0.05$), a decreasing trend was observed in the E-Se treated group relative to the control ($P = 0.08$; 1.94 vs. 2.45 nmol/mL, respectively). Total antioxidant capacity was significantly ($P = 0.01$) higher in the treatment group (605.70 μ mol/mL) compared to the control (518.31 μ mol/mL). Superoxide dismutase levels were not significantly affected by vitamin E-Se injection ($P > 0.05$), a tendency toward elevated SOD levels was observed in the E-Se treated ewes relative to the control group ($P = 0.07$; 1446.28 vs. 1254.06 U/gHb, respectively). The glutathione peroxidase concentration of ewes was significantly higher ($P = 0.03$) in the treatment group than in the control group (79.18 vs. 62.24 IU/gHb, respectively). No statistically significant difference was observed between groups in hemoglobin concentration ($P > 0.05$). In ewes that received vitamin E-Se, Se levels were greater ($P < 0.05$) than in control group (2.10 vs 1.27 μ mol/l) on the three weeks after ram exposure (Table 3).

Table 2 Effects of vitamin E-Se injection on hormonal profile in estrus-synchronized ewes

Hormonal profile	Experimental treatments		SEM	P-value
	Control	ESe		
Estradiol-17 β (pg/mL)	8.12	8.75	0.64	0.25
Progesterone (ng/mL)	4.15 ^b	5.48 ^a	0.32	0.01
T3 (nmol/L)	1.34 ^b	1.81 ^a	0.09	0.02
T4 (nmol/L)	70.28	73.50	3.23	0.32
T4/T3	52.47 ^b	40.76 ^a	2.84	0.008

T3: Triiodothyronine; T4: Thyroxine

a,b Means with different superscript letters in rows are significantly different, $P < 0.05$

Table 3 Effects of vitamin E-Se injection on antioxidant activity and selenium concentration in estrus-synchronized ewes

Parameters	Experimental treatments		SEM	P-value
	Control	E-Se		
MDA (nmol/mL)	2.45	1.94	0.27	0.08
TAC (μ mol/mL)	518.31 ^b	605.70 ^a	28.13	0.01
SOD (U/gHb)	1254.06	1446.28	92.73	0.07
GPx (IU/gHb)	64.24 ^b	79.18 ^a	5.36	0.03
Hb (g/dL)	13.82	14.55	0.91	0.25
Selenium (μ mol/L)	1.27 ^b	2.10 ^a	0.08	0.001

MDA: Malondialdehyde; TAC: total antioxidant capacity; SOD: superoxide dismutase; GPx: glutathione peroxidase; Hb: hemoglobin.

^{a,b} Means with different superscript letters in rows are significantly different, $P < 0.05$.

Discussion

In the present study, the significant increase in lambing rate observed in the vitamin E-Se treated group compared to the control group indicates a positive effect of antioxidant supplementation on reproductive efficiency in estrus-synchronized ewes. This finding aligns with previous studies demonstrating that vitamin E and selenium supplementation can enhance fertility outcomes by mitigating oxidative stress during critical reproductive phases. Vitamin E functions as a cellular antioxidant protecting lipid membranes from peroxidative damage, while selenium acts as an essential cofactor for glutathione peroxidase, an enzyme critical in detoxifying reactive oxygen species that otherwise impair folliculogenesis, ovulation, and corpus luteum function [12,13]. The lamb viability percentage was higher in the E-Se group, despite no significant difference being observed in the statistical analysis. This suggests that while antioxidant supplementation may contribute to slightly improved neonatal survival, other factors such as maternal care, environmental conditions, and genetic traits significantly influence lamb viability and may have overshadowed differences in this case. Similar observations were reported by studies in Yankasa and Awassi sheep, where selenium and vitamin E improved reproductive rates but neonatal mortality differences were non-significant

[14,15]. Selenium has been reported to improve uterine function and increase ovarian activity by reducing the incidence of ovarian cysts and enhancing uterine health, which in turn leads to an increased estrus rate [20]. It has been shown that selenium, by increasing the number of ovarian follicles and promoting nitric oxide production, enhances the stimulatory effect of gonadotropins on follicles. Additionally, selenium contributes to sperm transport and fertilization through increased myometrium and oviduct contractions, ultimately reducing the number of anestrous ewes [21]. The impact of vitamin E on ovulation rate and embryo-fetal survival has also been demonstrated in previous studies. Protection of gonadotropin receptors against oxidative damage can lead to enhanced estrogen secretion, and consequently improved fertility [22]. The increased lambing rate has been attributed to the stimulation of follicular growth and development and the associated rise in estrogen secretion following selenium treatment, which triggers strong positive feedback inducing a surge of luteinizing hormone (LH) [22]. These researchers concluded that ewes supplemented with selenium exhibited higher lambing percentages and greater lamb production efficiency compared to control groups [22]. In a study involving selenium and vitamin E deficient ewes, supplementation resulted in

increased lambing rates and twinning rates, as well as a reduction in anestrus ewes [23]. Furthermore, injectable barium selenate supplementation in ewes was reported to increase both the number of lambs born and their birth weights, while reducing embryonic mortality compared to controls [24]. Despite numerous reports indicating the positive effects of selenium and vitamin E on reproductive parameters, some investigations have found that supplementation with these nutrients does not produce significant changes in reproductive parameters. For instance, a study examining the effects of E-Se on Karacabey Merino ewes with low fertility found no significant differences among treatment groups in lambing rate, birth weight of lambs, and abortion rate [20]. In the experiment where female goats were administered 0.06 and 0.125 mg of selenium and 0.18 and 1.17 IU of vitamin E around the time of mating, no effects were observed on twinning rates, lamb production efficiency, and sex ratio [25]. Additionally, another study reported that three injections of vitamin E-selenium during estrus synchronization in Mehraban ewes did not influence fertility, prolificacy, lambing rate and lamb survival [12]. In this experiment, there was no significant influence of E-Se treatment on lamb birth weight or the production performance of lambs. However, lambs born to the group receiving vitamin E-selenium numerically exhibited higher average daily weight gain (Table 1). An association between low birth weight and reduced serum selenium concentration during early pregnancy has been reported [26]. It has been demonstrated that selenoproteins enhance the conversion of T4 to T3, thereby influencing maternal and fetal metabolism, ultimately leading to increased fetal weight [27]. Consistent with the results of the present study, several previous investigations have reported no significant effect of vitamin E-Se injection on lamb birth weight [10,20,22]. The average

daily gain of lambs in group E-Se was increased by 164.8 g and in control group by 151.4 g per day. Vitamin E and Se injection results in higher colostrum concentrations of these elements, and 1 month after parturition the treated ewes still have a higher milk concentration of these elements, therefore showing a 1-month carry-over effect [16]. In this study, the serum progesterone concentration three weeks after ram exposure was significantly increased in vitamin E-Se treatment compared to the control (Table 2). A previous study by Yildiz *et al.* [28] indicated that administering vitamin E-Se immediately prior to synchronization enhanced conception rate, antioxidant capacity, and plasma P4 concentration in cattle. Additionally, Kamada [29] reported that dietary supplementation with 0.5 ppm Se led to elevated plasma P4 levels in postpartum dairy cows. Farahavar *et al.* [12] also found that in vitamin E-Se supplemented ewes, the serum P4 levels were elevated in supplemented animals compared to those without supplementation, consistent with the findings of the present study. Progesterone is essential for fetal transfer from the oviduct to the uterus and for implantation, concurrently promoting increased endometrial thickness, histotroph secretion, and reduced uterine contractility [30]. It prepares the uterus for embryo implantation and supports pregnancy by supplying fetal nutrition. Across mammals, embryo loss during early gestation ranges from approximately 25% to 55%, with the majority attributed to corpus luteum (CL) dysfunction and insufficient progesterone production [30]. Reactive oxygen species (ROS) increase prostaglandin F2 α (PGF2 α) synthesis, and elevated PGF2 α concentrations can stimulate luteolysis and fetal loss [31]. ROS induce damage to luteinizing hormone (LH) receptors and impair the mitochondrial transport of cholesterol, thereby inhibiting progesterone biosynthesis [32]. Selenium enhances glutathione peroxidase (GPX)

activity in follicular fluid and reduces ROS levels [31]. A significant association exists between selenium-dependent glutathione peroxidase activity (specifically GPX-3) and the luteal capacity for progesterone production by the CL [32]. During luteal regression, an accumulation of hydrogen peroxide (H_2O_2) and lipid peroxides occurs within the corpus luteum, whereas antioxidant vitamins have been shown to mitigate ROS induced damage in cultured rat luteal cells [33]. Consequently, the antioxidant effect of E-Se on peroxide production could contribute to maintaining CL function. The present data demonstrated that the vitamin E-Se supplement groups had higher serum concentrations of T3 than the control group. This result is in agreement with other studies [34,35]. Many investigators agree with the present results and confirm the importance of Se in thyroid hormone metabolism in ewes [34]. Selenium is needed for the hepatic conversion of T4 to T3 and that type I iodothyronine deiodinase, identified as a selenocysteine containing enzyme, catalyzes deiodination of T4 to biologically active thyroid hormone T3. In this study, selenium supplementation also significantly influenced the T4/T3 ratio, resulting in lower values in supplemented lambs. It is known that selenium deficiency decreases hepatic 5'-deiodinase activity [35]. The reduced T4 to T3 ratio observed in the supplemented groups suggests an enhancement of 5'-deiodinase activity due to selenium supplementation. However, Kumar *et al.* [36] reported no changes in serum T3 and T4 levels, or T4/T3 ratios with supplementation of 0.15–0.3 mg Se/kg DM in lambs. Conversely, Rock *et al.* [37] found that dietary selenium in pregnant ewes increased plasma T3 and T4 concentrations but did not affect the T3 to T4 ratio, nor did selenium treatment alter thyroid hormone levels in newborn lambs. The results of current experiment demonstrate that the vitamin E-Se injection in estrus-synchronized

ewes significantly enhanced their TAC and GSH-Px activity. These findings are consistent with previous studies reporting that vitamin E and selenium supplementation improve antioxidant status by elevating enzymatic antioxidants such as GSH-Px, which plays a pivotal role in detoxifying peroxides and limiting oxidative damage during the reproductive period [12,38]. The antioxidant synergy of vitamin E, a lipid-soluble scavenger of ROS, and selenium, an essential cofactor for GSH-Px, likely contributed to the observed enhancement of TAC [39]. Although malondialdehyde (MDA) levels did not show a statistically significant decrease, the observed downward trend is consistent with other studies in which oxidative stress markers diminished or remained stable after antioxidant supplementation [40]. The tendency for increased SOD further complements the antioxidant system's improvement, indicating a broader enzymatic response. Vitamin E is commonly recognized for its role in fertility and participates in numerous selenium-dependent biological processes. Selenium contributes to the preservation of vitamin E levels and supports its stability within the plasma [41]. Vitamin E, a vital fat-soluble antioxidant in biological tissues, functions as a frontline defense against lipid peroxidation by scavenging free radicals and safeguarding cell membranes during the early phases of oxidative damage. Selenium plays a pivotal metabolic role in mammals through its incorporation into the active site of the selenoenzyme GSH-Px. Both selenium and vitamin E are essential micronutrients that function cooperatively, modulating a wide range of physiological processes, notably reproductive function [42]. Maintaining a balanced oxidative status is crucial during estrus synchronization and early pregnancy, as oxidative stress can compromise follicular development, fertilization, and embryo survival. The elevated GSH-Px levels in E-Se treated ewes

indicate improved cellular defense against oxidative stress, which may support better reproductive outcomes and fetal development [38]. The significant increase in plasma selenium after treatment confirms its bioavailability and incorporation, which is necessary for optimal selenoprotein function, including GSH-Px activity. The elevation of plasma selenium levels in treated ewes three weeks after ram exposure further supports efficient selenium uptake and its integration into antioxidant defense systems. Although superoxide dismutase (SOD) activity did not reach statistical significance, the observed trend toward higher SOD in the supplemented group indicates a favorable modulation of enzymatic antioxidants. The significant elevation in selenium aligns with findings from previous investigations [25,43]. In contrast, the administration of vitamin E-Se injections immediately following mating failed to elevate blood selenium concentrations in does [25] and during estrus synchronization and early pregnancy in ewes [15]. Variability among individual animals and potential confounding factors including nutritional status, parity, and environmental stressors should be accounted for in the interpretation of the results. The data imply that selenium incorporation into selenoproteins may occur efficiently in treated animals, reinforcing cellular defense mechanisms against oxidative stress.

Conclusion

It is concluded that the injections of vitamin E-Se in estrus-synchronized Moghani ewes on the days of sponge insertion and sponge withdrawal improved their reproductive performance by increasing lambing rate. Additionally, E-Se treatment improved antioxidant capacity and selenium levels in the ewes. Serum progesterone levels were significantly elevated post-mating, indicating a positive influence on luteal function. These findings suggest that administering vitamin E-

Se during estrus synchronization could enhance reproductive outcomes in ewes under field conditions.

Conflict of Interest

The authors declared no conflicts of interest in this work.

Authors' Contributions

Conceptualization: Vahid Vahedi; methodology: Vahid Vahedi and Morteza Behroozlak; statistical data analysis: Nemat Hedayat; investigation, Vahid Vahedi and Morteza Behroozlak; writing—original draft preparation: Vahid Vahedi; writing—review and editing: Vahid Vahedi, Morteza Behroozlak, and Nemat Hedayat; supervision: Vahid Vahedi; funding acquisition: Vahid Vahedi and Morteza Behroozlak. All authors have read and agreed to the published version of the manuscript.

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