



# Does Vitamin E or Vitamin E plus Selenium improve reproductive performance of rams during hot weather?

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## ABSTRACT

The objective of this study was to determine the effect of Vitamin E (Vit E) and Selenium (Se) on semen quality, sexual activity, packed cell volume (PCV), and white blood cell counts (WBC) of Awassi rams during the hot season.

Twelve Awassi rams were subdivided into three groups and treated for 90 days. Rams in the 1<sup>st</sup> group (T1) were treated twice weekly with 175 mg/ram vitamin E at intervals of 12h; rams in the 2<sup>nd</sup> group (T2) were treated on the same schedule with 70 mg/ram vitamin E plus 2800 mg selenium; and rams in 3<sup>rd</sup> group (T3) served as controls. Sperm quality (percentage of motile cells and percentage of morphologically normal cells) and quantity (sperm volume, and concentration) were recorded weekly. Sexual activity was tested by using the pen libido test at monthly intervals. Blood samples were taken before treatment and after the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> months. Semen quality was significantly affected by treatments: the ejaculate volume increased in T2 vs T1, and T3; mass activity and individual motility were increased in T1 and T2 vs T3. Sperm concentration was increased in T2 and T1 vs T3, and the percentage of dead and abnormal spermatozoa was reduced in T1 and T2 vs T3, though the differences were not statistically significant. Pen libido test showed reduced reaction time for the first mount in the 1<sup>st</sup> month in T2 and T1 vs T3. The number of serves was increased in groups T1 and T2 vs T3. There were no significant differences in PCV among groups. After 3 months from the beginning of treatments, the percentage of lymphocytes increased in T1 and T2 vs. T3, while the percentage of neutrophils was reduced in T2 vs T3 in the 2<sup>nd</sup> and 3<sup>rd</sup> months.

The results of this experiment indicate that treatments with vitamin E alone or in combination with selenium improved semen characteristics and reproductive performance of Awassi rams during the hot season.

*Key words:* Rams, Reproductive Performance, Selenium, Semen Characteristics, Vitamin E.

## RIASSUNTO

## PUÒ LA VITAMINA E, DA SOLA O CON SELENIO, MIGLIORARE LE PRESTAZIONI RIPRODUTTIVE DEGLI ARIETI DURANTE LA STAGIONE CALDA?

*Lo scopo di questo studio è stato quello di determinare l'effetto di Vitamina E (Vit E) e Selenio (Se) su qualità del seme, attività sessuale, valore ematocrito (PCV) e conteggio di leucociti (WBC) in arieti Awassi durante la stagione calda.*

*Dodici arieti Awassi vennero suddivisi in tre gruppi e sottoposti a trattamento per 90 giorni. Gli arieti del 1° gruppo (T1) furono trattati due volte alla settimana con 175 mg/ariete di vitamina E con un intervallo di 12h, gli arieti del 2° gruppo (T2) furono trattati allo stesso modo con 70 mg/ariete di vitamina E e 2800 mg di selenio, mentre gli arieti del 3° gruppo (T3) servirono da controllo. La qualità del seme (percentuale di cellule mobili e percentuale di cellule morfologicamente normali) e la quantità (volume e concentrazione spermatica) vennero registrate settimanalmente. L'attività sessuale venne valutata tramite "pen libido test" a cadenza mensile. Campioni di sangue vennero prelevati prima del trattamento e dopo il 1°, 2° e 3° mese. La qualità del seme venne significativamente influenzata dai trattamenti, con incremento del volume di eiaculato in T2 vs T1 e T3, di attività massale e di motilità individuale in T1 e T2 vs T3. La concentrazione spermatica incrementò in T2 e T1 vs T3, e la percentuale di spermatozoi morti e anormali si ridusse in T1 e T2 vs T3, sebbene le differenze non siano risultate statisticamente significative. Il "pen libido test" mostrò un ridotto tempo di reazione alla prima monta nel 1° mese in T2 e T1 vs T3. Il numero di accoppiamenti incrementò nei gruppi T1 e T2 vs T3. Non vi furono significative differenze tra i gruppi per quanto concerne il PCV. Dopo 3 mesi di trattamento, la percentuale di linfociti risultò aumentata in T1 e T2 vs T3, mentre la percentuale di neutrofili si ridusse in T2 vs T3 nel 2° e 3° mese.*

*I risultati di questo esperimento indicano che i trattamenti con vitamina E, da sola o in combinazione con selenio, migliorano le caratteristiche del seme e la performance riproduttiva di arieti Awassi durante la stagione calda.*

Parole chiave: Arieti, Prestazioni Riproduttive, Selenio, Caratteristiche Seminali, Vitamina E.

**Introduction**

Sheep are important animals in many countries because they can be successfully raised under harsh conditions and cost relatively little to maintain. The Awassi sheep is the most numerous and widespread type of sheep in southwest Asia, and has admirably adapted to the rigorous conditions found there. The libido and semen quality vary with season, and usually decline from May through August when the climate is dry, very hot and skies are cloudless.

Vitamin E (Vit E) and Selenium (Se) are two of the important nutrients that can affect several biological processes including spermatogenesis and semen quality (Marin-Guzman *et al.*, 1997; Yousef *et al.*, 2003), reproduction (Koyuncu and Yerlikaya, 2007), metabolism (Awadeh *et al.*, 1998), immunity

(Hernken *et al.*, 1998), and protecting against oxidative stress (Bernabucci *et al.*, 2002).

Selenium (Se) is an essential dietary trace element and is always of research interest because it is required for the maintenance of male fertility. This element is also required for testosterone biosynthesis and the formation and normal development of spermatozoa (Brown and Arthur, 2001). Both testis and epididymis require exogenously supplied Se in order to synthesize a variety of known selenoproteins, whose precise role in spermiogenesis and post testicular sperm maturation are not clearly defined. In many areas of the world plants do not provide levels of this element adequate to meet dietary requirements (Hogan *et al.*, 1993). Insufficient dietary Se leads to oxidation, rupture of muscle membranes and leakage of cellular enzymes into circulation, and the animals differ in their re-

quirements for Se and in their susceptibility. Moreover, deficiency in Vit E and Se in sheep flocks can lead to stiff lamb disease or nutritional muscular dystrophy.

Vitamin E compounds in plant tissues are a mixture of four tocopherols and four tocotrienols, both forms in the "D" configuration, of which D- $\alpha$ -tocopherol (RRR-  $\alpha$ -tocopherol) has the highest biological activity in animals. These eight isomers constitute the Vit E product commonly known as DL- $\alpha$ -tocopherol, also denoted as all *rac*- $\alpha$ -tocopherol (Mahan *et al.*, 2000). The association of Vit E deficiency with impaired male reproduction was established more than three decades ago, and traditionally it is called the "Anti-sterility Vitamin". Nutritional deficiency of Vit E has also been linked to liver necrosis in swine and rats, foetal resorption in rats, and encephalomalacia in poultry.

Furthermore, many studies have been investigated for their effects on immunological responses in various animal species. In mice and chickens, Spallholz *et al.*, (1975) demonstrated that both dietary and injectable Se enhanced the primary immune response, and increased serum IgG antibody titers in mice supplemented with 60 to 180 mg Vit E/kg diet and antigenically challenged with sheep red blood cells (RBC) or tetanus toxoid. Similarly, Smith *et al.* (1979) reported the direct evidence that Vit E and Se are related to mammary health. Vit E has been implicated in stimulation of antibody synthesis, particularly IgG. Reffett *et al.* (1988) demonstrated that Se supplementation of lambs enhanced serum IgM ( $P < 0.05$ ) and mean immunoglobulin concentration by increasing the number of IgM-producing cells, and Vit E supplementation appeared to stimulate the secondary immune response, generally associated with IgG antibody production.

Several researchers supported the hypothesis that seasonal alterations of the oxidative status depend more on weather

conditions such as temperature and humidity (Bernabucci *et al.*, 2002). Oxidative stress resulting from increased production of free radicals and reactive oxygen species, and/or a decrease in antioxidant defences, leads to damage of biological macromolecules and disruption of normal metabolism and physiology, and when reactive forms of oxygen are produced faster than they can safely be neutralized by antioxidant mechanisms, oxidative stress results (Tervisan *et al.*, 2001). In response to heat stress, rams secrete ACTH from the pituitary gland which causes increased adrenal cortisol release into the circulation, and the stress augments the autonomic nervous system release of norepinephrine and the adrenal release of norepinephrine and epinephrine into circulation. Studies on the effect of heat stress on oxidative status in ovine are lacking.

Sconberg *et al.* (1993) demonstrated that stressed rams or rams given a stress treatment of ACTH and epinephrine injections were found to have reduced circulating neutrophils and RBC, and reduced plasma  $\alpha$ -tocopherol levels. Similarity, Nockels *et al.* (1996) demonstrated that stressed cattle may have reductions in  $\alpha$ -tocopherol concentrations in certain tissues, and supplemental Vit E may be required to restore  $\alpha$ -tocopherol in tissues following stress, and Calamari *et al.* (1999) observed negative effects of heat stress on some plasma markers of oxidative status in midlactating cows. While, Zhu and Setchell (2004) demonstrated that increasing the temperature of testes causes a derangement in the spermatogenesis, which leads to a decrease in sperm number, motility and normality, and reduces the ability of sperm to produce normal offspring. Both Vit E and Se-containing enzyme glutathione peroxides (GPx4), are an integral part of the antioxidant system present in most mammalian cells, and play an important role in protecting cells again-

st oxidative stress and toxic agents (Flohè, 2007). Furthermore, Burk *et al.* (2007) observed a strong association of low sperm GPx4 with infertility.

To date, there have no long-term studies evaluating the connection between Vit E, Se and hot weather stress on reproductive performance and hematologic parameters in Awassi rams. Therefore, the aim of this experiment was to evaluate the effects of Vit E alone or in combination with Se on changes in semen quality, sexual activity, and some hematologic parameters caused by hot weather stress in Awassi rams.

## Material and methods

### *Animal and semen collection*

The experiment was conducted from May through July (in Iraq, the hot season runs from May to October) and air temperatures hovered between 43-54°C in the shade (Source: U.S. National Climatic Data Center, Asheville, N.C.). On a farm located in the Agriculture College/Baghdad University, twelve adult Iraqi Awassi rams (63±2.2 kg BW, and 30 - 36 mo of age) were kept in semi open pens. The daily hours devoted to outdoor feeding - related activity supplemented with hay and some barley were randomly assigned to three groups. Rams in the 1<sup>st</sup> group (T1) were injected twice a week 175 mg/ram Vit E at intervals of 12h, whereas rams in the 2<sup>nd</sup> group (T2) were injected twice a week with 70 mg Vit E plus 2800 mg Se /ram at intervals of 12h. Rams in 3<sup>rd</sup> group (T3) served as controls. Semen was collected using an artificial vagina at weekly intervals, and the ejaculates were immediately immersed in a warm water bath at 35°C until their assessment. Semen assessment was performed in approximately 25 min.

### *Analysis of standard semen parameters*

Semen samples were analyzed for volu-

me, pH, colour, and concentration. Progressive sperm motility was subjectively evaluated by using the standard method. Motility estimations were carried out from three different microscope fields in each sample at 35°C. The mean of the three estimations was used as final motility score. The viability of spermatozoa in samples was assessed by mean of the nigrosen - eosin stain method. The final composition of the stain was eosin - Y 1.67 g, and sodium citrate 2.9 g, dissolved in 100 ml distilled water.

Sperm suspension smears were prepared by mixing a drop of sperm sample with two drops of stain on a warm slide and spreading the stain with a second slide; viability was assessed by counting at least 200 cells under phase-contrast at 1000x magnification. Sperm displaying partial or complete purple staining were considered nonviable; only sperm showing strict exclusion of stain were counted as viable. For abnormal sperm assessment, at least three drops of each sample were added to Eppendorf tubes containing 1 ml of Hancock solution [62.5 ml formalin (37%), 150 ml sodium saline solution, 150 ml buffer solution, and 500 ml of double-distilled water]. One drop of this mixture was put on a slide and covered with a cover slip. The percentage of total sperm abnormality (acrosomal abnormality, detached heads, abnormal mid-pieces and tail defects) was determined by counting at least 200 spermatozoa under phase contrast microscopy (magnification 1000 x, oil immersion).

### *Sexual activity and blood sample test*

In the pen and flock mating test Iraqi Awassi ewes ≥2.5 years old were used. Oestrus was induced in these ewes by two injections of 25 mg progesterone on d 1 and 3, and an injection of 200 µg oestradiol benzoate on d 5. Ewes displayed behavioural oestrus 18 - 22 h after the injection of oestradiol and remained in oestrus for at least 27h.

In the sexual activity test, each ram was placed in a pen (16 m<sup>2</sup>) with four oestrus ewes, and usually each mount served timed close to 5 sec. Mounts were defined as the ram straddling and clasping the ewe, and contacting her rump with his brisket. A serve was defined as a mount accompanied by intromission and ejaculation, characterized by distinct pelvic thrust with head thrown back, followed by a short period during which the ram showed no interest in the ewes. All rams were given a 20 min pen mating test; reaction time for the 1<sup>st</sup> mount, time for 1<sup>st</sup> service, number of mounts, and number of services were recorded. In all groups, the test was carried out at the end of month 1, 2, 3, and before treatments.

Blood samples were taken from the jugular veins of all rams before treatment and at the end of month 1, 2, and 3. The PCV, and WBC counts were measured on all animals.

#### *Statistical analysis*

The results were expressed as Ls mean  $\pm$ SE. The effects of the treatment on semen quality were analyzed by using the PROC MIXED procedure of SAS (1992) according to the following model:

$$Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + R_k + E_{ijk}$$

where  $Y_{ijk}$  is the observation,  $\mu$  is the overall mean,  $T_i$  is the treatment effect ( $i=1$  and  $2$ ),  $W_j$  is the time effect ( $j=1, 2$  and  $3$ ),  $(TW)_{ij}$  is their interaction,  $R_k$  is the ram ( $k=1, 2, 3$  and  $4$ ), and  $E_{ijk}$  the residual error.

The effects of treatment on sexual activity and hematologic parameters were analyzed by using the general linear model procedure of SAS (1992) according to the following model:

$$Y_{ij} = \mu + T_i + R_j + E_{ij}$$

where  $Y_{ij}$  is the observation,  $\mu$  is the overall mean,  $T_i$  is the treatment effect ( $i=1$  and  $2$ ),  $R_j$  is the ram effect ( $k=1, 2, 3$  and  $4$ ), and  $E_{ij}$  the residual error.

Significant differences between means

were tested using the Duncan multiple range test.

## **Results**

Awassi rams treated with Vit E alone or in combination with Se responded positively on several parameters as compared with the control group. Ejaculate volume increased significantly ( $P < 0.001$ ) in the T2 ( $0.87 \pm 0.03$  ml) and T1 ( $0.68 \pm 0.04$  ml) groups as compared with T3 ( $0.62 \pm 0.04$  ml), and the positive effects of Vit E and Vit E + Se were noted starting after the 4<sup>th</sup> week of treatments and were still present at the end of the experiment (Figure 1). Mass activity and individual motility increased significantly ( $P < 0.001$ ) in the T2 and T1 groups as compared to T3 after the 3<sup>rd</sup> week of treatment, continuing until the end of the experiments (Figures 2 and 3). Furthermore, sperm concentration increased insignificantly in T2 ( $7.140 \times 10^9/\text{cm}^3$ ) and T1 ( $6.762 \times 10^9/\text{cm}^3$ ) when compared with T3 ( $6.595 \times 10^9/\text{cm}^3$ ) (Figure 4). The percentage of dead and abnormal sperm were reduced significantly ( $P < 0.001$ ) in T2 and T1 compared to the control group after the 3<sup>rd</sup> week of treatment and this effect continued until the end of the experiment (Figures 5 and 6).

Pen libido test data are presented in Table 1. At the beginning of the experiment, no differences observed among all rams in three groups in the pen libido test. Reaction time for the first mount was reduced in the 1<sup>st</sup> month insignificantly in T2, and T1 *vs.* T3 while the number of serves increased significantly ( $P < 0.05$ ) in T1, and T2 *vs.* T3. Pen libido test data were presented in Table 2. In the 3<sup>rd</sup> month of treatment, the percentage of lymphocytes increased significantly ( $P < 0.05$ ) in T1 and T2 *vs.* T3 while, the percentage of neutrophils was reduced significantly ( $P < 0.05$ ) in T2 *vs.* T3 in the 2<sup>nd</sup>, and 3<sup>rd</sup> mo of treatment. No significant differences

Figure 1. Effect of Vit E alone (T1) or in combination with Se (T2) on ejaculate volume.

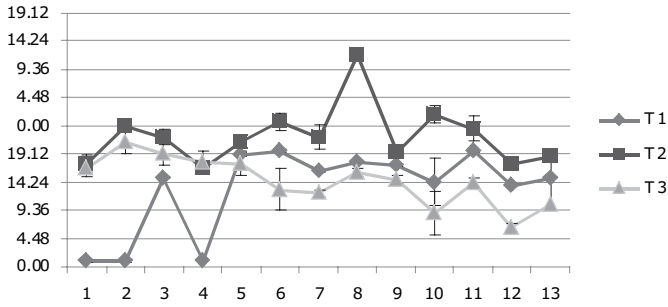


Figure 2. Effect of Vit E alone (T1) or in combination with Se (T2) on mass activity.

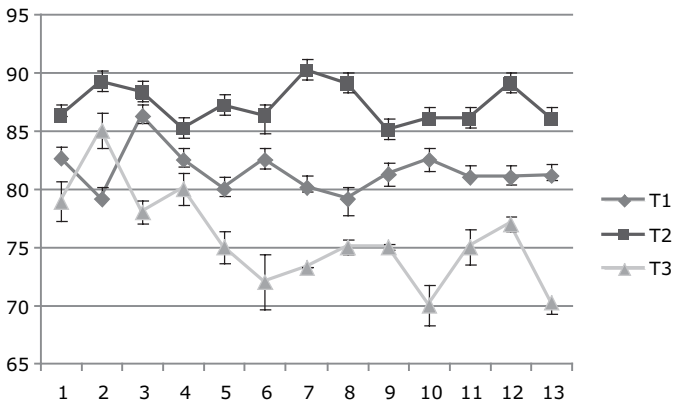


Figure 3. Effect of Vit E alone (T1) or in combination with Se (T2) on individual motility.

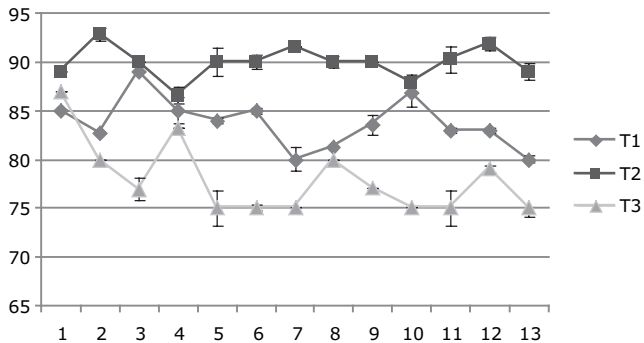


Figure 4. Effect of Vit E alone (T1) or in combination with Se (T2) on sperm concentration.

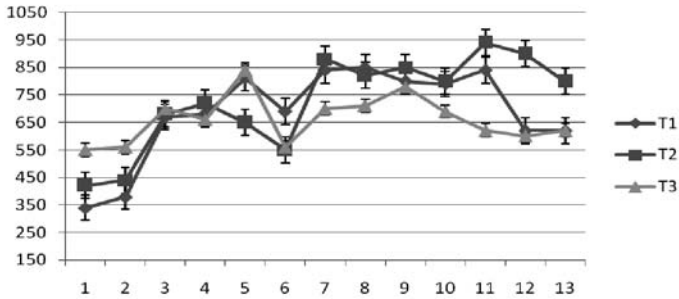


Figure 5. Effect of Vit E alone (T1) or in combination with Se (T2) on percentage of dead sperm.

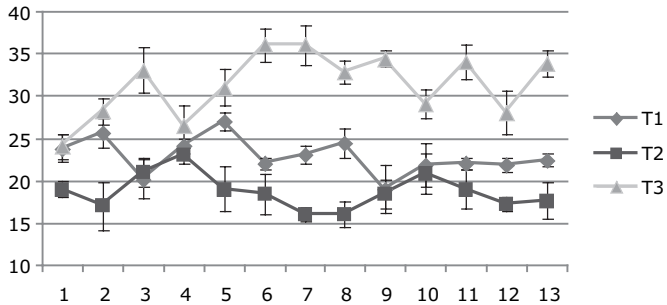


Figure 6. Effect of Vit E alone (T1) or in combination with Se (T2) on abnormal sperm.

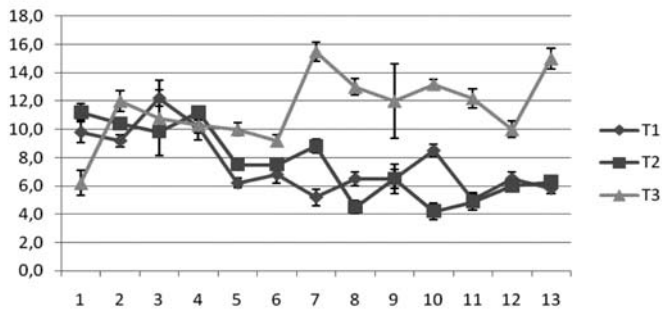


Table 1. Pen libido test of Awassi rams in different treatment groups.

Months	Treat.	Time for first mount (min.)**	Time for first service (min.)	Number of mounts	Number of services*
1	T1	0.500 ± 0.05	4.750 ± 1.07	39.75 ± 4.89	1.250 <sup>ab</sup> ± 0.12
	T2	0.563 ± 0.15	6.375 ± 2.19	44.50 ± 5.72	1.500 <sup>b</sup> ± 0.25
	T3	1.167 ± 0.31	0.00 ± 0.00	43.33 ± 1.96	0.100 <sup>a</sup> ± 0.00
2	T1	0.668 ± 0.18	2.562 ± 0.73	20.75 ± 3.99	1.520 <sup>b</sup> ± 0.31
	T2	0.563 ± 0.12	4.375 ± 1.04	27.75 ± 4.86	1.750 <sup>b</sup> ± 0.24
	T3	1.083 ± 0.08	0.00 ± 0.00	27.00 ± 4.77	0.100 <sup>a</sup> ± 0.00
3	T1	0.563 <sup>ab</sup> ± 0.15	4.187 ± 0.91	23.750 ± 1.42	2.500 <sup>a</sup> ± 0.32
	T2	0.250 <sup>a</sup> ± 0.00	1.625 ± 0.31	29.500 ± 1.20	2.250 <sup>a</sup> ± 0.12
	T3	1.000 <sup>b</sup> ± 0.00	1.530 ± 0.50	29.333 ± 5.90	0.333 <sup>b</sup> ± 0.22

\* a-b: Means in the same column with different superscripts differ ( $P < 0.05$ ).

\*\* a-b: Means in the same column with different superscripts differ ( $P < 0.01$ ).

Table 2. Haematologic indices of Awassi rams in different treatment groups.

Months	Treat.	PCV (%)	Different White Blood Cell Count				
			Lymphocyte (%)*	Monocyte (%)	Neutrophil (%)*	Acidophil (%)	Basophile (%)
0	T1	29.3 ± 0.55	38.2 ± 0.82	12.0 ± 1.38	40.3 ± 2.07	8.4 ± 0.14	1.0 ± 0.00
	T2	26.0 ± 0.73	40.4 ± 0.98	11.0 ± 1.06	37.1 ± 1.50	10.0 ± 1.97	1.5 ± 0.30
	T3	27.0 ± 0.29	36.8 ± 1.54	10.9 ± 0.82	40.5 ± 3.33	11.1 ± 3.46	0.7 ± 0.18
1	T1	28.5 ± 0.52	40.2 ± 0.34	12.1 ± 0.68	37.9 <sup>a</sup> ± 2.60	8.2 ± 3.35	1.6 ± 0.23
	T2	26.0 ± 0.63	41.7 ± 1.80	11.40 ± 0.85	39.1 <sup>a</sup> ± 1.39	6.2 ± 0.95	1.6 ± 0.16
	T3	26.7 ± 0.72	45.5 ± 0.00	15.70 ± 1.74	29.5 <sup>b</sup> ± 2.46	8.0 ± 1.04	1.3 ± 0.22
2	T1	27.0 ± 0.35	42.0 ± 0.68	12.00 ± 1.36	41.0 <sup>ab</sup> ± 1.21	3.4 ± 0.68	1.6 ± 0.34
	T2	25.3 ± 0.44	41.5 ± 0.50	13.50 ± 0.73	36.3 <sup>b</sup> ± 0.88	7.8 ± 1.64	0.9 ± 0.12
	T3	25.5 ± 0.87	41.0 ± 1.26	13.30 ± 1.92	42.0 <sup>a</sup> ± 0.57	3.3 ± 0.93	0.4 ± 0.08
3	T1	27.5 ± 0.85	32.1 <sup>a</sup> ± 1.52	11.10 ± 0.84	51.0 <sup>ab</sup> ± 2.59	4.7 ± 0.51	1.1 ± 0.06
	T2	26.8 ± 0.31	37.6 <sup>a</sup> ± 1.38	9.70 ± 0.82	48.3 <sup>b</sup> ± 2.46	3.9 ± 0.68	0.5 ± 0.17
	T3	26.7 ± 0.33	25.8 <sup>b</sup> ± 1.04	13.8 ± 0.84	56.2 <sup>a</sup> ± 2.08	3.5 ± 1.37	0.7 ± 0.16

\* a-b: Means in the same column with different superscripts differ ( $P < 0.05$ ).



in the percentage of monocytes, acidophils or basophils were seen among all groups through the experiment. Hematologic indices data are presented in Table 2.

## Discussion

In our study, the treatment with Vit E and Se have a positive effect on the characteristics of semen, and some fertility traits in Iraqi Awassi rams during hot and dry weather conditions. After the first month, the treatment with Vit E and Se increased semen volume and the positive effect continued until the end of experiment. Both Vit E and Se are necessary for the development of spermatozoa and sperm production. Additionally, Bearden and Fuquay (1997) showed that treatment with Vit E and Se leads to an increased testosterone level that has a direct effect on secondary sexual activity. In rats, Marin-Guzman *et al.* (2000) suggested that the effect of Vit E occurs through intracellular factors that regulate steps in the development of germ cells, although the effects might be different in other animals since dietary supplementation with Vit E does not influence testicular sperm reserves or Sertoli or germ cell populations in boars. In addition, Se is necessary for the development of germ cells in testes during development of spermatozoa and has positive effects on the number of germ cells in adults (Liu *et al.*, 1982). Furthermore, Se seems to be essential for Sertoli cell development and ultimately the number of Sertoli cells in the developing testes. This effect becomes more pronounced as animals become older, suggesting that Se is important during testicular development (Marin-Guzman *et al.*, 2000). Burk *et al.* (2007) observed that normal sperm development and function critically depend on testicular supply with Se via selenoprotein-P, and, as expected, Se-deficient sperm is not able to reach its target (the ovum) in the female reproductive tract.

Moreover, the deficiency of Vit E caused testicular degeneration in chickens, rats, hamsters, rabbits, guinea pigs, dogs, cats, pigs and monkeys, and resulted in a lower number of germ cells and reduced sperm production. All of the aforementioned processes have an additive effect on sperm production. A study by Liu *et al.*, (1982) indicated that prolonged Se deficiency in boars resulted in low sperm concentrations. Brezinska-Slebodinska *et al.* (1995) observed that supplementation with Vit E increased the concentration of spermatozoa in semen, while Scott *et al.* (1998) showed no effect on sperm density by supplementing the subjects with Se for 3 mo. This may be related to the effect of Vit E and Se on sperm metabolism and conformation (Marin-Guzman *et al.*, 1997), as sperm motility increased in selenium treated groups. Cooper *et al.* (1987) demonstrated that dietary deficiencies of Vit E in growing males causes degenerative spermatogonium.

Vitamin E and Se act as antioxidants which in turn may provide direct protection of sperm cells from morphological damage by preventing free radical oxygen from damaging sperm cells (Brezinska-Slebodinska *et al.*, 1995). Vit E helps protect the sperm cell membrane from damage, while Se prevents abnormalities in sperm tails. Burk *et al.* (2007) observed a strong association of low sperm GPx4 with infertility. Thus, impaired GPx4 biosynthesis, due to Se deficiency, or to genetic defects in GPx4 itself or in proteins involved in Se distribution and selenoprotein biosynthesis, may cause male infertility, but can also be an epiphenomenon due to any perturbation of testicular function (Flohè, 2007). Moreover, the morphology and the motility of sperm cells would be preserved by the binding of Vit E and Se to endoperoxides (Marin-Guzman *et al.*, 2000). Liu *et al.* (1982) showed that prolonged Se deficiency in boars resulted in sperm with a high incidence of cytoplasmic

droplets, while Bearden and Fuquay (1997) demonstrated that a deficiency in Vit E resulted in a degeneration of the testes and permanent sterility. Vit E tended to improve semen quality in chickens by increasing concentrations of spermatozoa and cell viability (Franchini *et al.*, 2001).

It is known that there is a positive correlation between testosterone concentration and sexual activity in males. Under the conditions of our experiment, sexual activity increased after treatment with Vit E alone or in combination with Se. This increase in sexual activity combined with increased testosterone concentration leads to a direct effect on semen volume. Kaur and Bansal (2004) demonstrated that the levels of testosterone, FSH, LH were significantly reduced during Se deficiency, which has been implicated in testicular dysfunction. Yousef *et al.* (2003) demonstrated that the treatment of rabbits with Vit E significantly increased reaction time in the libido test. While Lees *et al.* (1982) demonstrated that levels of plasma testosterone and corticosterone in male rats given a diet deficient in Vit E for 130 d were significantly lower than those in rats given the same diet supplemented with Vit E. Our findings are in agreement with those of other authors who showed that Vit E and Se supplementation leads to improved sperm motility and increased percentage of normal sperm (Yousef *et al.*, 2003). Contrary to our findings, other authors have reported that the addition of inorganic Se to the diets of rams, boars, and dairy or beef bulls did not improve their semen quality (Audit *et al.*, 2004). This discrepancy may be related to differences among species or treatments.

Several studies have shown that Vit E, Se or a combination of the thereof can increase the viscosity of phagocyte cell membranes, leading to improved phagocytosis of foreign bodies, and may also increase production of immunoglobins (IgM and IgG) (St-Laurent

*et al.*, 1990). Vit E and Se can also affect lymphocyte production in the bone marrow (Hogan *et al.*, 1993). Brzezinska-Slebozinska *et al.* (1995) suggested that dietary Vit E may serve as an antioxidant in boar semen. Injection of Vit E and Se were found to be necessary to attain maximum immunologic responses (Nemec *et al.*, 1994). Wuryastuti *et al.* (1993) suggested that both Se and Vit E may be necessary to enhance the immunogenic capability of reproducing sows. All the above factors would improve immune responses. In our study, there were no significant differences among all groups in PCV until the end of experiment. However, in the 3<sup>rd</sup> mo of treatments with Vit E and Vit E + Se, the percentage of lymphocytes increased significantly in treated groups. While there was no differences among them, and in the 2<sup>nd</sup> and 3<sup>rd</sup> mo of treatments, the percentage of neutrophils was reduced significantly in T2 *vs* T3. Reffett *et al.* (1988) demonstrated that serum immunoglobulin M (IgM) concentration was enhanced significantly by Se supplementation, while Se and/or Vit E did not affect serum immunoglobulin G levels. Hernken *et al.* (1998) noted that a deficiency in Vit E and/or Se resulted in a reduced immunologic response. Fry *et al.* (2006) demonstrated that Se levels had minimal effects on the immunity of weaned beef calves, and Garner *et al.* (2006) demonstrated that treatment with Vit E and Se over the course of a month did not improve the immune response of calves.

## Conclusions

In conclusion, our results demonstrated a clear positive effect of Vit E and Vit E + Se on semen characteristics that could improve the reproductive performance in native Iraqi Awassi rams during the hot season. A search for new ways to supplement feed with vitamins and minerals without the need for special tools would be most bene-

ficial. Further studies using different dosages of Vit E and Se are necessary to improve the immune response in animals during hot weather.

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