



## Reproductive Performance and Physiological Responses in Awassi Ewes Under Intravaginal Sponges Application and Fed Selenium and Vitamin E

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### ABSTRACT

This study was carried out to investigate the impact of feeding selenium (Se) and vitamin E on the physiological and reproductive performance of 18 multiparous Awassi ewes through the breeding season. The ewes received three levels of oral selenium-vitamin E for 60 days until the study was completed. The experimental ewes were assigned into three groups; the control group (SE0) received 2 mL of sterile saline per head, the second group (SE1) received 1.5 mL/head Se and vitamin E (150 mg  $\alpha$ -Tocopherol acetate + 0.15 mg Sodium selenite), while the third group (SE2) received 3 mL/head (150 mg  $\alpha$ -Tocopherol acetate + 0.15 mg Sodium selenite). The SE1 and SE2 groups demonstrated significant highest ( $p < 0.05$ ) rate of conception and overall pregnancy. The study results showed the lambing rate in the SE2 group increased significantly ( $p < 0.05$ ) compared to the other groups. All groups had low plasma progesterone levels on day 14, even though concentrations increased in SE0 and SE1, which had higher progesterone levels than SE2. SE1 had the highest estrogen concentration on day 16 compared to SE0 and SE2. On day 14, the SE0 group had a significantly lower ALT enzyme compared to the SE1 and SE2 groups. SE1 had significantly lower mean corpuscular volume (MCV), white blood cells (WBC), and platelets (PLT) concentrations than the other two groups. Overall, the estrus synchronization program and the administration of Se and vitamin E resulted in a significant improvement in pregnancy and lambing rates among Awassi ewes. Physiological and reproductive potential of sheep can be increased by improving their diet throughout the period of reproduction by including Se and vitamin E.

**Keywords:** Awassi ewes; intravaginal sponges; selenium; sexual hormones; vitamin E

### INTRODUCTION

The constant increase in human population and consumption means that the ruminant industry is insufficient to meet local demand. Although sheep are important to the world's food supply, the reproductive habits of different sheep breeds vary with the changing of the seasons, latitude/longitude, the length of the photoperiod, and other environmental factors (Carvajal-Serna *et al.*, 2021; Saeed *et al.*, 2023a; van Rosmalen *et al.*, 2022). According to a number of studies, most breeds of sheep are asexual from late June through early September (Ozyurtlu *et al.*, 2010; Tabbaa *et al.*, 2018; Talafha & Ababneh, 2011). Sheep farmers have responded by adopting cutting-edge artificial reproduction methods like estrus synchronization. Synchronizing ewes' estrus cycles is an intriguing method for improving their fertility. Reproductive efficiency is increased by estrus synchronization, and production gains are realized through the ability to

choose when births occur (Hasani *et al.*, 2018). Although excess or deficiency of energy and protein can be seen in the body condition and weight of the animals, vitamins and mineral imbalances are not always as obvious (Asin *et al.*, 2021). However, deficiencies and micronutrient imbalances are often only discovered after health issues have already been manifested (Masters, 2018). The rumen environment and the activity of rumen microbes that convert selenium (Se) to an insoluble form reduce the absorption potential of Se in ruminants compared to non-ruminants (Xun *et al.*, 2012). Compared to the nearly 85% absorbed by non-ruminants, Se absorption is only around 35% in ruminants (Galbraith *et al.*, 2016). Intestinal absorption of Se occurs in the same location for both classes. According to several studies, ewes that give birth to lambs in the autumn may be susceptible to Se and vitamin E deficiency. This is due to the heightened requirements for these necessary nutrients throughout gestation and the inadequate levels of these nutrients found in grain supplements, dry pasture,

and crop stubbles (Farahavar *et al.*, 2020; Sterndale *et al.*, 2018; Yazlik *et al.*, 2020). Selenium has been linked to an increase in ovarian structures, ovarian size, and the number of corpora lutea, all of which should be reflected in greater fertility because it is believed that Se helps keep the corpus luteum functioning normally (Cabrera-Mora *et al.*, 2019). In this regard, vitamin E optimizes and enhances the immune response by virtue of its potent lipid-soluble antioxidant activity, which prevents lipid peroxidation and eliminates free radicals (both of which are immunosuppressive and cause cell and tissue damage) (Cabrera-Mora *et al.*, 2019; Wu *et al.*, 2019). The impact of Se and vitamin E supplementation on the reproductive performance of sheep subjected to intravaginal sponges remains uncertain. Therefore, this study was conducted to examine the influence of Se and vitamin E on the physiological response, reproductive performance, and hormonal profile of sheep that were administered intravaginal sponges.

## MATERIALS AND METHODS

### Ethics Approval

The research was approved by the Committee of Research Ethics, which established guidelines for animal experiments (Approval No. 674/2024). The ethical standards established by Anbar University were strictly complied with in all experiments performed during this research.

### Experimental Design

The study was conducted at a privately owned sheep farm located in the Anbar Province of Iraq (33°00'0.00" N 41°45'0.00" E). The data of meteorological measures during the study period were obtained from the Meteorology Station, indicating that the average daily temperature and humidity were 30 °C and 23%, respectively. Eighteen (n=18) multiparous Awassi ewes (mean body weight: 26±0.78 kg), with age 3±0.7 years and with a mean body score of three (on a scale of one to five), were included in the research, which was carried out from the breeding season in May to June. The ewes were housed in groups under a roof in an open-sided barn. The ewes were allowed to graze in the pasture through the daytime and were housed in the farm's housing facilities at night. During the study, ewes were fed a 60:40 ratio of commercial concentrate to roughage. At all times, water was provided *ad libitum*.

### Animal Management and Treatments

The ewes were all checked for abnormalities in their reproductive systems and for pregnancies using ultrasonography before the trial began. In addition, three rams were utilized for breeding purposes and were in good health, devoid of any major contagious ailments. All ewes were treated to the procedure of estrus synchronization using intravaginal sponges (Spongovet/Hipra, Spanish), impregnated with 40 mg of progesterone for 14 days with 400 intramuscular IU

eCG, 24 hours before sponges were removed. The ewes were randomly allocated into three groups, each with six ewes with similar body weights and ages. The ewes were treated orally on three levels of mixture Se plus vitamin E for 60 days. The first group (SE0) was used as a control and was given 2 mL of sterile saline solution per head. The second group (SE1) was administered with Se and vitamin E at a level of 1.5 mL/head (containing 150 mg of  $\alpha$ -Tocopherol acetate + 0.15 mg of Sodium selenite), whereas the third group received 3 mL/head (containing 150 mg of  $\alpha$ -Tocopherol acetate + 0.15 mg of Sodium selenite).

### Blood Sampling

Blood samples (5 mL each) were obtained via the jugular vein of all ewes into plain vacutainer tubes (BD Franklin Lakes NJ, USA). The first blood sample was taken at the beginning of the study (0 day); the second blood sample was collected at the time of intravaginal sponge removal (14<sup>th</sup> day); the third blood sample was taken on day of insemination (16<sup>th</sup> day) and for hematology determination 1 mL tubes containing EDTA (Ethylenediamine tetra acetic acid). The serum was centrifuged at 2500 rpm for 15 minutes, then transferred to 1.5 mL Eppendorf tubes and kept at -20 °C until analysis. The concentrations of estradiol and progesterone in the blood were determined using commercial ELISA kits (Demeditec Diagnostics GmbH, Kiel-Wellsee, Germany). An ELISA (enzyme-linked immunosorbent assay) technology from the Immunotech – Beckman Coulter Company was used to detect IgG in blood serum. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using commercially available diagnostic kits from Roche Diagnostics GmbH in a Roche Hitachi Diagnostic Modular Analyzer P-800 (D-68298, Mannheim, Germany). A hematology analyzer was used to assess hematological parameters (CELL- DYN 3700 Abbott, USA).

### Reproductive Performance

All animals were monitored for estrus signals every 12 hours using a teaser ram for three consecutive days after intravaginal sponge removal. Estrus response (%), time to onset of estrus (h) (estimated as the interval (h) from the removal of the intravaginal sponge to the time when the ewe starts to express standing estrus), duration of estrus (h) (from the start of standing estrus to the end of sexual receptivity), and pregnancy/ conception rate (%) were all evaluated within the study. After parturition, the litter size was noted, and further parameters including the pregnancy, lambing, and mortality rates (lamb death after birth) as well as the mastitis rates, were measured (Kuru *et al.*, 2022). The conception and pregnancy rate were calculated as follows:

Conception rate (%) = (number of ewes that conceived)/ (number of inseminated ewes) × 100

Pregnancy rate (%) = (number of pregnant ewes)/ (number of mated ewes) × 100

The rate of vaginal discharge was measured in ewes that exhibited vaginal discharges after the removal of an intravaginal sponge. This was done to evaluate the quantity, purulence, and odor of the discharge in the ewes.

The mastitis detection was performed using the DeLaval cell counter (DeLaval International AB, Sweden) by sampling 2 mL of milk in a sterile tube from each half udder of the animal.

**Statistical Analysis**

The samples were statistically analyzed in triplicate, and the results were calculated and presented as means of averages utilizing the analysis of variance (ANOVA) technique with Statistical Analysis Software (version 9.4) for a completely randomized design. The reproductive variables were evaluated using a chi-squared test to examine descriptive data. The analysis of variance and Duncan’s multiple range tests was used, while a statistically significant difference (p<0.05) was identified between the treatments. The subsequent model was employed to assess the differences among treatments:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where  $Y_{ij}$  is the dependent variable,  $\mu$  is represented overall mean,  $\alpha_i$  is the fixed effect for levels of mixture Se plus vitamin E (1.5 and 3 mL/head), and  $e_{ij}$  is the experimental error assumed to be NID with (0, 2e).

**RESULTS**

The study results indicated that increased administrations of Se and vitamin E showed the effect on the diverse physiological and biochemical characteristics of female sheep. Table 1 shows the alterations in the values of liver enzymes, specifically AST and ALT, in response to various treatment interventions. No significant impact was observed on the liver AST enzyme activity across all periods for experimental groups. The results of the current study

showed that the activity level of the ALT enzyme was significantly lower in the SE0 group, while it was higher (p<0.05) in both the SE1 and SE2 groups on day 14 of the study period. However, the level of IgE was significantly increased (p<0.05) on the 14th and 16th days in the SE2 group compared to the SE0 and SE1 groups.

The evaluation of hemolytic variables in the ewes revealed that as Se and vitamin E administration increased, there was a linear decrease (p<0.05) in the red cell distribution width (RDW), red blood cells (RBC), and mean platelet volume (MPV) in SE2 (Table 2). The SE1 groups exhibited significantly reduced concentrations of Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), and platelet (PLT) in comparison to the other two groups (p<0.05). The concentration of lymphocytes in the SE2 group was considerably greater (p<0.05) than in the SE0 and SE1 groups. The procalcitonin (PCT) concentrations in the SE0 and SE2 groups were lower significantly (p<0.05) than SE1. There were no significant differences in hemoglobin (Hb), packed cell volume (PCV), or mean corpuscular hemoglobin (MCH) that were statistically significant between the SE0, SE1, and SE2 groups.

The progesterone ( $P_4$ ) and estradiol ( $E_2$ ) concentrations in the serum of the ewes on days 0, 14, and 16 after the treatment indicated that days 14 and 16 did not exhibit any discernible impact on any of the three treatment groups (Table 3). While  $P_4$  concentrations increased on day 14<sup>th</sup>, the average serum  $P_4$  levels remained low across all groups. SE0 and SE1 exhibited elevated  $P_4$  concentrations in comparison to SE2. The concentration of  $E_2$  increased in SE1 on day 16, but remained unchanged between SE0 and SE1.

There were no significant changes detected in the colors of the mucus, whether purulent or healthy (Table 4). A significant variance (p<0.05) was shown among the SE0, SE1, and SE2 groups regarding mucus quantity. The SE2 group exhibited the highest average quantity of 0.8 mL, followed by 0.7 mL for the SE0 group and 0.5 mL for the SE1 group (Table 4).

Table 1. Serum biochemical concentration of Awassi ewes administrated with selenium and vitamin E

Variables	Treatments			SEM	Probability
	SE0	SE1	SE2		
AST (U/L)					
0 Day	93.50	88.50	103.00	5.25	0.585
14 Day	103.50	97.50	81.00	5.92	0.310
16 Day	77.50	81.00	88.50	3.49	0.482
ALT (U/L)					
0 Day	27.50	21.50	22.50	1.43	0.194
14 Day	21.50 <sup>b</sup>	25.50 <sup>a</sup>	24.50 <sup>a</sup>	0.70	0.019
16 Day	37.50	25.50	23.00	2.86	0.058
IgE (IU/mL)					
0 Day	2.68	4.25	4.39	0.64	0.552
14 Day	1.88 <sup>b</sup>	2.71 <sup>b</sup>	5.04 <sup>a</sup>	0.54	0.015
16 Day	2.64 <sup>b</sup>	1.38 <sup>b</sup>	3.86 <sup>a</sup>	0.41	0.019

Note: SE0= control group, SE1= Administrated with Se and vitamin E at 1.5 mL/head, SE2= Administrated with Se and vitamin E at 3 mL/head. <sup>a,b</sup>Means in the same row with different superscripts differ significantly at p<0.05. AST= Aspartate aminotransferase, ALT= Alanine transaminase, IgE= Immunoglobulin E.

Table 2. Hematological parameters of Awassi ewes administration with selenium and vitamin E after 16th day of treatment

Variables	Treatments			SEM	Probability
	SE0	SE1	SE2		
RBC ( $10^6/\mu\text{L}$ )	9.02 <sup>b</sup>	9.59 <sup>a</sup>	7.69 <sup>c</sup>	0.28	0.001
Hb (g/dL)	8.30	8.40	8.16	0.05	0.263
PCV (%)	27.00	26.45	27.56	0.23	0.147
MCV (fL)	30.70 <sup>b</sup>	27.25 <sup>c</sup>	35.06 <sup>a</sup>	1.13	0.001
MCH (pg)	2.02	2.10	2.06	0.28	0.980
MCHC (g/dL)	9.20 <sup>b</sup>	8.60 <sup>c</sup>	10.26 <sup>a</sup>	0.25	0.002
RDW (%)	30.20 <sup>b</sup>	32.30 <sup>a</sup>	29.70 <sup>c</sup>	0.40	0.001
WBC (cells/ $\mu\text{L}$ )	16.50 <sup>a</sup>	11.60 <sup>c</sup>	14.06 <sup>b</sup>	0.70	0.001
Lymphs (%)	51.40 <sup>c</sup>	58.60 <sup>b</sup>	62.70 <sup>a</sup>	1.65	0.001
PLTs ( $10^3/\mu\text{L}$ )	17.70 <sup>a</sup>	15.10 <sup>c</sup>	17.06 <sup>b</sup>	0.39	0.001
MPV (fL)	428.00 <sup>b</sup>	568.66 <sup>a</sup>	203.50 <sup>c</sup>	53.17	0.001
PCT (mL/L)	5.70 <sup>b</sup>	6.25 <sup>a</sup>	5.70 <sup>b</sup>	0.11	0.032

Note: SE0= control group, SE1= Administrated with Se and vitamin E at 1.5 mL/head, SE2= Administrated with Se and vitamin E at 3 mL/head. <sup>a,b,c</sup> Means in the same row with different superscripts differ significantly at p<0.05. RBC= Red blood cells, Hb= Hemoglobin, PCV= Packed cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, RDW= Red cell distribution Width, WBC= White blood cells, Lymphs= Lymphocytes, PLTs= Platelets, MPV= Mean platelet volume, PCT= Procalcitonin.

Table 3. Reproductive hormone concentrations of Awassi ewes administration with selenium and vitamin E

Variables	Treatments			SEM	Probability
	SE0	SE1	SE2		
Progesterone (ng/mL)					
0 Day	1.24	1.35	1.28	0.01	0.062
14 Day	1.36 <sup>a</sup>	1.42 <sup>a</sup>	1.27 <sup>b</sup>	0.02	0.006
16 Day	1.48	1.17	1.25	0.12	0.702
Estradiol (pg/mL)					
0 Day	35.77	46.89	29.53	2.92	0.115
14 Day	38.84	34.98	34.55	2.09	0.715
16 Day	178.03 <sup>b</sup>	270.90 <sup>a</sup>	175.70 <sup>b</sup>	18.09	0.002

Note: SE0= control group, SE1= Administrated with Se and vitamin E at 1.5 mL/head, SE2= Administrated with Se and vitamin E at 3 mL/head. <sup>a,b</sup> Means in the same row with different superscripts differ significantly at p<0.05.

Table 4. Mucus vaginal discharge traits of Awassi ewes with administration of selenium and vitamin E

Treatments	Mucus color		Mucus quantity (mL)
	Purulent	Healthy	
SE0	3	3	0.7±0.02 <sup>ab</sup>
SE1	1	5	0.5±0.01 <sup>b</sup>
SE2	2	4	0.8±0.05 <sup>a</sup>
Probability	0.603	0.776	0.027
x <sup>2</sup> value	1.011	0.506	--

Note: SE0= control group, SE1= Administrated with Se and vitamin E at 1.5 mL/head, SE2= Administrated with Se and vitamin E at 3 mL/head. <sup>a,b</sup> Means in the same column with different superscripts differ significantly at p<0.05.

There were no significant differences among the three groups in estrus response, as indicated in Table 5. The impact of Se and vitamin E administration on the duration of estrus response in Awassi ewes remained unaffected.

The study examines the effects of administering Se and vitamin E to Awassi ewes for 60 days, specifically focusing on their reproductive performance, as presented in Table 6. The SE1 and SE2 groups exhibited the highest (p<0.05) conception and total pregnancy rates. The lambing rate in the SE2 group was significantly

Table 5. Time of estrus response in Awassi ewes with administration of selenium and vitamin E

Treatments	Time of estrous response		
	12 h	24 h	36 h
SE0	3	3	0
SE1	3	3	0
SE2	5	1	0
Probability	0.692	0.561	-
x <sup>2</sup> value	0.735	1.155	-

Note: SE0= control group, SE1= Administrated with Se and vitamin E at 1.5 mL/head, SE2= Administrated with Se and vitamin E at 3 mL/head.

higher (p<0.05) than in the other groups. The treatment groups revealed the most minimal rates of mortality and mastitis. However, no statistically significant disparities were noted among the groups regarding estrus response, time to onset, and duration of estrus.

DISCUSSION

The supplementation of different amounts of Se and vitamin E did not result in any notable variations in serum AST levels. This result suggests

Table 6. Reproductive performance of Awassi ewes with administration of selenium and vitamin E

Variables	Treatments			Probability	$\chi^2$ value
	SE0	SE1	SE2		
Estrus response (%)	100	100	100	1.00	0.00
Time to onset of estrus (h)	36	36	36	1.00	0.00
Duration of estrus (h)	36	36	36	1.00	0.00
Conception rate (%)	83 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	0.03	5.93
Total pregnancy rate (%)	83 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	0.03	5.93
Lambing rate (%)	83 <sup>b</sup>	83 <sup>b</sup>	100 <sup>a</sup>	0.03	5.86
Litter size	1.33 <sup>a</sup>	1.00 <sup>b</sup>	1.25 <sup>b</sup>	0.02	---
Mortality (%)	16.67 <sup>a</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0.03	5.93
Mastitis (%)	16.67 <sup>a</sup>	16.67 <sup>a</sup>	0 <sup>b</sup>	0.03	5.86

Note: SE0= control group, SE1= Administrated with Se and vitamin E at 1.5 mL/head, SE2= Administrated with Se and vitamin E at 3 mL/head. <sup>a,b</sup>Means in the same row with different superscripts differ significantly at  $p < 0.01$ .

that the quantities of Se and vitamin E administered in this research did not negatively impact the liver's normal functioning. Similarly, sheep that received Se supplementation showed an unchanged result in serum AST enzyme activity (Soliman, 2015). The supplementation of Se and vitamin E to sheep did not significantly affect ALT levels at day 0. However, the majority of these values fell within the normal range. Animals that receive doses of Se and vitamin E (SE1) over an extended period exhibit lowered ALT enzyme activity. The interesting outcome could be clarified by the fact that the decrease in ALT activity observed on day 14 might be attributed to Se involvement in removing hydrogen peroxide. This elimination process leads to tissue degradation, aided by the enzyme glutathione peroxidase. As a result, a decline in ALT enzyme activity was noticed in the blood serum (Ali *et al.*, 2023). Se and vitamin E administration displayed a relationship with serum IgE levels at SE2. No similar findings were observed at SE1. These associations can partly be explained by sheep having IgE levels showcasing a stronger immune response against parasites within their uterine cavity (Silva *et al.*, 2012; Shareef *et al.*, 2023). This finding is consistent with that of Alba-Hurtado & Muñoz-Guzmán (2013), who reported that increased IgE levels are linked to immunity against gastroenteric nematodes in sheep and goats.

A study showed that the combination of Se and vitamin E had no effect on the levels of Hb, PCV or MCH in sheep (Khalili *et al.*, 2019). These results reflect those of Novoselec *et al.* (2022), which also reported no impact of Se and vitamin E on these indicators. However, in this study, the group SE2 values for MCV and MCHC were higher than the control group. Acute infections often lead to an increase in reactive lymphocytes, which can be observed during the recovery period (Khalili *et al.*, 2019). In the present study, there was a decrease in the number of RBC in SE2 group, which contradicted studies that suggested lambs fed a diet containing 0.3 ppm Se in dry matter as Se yeast experienced both an increased quantity and resilience of RBC (Faixova *et al.*, 2007). The observed alterations in the parameters of lymphocytes, PLT, PCT, and MPV in the current investigation fell within

the established physiological value ranges that are considered normal for sheep (Soliman, 2015). Therefore, the post-supplement response of blood RBCs and PCV levels may be enhanced by Se and vitamin E supplementation.

The pattern of serum  $P_4$  profiles was observed when ewes were fed on Se and vitamin E treatments. However, within 48 hours (on day 16), the average serum  $P_4$  concentration decreased significantly after removing the device. Comparing the levels of Se and Vitamin E in groups SE0 and SE2 with the findings of Kuru *et al.* (2017) reveals that Se therapy in ewes could potentially be advantageous. This therapy may enhance fertility rates before estrus synchronization with progesterone, particularly in regions where Se deficiency is prevalent. These results corroborate the findings of a great deal of the previous work on using intravaginal devices to decrease  $P_4$  levels over extended periods promoting negative conditions that contribute to reduced fertility (Silva *et al.*, 2020). The current study demonstrated that an increase in serum  $E_2$  levels in SE1 on day 16<sup>th</sup> after intravaginal sponge's withdrawal might lead to the synchronization of a new follicular wave, which consists of potential ovulatory follicles. These results are in agreement with Hashim & Sembiring (2013), who reported that the concentration of  $E_2$  showed a positive correlation with the quantity of large follicles observed during the estrus cycle. The  $E_2$  is widely regarded as an effective indicator of follicle qualities.

The study results suggested that varying doses of oral Se and vitamin E supplements can impact the properties of vaginal mucus secretion. This result differed from others, who demonstrated that approximately 80% of the sheep treated with traditional long-term treatments (lasting 14 days) exhibited purulent and bloody vaginal discharges (Martinez-Ros *et al.*, 2018). A possible explanation for this might be Se and vitamin E are linked to blood alkalinity; elevated vaginal pH increases vulnerability to infections, which may also have an indirect impact on fertility. This finding was contrary to that of Mansoor *et al.* (2020), who reported that following the removal of sponges from ewes, there were signs of vaginal inflammation characterized by the secretion of mucus accompanied

by an unpleasant odor. The quantity of mucus increased gradually between treatment groups. Due to its intravaginal sponge-like properties, it effectively retained vaginal secretions without adhering to the mucus of the vaginal wall (Swelum *et al.*, 2019). There are several possible explanations for this result, but the quantity, composition, and physical characteristics of vaginal mucus may influence the semen that travels through the cervix.

The utilization of intravaginal sponges and the administration of Se and vitamin E proved highly effective as P<sub>4</sub> synchronizing agents in this study. The concurrent utilization of Se and vitamin E (SE1 and SE2), along with P<sub>4</sub> has been proven to improve the proportion of sheep displaying a response and timing of estrus. The level of P<sub>4</sub> in the bloodstream significantly increases after the insertion of sponges. Then, it decreases swiftly upon removal without an accumulative effect (Swelum *et al.*, 2019). In the study by Cadena-Villegas *et al.* (2018), no significant differences in estrus response were observed among sheep.

The reproductive performance of ewes shows variable enhancements when supplemented with Se and vitamin E. Several authors have noted this phenomenon (Daghigh Kia *et al.*, 2019; Mohammed *et al.*, 2020; Saeed *et al.*, 2023b). Various factors influence the variation of reproductive performance, including the type of supplement, dosage, method of administration, timing, frequency of supplementation, and the initial Se and vitamin E status of the ewes (Awawdeh *et al.*, 2019). The present study results showed that Se and vitamin E administration enhances conception rates in sheep. A previous study reported that vitamin E and Se supplemented for a period there is an enhancement in pregnancy rates due to the increased concentration of the  $\beta$  carotene molecule in tissues (Özar *et al.*, 2022). This finding was also reported by Naikoo *et al.* (2016), where elevated levels of vitamin E improve antioxidant activity, creating a conducive environment for implantation and embryonic development in the uterine as a result of enhancement pregnancy rates and fertility. Additionally, research has shown that administering hormone injections containing vitamins and minerals to sheep during mating does not affect pregnancy or lambing rates (Gonzalez-Rivas *et al.*, 2023). The most obvious finding to emerge from the analysis is that SE0 only appeared in the mortality rate among other groups, which might be due to a lack of some trace element such as Se or Cu that can support the immunity of the body's against diseases. Inadequate levels of these elements can hinder ovulation, leading to embryo loss and possibly even mortality (Kumbhar *et al.*, 2021). A significant finding of this research was that supplementing Se and vitamin E did not impact reproductive factors. This result aligns with Ziaeis (2015) study, which found that giving goats either Se or vitamin E separately did not cause any changes in their parameters. It is widely acknowledged that Se and vitamin E levels during parturition can influence the performance of ewes, which may have implications for the health of the udder. It seems possible that these results are indicated by the supplementation of 1000 IU of vitamin E and

2.5 mg of Se per 1 kg of dry matter, resulting in a 5% increase in milk production in cows (Netto *et al.*, 2022).

## CONCLUSION

The reproductive performance of Awassi ewes was enhanced by administering Se and vitamin E alongside the estrus synchronization program, resulting in improved immune body response and hormones related to the reproductive performance, especially IgE, progesterone, and estrogen, with a consequent enhancement in their lambing rate compared to the control group. Supplementing ewes with Se and vitamin E at level 3 mL/head in areas where the breeding season aligns with animal grazing can enhance their physiological response and reproductive performance.

## CONFLICT OF INTEREST

A. A. Samsudin serves as an editor of the *Tropical Animal Science Journal* but has no role in the decision to publish this article. The authors also certify that there are no conflicts of interest with any other issue related to this study.

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