



EFFECT OF THE INJECTION OF VITAMIN E AND SELENIUM ON SEMEN CHARACTERISTICS AND SOME TESTICULAR MEASUREMENTS ON KARADI RAMS

Abdulnasir T.H.M. Alkhashab* and Mohannad Mahdi Hameed

Department of Animal Production, College of Agriculture and Forestry, Mosul University, Mosul, Iraq.

Email: doctornasir975@yahoo.com

Abstract

The objective of this study was to determine the effect of vitamin E and selenium (Se) on physiological semen characteristics and some testicular measurements of Karadi rams. Fifteen (15) Karadi rams with average body weight 63 kg and 3-4 years of age were randomly divided into three groups (5 rams / group) and the rams were fed a standard diet equal in energy and protein and treated as follows. The first group (T1) was regard as control: rams were injected with 1ml normal saline once weekly, The second group (T2): rams were injected with 2 ml (2.5 mg vitamin E and 50 µg sodium selenite / kg body weight) once weekly ,The third group (T3) animals were injected with 4 ml (5 mg vitamin E and 100 µg sodium selenite /kg body weight) twice in week (2ml /72 h.) and the experiment continue for 3 months. Results revealed that treatment with vitamin E and selenium Se led to a significant improvement ($P \leq 0.05$) on some semen characteristics and a significant ($P \leq 0.05$) increase in testicular circumference and volume of Karadi Rams.

Keywords : Vit. E & Selenium, semen characteristics, Karadi Rams.

Introduction

Livestock is an essential part of the agricultural production sector, which suffers from a decline in animal production and productivity in Iraq. In order to raise the productive efficiency of farm animals, it must improve reproductive efficiency in the animals (Al-Sayegh, et al., 1992). So Some vitamins and minerals are important and essential elements in the reproduction of farm animals (Yousif *et al.*, 2003).

Vitamin E and selenium are essential nutrients with complementary biological functions as antioxidants for minimizing cellular damage caused by endogenous peroxides (Kolb *et al.*, 1997). Vitamin E prevents oxidative damage to sensitive membrane lipids by suppressing hydro peroxide formation (Chow, 2001) and protects cellular membranes thus maintaining membrane integrity and reducing oxidative stress (Hogan *et al.*, 1993). There is physiological synergism between selenium and vitamin E. Previous reports have suggested that vitamin E and selenium (Se) are important nutrients that act synergistically and can affect many biological process including spermatogenesis and semen quality (Marin- Guzman *et al.*, 1997 and Yousef *et al.*, 2003). Research suggests the role of vitamin E and selenium in maintaining libido and improving semen quality (AL-Haboby *et al.*, 2004, Zubair, 2017). The improvement of the reproductive efficiency of genetically modified rams is to increase the living sperm free of deformities that are able to reach the fertilization site actively, depending on the quality and quantity of the semen produced (Saacke *et al.*, 1994). The objective of the study is the possibility of injecting the mixture of vitamin E and selenium for the Karadi rams and study its effect on semen characteristics and measurements of the testicles dimensions in the local Karadi rams.

Materials and Methods

The present study was carried out in animal field of animal resources, College of Agriculture and Forestry, University of Mosul. This study was conducted during January and March 2014, Fifteen karadi rams, aged 3-4 years and mean weight 63 kg, were placed in semi-enclosed pens. These rams were supervised by veterinarians throughout the

experiment. The animals were fed a standardized diet. Concentrated in the field was provided by 1 kg in two morning and evening meals, and the diet consisted of 50% barley 38% bran 5% yellow corn 5% soybean 1% limestone, 1% salts with 13.32% raw protein and 2465 kcal. Me. (Khawaja *et al.*, 1978) and gave hay as roughage at the rate of 500 g / animal / day, water and molds of mineral salts were available daily in front of animals, The rams were divided into three groups. The first as control group (T₁) was left untreated. The second group (T₂) was injected with (2 ml) vitamin E and selenium mixture (2.5 mg + 50 µg of vitamin and selenium / kg live weight) as intramuscular. The third group (T₃) was injected with 4 ml of the same mixture (5 mg + 100 µg of vitamin and selenium / kg weight as intramuscular and the dose was divided twice every 72 hours during the week. Semen was collect by using the electroejaculator of sheep and goats at a rate of once every two weeks to study the effect of vitamin E and selenium injection on the semen characteristics of the rams in the experimental groups, semen was collected from the animals (one ram per day) of each group during the week of collection and continued collection during the duration of the experiment, which lasted three months. Semen was evaluated immediately upon collection for general characters (semen ejaculated volume, concentration, pH , mass motility, live, dead sperm and abnormal spermatozoa). Semen ejaculated volume (ml) was measured using a graduated collection tube to the nearest 0.1 ml. Mass motility was estimated according to the recommendations of Evans, Maxwell, (1990) and Chemineau *et al.*(1991) respectively, and the promise of sperm concentration using the hemocytometer counting chamber (Mayer, 1955). The percentage of live, dead and sperm abnormality was calculated by method of (Chemineau *et al.*, 1991). Initial (pH) of semen was measured using a digital pH-meter device immediately after collection by immersing the end of the electrode in the semen directly from the collection tube.

Blood samples were collected to measure Serum testosterone concentration every 15 days from the jugular vein into 10 ml tubes. The samples were immediately transported to the laboratory and centrifuged at 2500×g for

min and harvested sera stored at -20°C until the testosterone estimation. Serum testosterone was measured using several analyzes (Kit) produced by Elisa Microwells of America, established by Elisa, according to the company's recommendations.

Scrotal circumference was measured by using a plastic measuring tape at the widest of the paired testes and testes size was measured by immersing both testicles and scrotum in lukewarm water in plastic pot (2 liters size) and then calculate the amount of removed water (Aquirre *et al.*, 2007).

Statistical Analysis

Data obtained were subjected to analysis of variance in completely randomized design and means separated by Duncan multiple test (1955). The results analyzed using a two-way analysis of variance in SAS (2001) package were expressed as the means \pm SEM in SAS (2001) package using the model below:

$$Y_{ijk} = \mu + T_i + S_j + TS(i)j + e_{ijk}$$

As:

Y_{ij} = The transaction value of j for transaction i

M = The general mean of the studied character

T_i = Effect of the studied treatments (the study included three treatments)

S_j = Effect of the month on the characteristics of raw semen studied.

$TS(i)j$ = Effect of Interaction between Transactions and Month.

E_{ijk} = Random error which is distributed naturally at an average of zero and a variance of $2e\sigma$.

Results and Discussion

The result of this study (Table 1) showed a significant ($p \leq 0.05$) effect of vitamin E and selenium on semen characteristics (volume, sperm concentration, mass and individual motility of the sperm, percentage of live, dead and abnormal sperm). The second group witch treated with (5mg / 50 μg of vitamin and selenium mixture/kg live weight) gave highest dose of semen volume (1.68 ml) followed by (1.55 ml) in third group treated with (5 mg/100 μg of vitamin E and selenium mixture/kg live weight) and were significantly different from control group (first group) which gave 1,27 ml of semen volume .This result is similar to the finding of yue *et al.* (2010) and Dulaimi (2010) and in agreement with finding of Li-quanq Shi *et al.* (2010), Anita & Jacyno (2005).

The results of the analysis (Table 2) showed a significant effect ($p \leq 0.05$) of month on semen ejaculation volume, which increased from 1.14 ml in the first month to 1.89 ml in the third month of the experiment. The results agrees with Daham (2002) and Taha *et al.* (2000a) who obtained a significant difference in semen volume between the months of the experiment, and did not agree with Hussain *et al.* (2012), who did not have a significant effect of winter months on semen volume of the male goats. There was in significant effect of the treatments \times month on the pH values

between the experimental groups. The results were agrees with Li-quanq shi *et al.* (2010) Tajangookeh *et al.* (2007) and Daham (2002) while Hussein *et al.* (2012) had a significant effect for the month on the pH values of semen.

A significant increase ($p \leq 0.05$) in sperm concentration in second and third groups which gave the highest concentration of sperm compared to control group. These results were agreed with Ammar *et al.* (2009), Anita & Jacyno (2005) and in agreement with (Hodgson *et al.*, 2001). Also a significant ($p \leq 0.05$) effect of month in sperm concentration with the highest concentration in the third month and was significantly ($p \leq 0.05$) different than sperm concentration in the first month, while there was no difference observed between second and first, third months, these results were agreed with Tajangookeh *et al.* (2007) and Taha *et al.* (2000a) and in agreement agree with Deham (2002).

A significant effect ($p \leq 0.05$) of vitamin and selenium treatments on mass motility of sperms, witch the second and third groups gave the highest mass motility compared to the control group, these results were agreed with Mahmoud *et al.* (2013) And Soleimani *et al.* (2009) and in agreement with Li-quanq Shi *et al.* (2010), Anita & Jacyno (2005) while the results (Table 2) did not show any significant effect of months on mass motility and agree with Hussein *et al.* (2012) and Daham (2002). The treatment with vitamin and selenium gave a significant effect ($p \leq 0.05$) on the percentage of individual motility of sperms in raw semen and it was 91% in second group followed by 83% in third group compared to 77% in control group (Table 1). These results were agreed with Mahmoud *et al.* (2013), Dulaimi (2010) and Ammar *et al.* (2009) and in agreement with Anita & Jacyno (2005). While Statistical analysis did not show any significant effect of months on the individual motility percentage in raw semen, these results were agrees with Hussein *et al.* (2012), Daham (2002), Taha *et al.* (2000a). Also a significant effect ($p \leq 0.05$) of the treatments (Table 1) on live and dead sperms, with the highest percentage 84% of live sperm in second group, which differed significantly from 76% in third group to 70% in control group. In contrast dead sperm decreased from 30.4% in control group to 23.9% in third group and 15.8% in second group, these results were agreed with Mahmoud (2013) Dulaimi (2010) And Soleimani *et al.* (2009). A significant effect of month on the percentage of live and dead sperm (Table 2), the results were agrees with Hussein *et al.* (2012) and Daham (2002) and in agreement with Taha *et al.* (2000 a) who did not get a significant effect of month on the percentage of live and dead sperms. Also a significant decrease ($p \leq 0.05$) in abnormal spermatozoa from 5.83% in control group to 4.6 % in third group and 3.9 % in second group (Table 1) these results were similar to the finding of Hussein *et al.* (2012), Dulaimi (2010) and Daham (2002) who gets an increase in sperm abnormalities during the winter months, while Taha *et al.* (2000a) did not find a significant effect of month in the percentage of abnormal spermatozoa. Moreover the results of vitamin E, selenium and month interaction (Table 3) showed a significant ($p \leq 0.05$) effect on semen characteristics of rams.

Table 1: Effect of treatment of vitamin and selenium mixture on semen characteristics in rams (mean \pm standard error)

Item Treat.	Semen volume ml	ph	Sperm conc. $10^6 \times$	Mass motility (Degree)	Individual motility %	Live Sperm %	Dead sperm %	Abnormal sperm %
T ₁	1.27 \pm 1.14 b	6.8 \pm 0.06	2.61 \pm 0.29 c	2.76 \pm 0.15 c	77 \pm 2.00 c	70 \pm 0.72 c	30.40 \pm 0.84 a	5.83 \pm 0.37 a
T ₂	1.68 \pm 0.10 a	6.87 \pm 0.05	4.22 \pm 0.52 a	3.89 \pm 0.13 a	91 \pm 1.15 a	84 \pm 0.68 a	15.86 \pm 0.69 c	3.90 \pm 0.71 c
T ₃	1.55 \pm 0.14 a	6.79 \pm 0.06	3.28 \pm 0.57 b	3.42 \pm 0.11 b	83 \pm 0.77 b	76 \pm 0.52 b	23.93 \pm 0.52 b	4.60 \pm 0.44 b

a.b.c. means in the same column have the different superscript are significantly different at ($p \leq 0.05$)

Table 2: Effect of months of experiment on semen characteristics in rams (mean \pm standard error)

Item Month	Semen volume MI	ph	Sperm conc. $10^6 \times$	Mass motility (Degree)	Individual motility %	Live Sperm %	Dead sperm %	Abnormal sperm %
1	1.14 \pm 0.09 c	6.86 \pm 0.05	2.838 \pm 0.58 b	3.21 \pm 0.15	82.13 \pm 1.70	75.06 \pm 1.45 b	25.13 \pm 1.84 a	4.83 \pm 0.75
2	1.47 \pm 0.08 c	6.82 \pm 0.05	3.612 \pm 0.77 ab	3.54 \pm 0.13	84.60 \pm 1.42	76.66 \pm 1.55 ab	23.33 \pm 1.55 ab	4.73 \pm 0.77
3	1.89 \pm 0.12 a	6.80 \pm 0.06	3.853 \pm 0.76 a	3.32 \pm 0.23	83.66 \pm 2.85	78.26 \pm 1.68 a	21.73 \pm 1.68 b	4.76 \pm 0.91

a.b.c. means in the same column have the different superscript are significantly different at ($p \leq 0.05$)

The results of this study showed a significant ($p \leq 0.05$) improvement in semen characteristics of rams in treatment groups. The superiority of the semen ejaculated volume of rams that were injected with vitamin E and selenium may be due to the increase in secretion of testosterone (Fig. 1), which affects on the effectiveness of the accessory sex glands (Bearden and Fuquay, 1997). Underwood (1981) reported that there is a contribution to some minerals, including selenium, to increase the effectiveness of the accessory sex glands of the male reproductive system, including prostate

gland and seminal vesical gland. Also Smith *et al.* (1979) found highest concentration of selenium in sex glands (prostate, seminal vesical, and cowper gland) respectively, which increases the efficacy of glutathione peroxidases (GSH_px) and its ability to eliminate free radicals produced internally by metabolic processes and thus increasing the secretion of these glands for the Selenium element is essential in increasing the effectiveness of GSH_px (Flohe, 1976).

Table 3: Effect of the interaction of treatment \times months on semen characteristics in rams (mean \pm standard error)

Treat.	Month	Semen characteristics							
		Semen volume MI	ph	Sperm conc. $10^6 \times$	Mass motility (Degree)	Individual motility %	Live Sperm %	Dead sperm %	Abnormal sperm %
T ₁	1	0.96 \pm 0.124 C	6.82 \pm 0.03	2.636 \pm 0.73 \pm d	2.82 \pm 0.24 c	77.60 \pm 3.61 de	69.20 \pm 1.68 d	32.80 \pm 1.74 a	5.70 \pm 0.97 a
	2	1.34 \pm 0.06 bc	6.84 \pm 0.09	2.686 \pm 0.52 d	3.26 \pm 0.20 bc	80.00 \pm 2.32 cd	70.20 \pm 0.80 d	29.80 \pm 0.80 ab	5.80 \pm 0.37 a
	3	1.52 \pm 0.27 B	6.82 \pm 0.09	2.506 \pm 0.29 d	2.20 \pm 0.14 d	72.40 \pm 4.03 e	71.40 \pm 1.20 d	28.60 \pm 1.20 b	6.00 \pm 0.54 a
T ₂	1	1.34 \pm 0.07 bc	6.94 \pm 0.09	3.586 \pm 0.48 ab	3.54 \pm 0.29 ab	86.80 \pm 1.93 bc	81.20 \pm 0.80 b	17.40 \pm 1.46 d	3.80 \pm 0.80 Cd
	2	1.62 \pm 0.15 ab	6.88 \pm 0.11 \pm	4.232 \pm 0.98 ab	4.06 \pm 0.13 a	90.60 \pm 0.67 ab	83.80 \pm 1.06 ab	16.20 \pm 1.06 de	4.00 \pm 1.50 cd
	3	2.10 \pm 0.08 A	6.80 \pm 0.08	4.832 \pm 1.16 a	4.08 \pm 0.17 a	95.40 \pm 0.74 a	86.00 \pm 0.54 a	14.00 \pm 0.54 e	3.90 \pm 1.46 d
T ₃	1	1.14 \pm 0.24 bc	6.84 \pm 0.02	2.294 \pm 0.04 d	3.28 \pm 0.18 bc	82.00 \pm 1.76 cd	74.80 \pm 0.86 c	25.20 \pm 0.86 c	5.00 \pm 0.58 ab
	2	1.46 \pm 0.16 bc	6.76 \pm 0.06	3.920 \pm 1.23 cd	3.30 \pm 0.18 bc	83.20 \pm 0.80 cd	76.00 \pm 0.54 c	24.00 \pm 0.54 c	4.40 \pm 0.50 bc
	3	2.06 \pm 0.13 A	6.78 \pm 0.14	3.622 \pm 0.73 bc	3.68 \pm 0.19 ab	83.20 \pm 1.52 cd	77.40 \pm 1.02 c	22.60 \pm 1.02 c	4.40 \pm 0.80 bcd

a.b.c. means in the same column have the different superscript are significantly different at ($p \leq 0.05$)

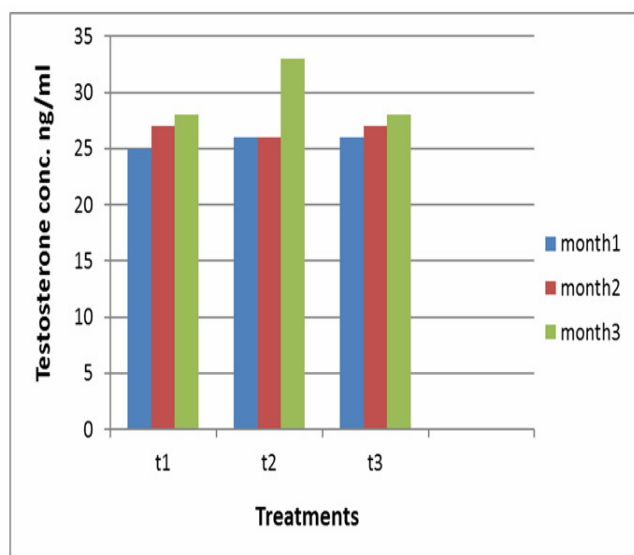


Fig. 1: Effect of vitamin E and selenium \times month on testosterone conc.

Several studies confirmed the results of this experiment in the role of vitamin E and selenium in improving the quality of semen due to the role of vitamin E for protection of the plasma membrane of sperm from unsaturated fatty acid peroxides, while Selenium prevents the occurrence of distortions in the tail of sperm and maintain the integrity of sperm and its activity and metabolic function (Zubair, 2017, Burk *et al.*, 2007, Guzman-Marin, 1997). Mc Dowell (1989) also mentioned that Vitamin E binding biologically in the structure of the plasma membrane and mitochondrial membrane and maintaining its integrity.

Zubair,(2017) reported about the role of vitamin E in reducing the percentage of abnormalities that occur in sperm acrosome through the action of vitamin E on the two axes , first increase the effectiveness of the enzyme superoxide dismutase (SOD), and the second disposal of the root of Superoxide anions, which promotes the production of peroxides causing deformation of the plasma membrane and sperm acrosome. The presence of selenium in the structure of the main protein (Keratinoid proteins) in composition of sperm tail, which is the movement of the sperm, so its presence is necessary in the formation of sperm tail, especially during the transformation of Spermatids to Spermatozoa (Calvin, 1987). The lack of selenium causes an increase in the number of sperm containing cytoplasmic droplets (Marin-Guzman *et al.*, 1997). The principle of the work of vitamin E and selenium directly as antioxidants of free radicals that cause damage of the sperm (Brzezinska and Slebodzinska, 1995). Burk *et al.* (2007) indicate that selenium acts as a co-factor of glutathione peroxidase enzyme (GPx4), which directly destroyed unsaturated fatty acid peroxides and the highest activity of the GPx4 enzyme is through differentiation of Spermatogonia cell occurs. Therefore, the lack of selenium leads to a rupture of the testicular tissue, affecting the shape, concentration and sperm motility (Flohe, 2007). Also Selenium helps to develop testicular tissue naturally and improves sperm Spermatogenesis (Behne *et al.*, 1996).

The production of good sperms depends on the extent to which the testicular tissue is supplied with selenium, which is often exist as a form of Selenoprotein-P, because the lack of selenium leads to product sperms that cannot continue to fertilize the egg in the female reproductive system (Burk *et al.*, 2007). Selenium deficiency also leads to a decrease in the concentration of sperm in pigs (Liu *et al.*, 1982). While Udala *et al.* (1995) injected bulls with (0.75mg Vit.E) or (0.05mg Se / kg body weight) observed a significant increase in sperm concentration. Cooper *et al.* (1987) showed that vitamin E deficiency led to a decrease in germ cells and thus reduced in sperm production. also the results of this study were agrees with the finding of Yousef *et al.* (2003) and Hedayati *et al.* (2009) in the role of vitamin E and selenium to increase the concentration of sperm.

Our current study has shown that vitamin E and selenium have a positive effect in improving alive of sperm and reducing the percentage of dead sperm. These results confirm the findings of Lodhi *et al.* (2008) who reported that there is appositive correlation with high alive sperm and negative with dead and sperm abnormality. While Bartle *et al.* (1980) and Daramola *et al.* (2016) did not observe any effect of vitamin E and selenium in improving semen quality, this may be due to experimental conditions on one hand and to experimental animals on the other, which may not be deficient in vitamin E and selenium, moreover the results showed a positive improvement of month in semen characteristics (semen ejaculate volume, sperm concentration, live and dead sperm percentage) during the months of experiment, Possibly due to the increase in sperm vitality and the decrease of dead sperm (Taha *et al.* 2000a) or as a result of vitamin and selenium mixture treatment, which was useful and led to a positive effect and improved semen characteristics during experimental months.

The results of this study (Table 4) showed that the treatment with vitamin E and selenium Se significantly improved the testicular volume of Karadi rams to (1009 cm³) in the second group compared with (811.6 cm³) in control group, While insignificant improvement was found between the second and third group, or the third group with (844.6cm³) testicular volume compared to control group. also the results of the statistical analysis showed that a significant improvement ($p \leq 0.05$) in scrotal circumference of Karadi rams in treatment groups compared to the control group (Table 4), these results were agrees with Shi Li-quauq *et al.* (2010) and Soleimani *et al.* (2009) and in agreement with Mahmoud *et al.* (2013) and Anita and Jacyno (2005) who did not found significant improvement in measurements of testicular dimensions, and there was no significant effect for months on measurement of testicular dimensions (Table 5).

The increase in testicular volume of Karadi rams was associated with the formation of sperm (spermatogenesis) where the volume increases with increasing sexual effectiveness and the increase is mainly due to the total increase in the length and diameter of seminiferous tubules (Yarney and Sanford, 1986) and reduction or decrease in selenium may reduce diameter of seminiferous tubules and small testicular volume (Behne *et al.*, 1996, Marin-Guzman *et al.*, 2000).

Table 4: Effect of treatment with vitamin and selenium mixture on testicular volume (cm³) of karadi rams (mean \pm standard error)

Item	Months of experiment			Average of treatments
	Fist month	Second month	Third month	
T1	801.20 \pm 62.77	813.60 \pm 64.94	63.93 \pm 820.00	811.60 \pm 34.21B
T2	996.20 \pm 101.25	1011.20 \pm 104.71	104.16 \pm 1020.00	1009.13 \pm 55.32A
T3	841.20 \pm 154.17	845.20 \pm 152.94	847.40 \pm 152.12	844.60 \pm 81.82AB
Average of months	879.53 \pm 64.19	890.00 \pm 64.88	895.80 \pm 64.70	

A.B. means in the same column have the different superscript are significantly different at ($p \leq 0.05$)

Table 5: Effect of treatment with vitamin and selenium mixture on scrotal circumference (cm) (mean \pm standard error)

Item	Months of experiment			Average of treat.
	Fist month	Second month	Third month	
T1	28.60 \pm 1.20b	28.80 \pm 1.35b	28.80 \pm 1.35b	28.73 \pm 0.70 C
T2	36.60 \pm 1.28a	37.20 \pm 1.49a	37.80 \pm 1.71a	37.20 \pm 0.81A
T3	30.60 \pm 0.87b	31.00 \pm 0.83b	31.40 \pm 0.87b	31.00 \pm 0.46 B
Average of months	31.93 \pm 1.09	32.33 \pm 1.16	32.66 \pm 1.24	

a.b.c. means in the same column have the different superscript are significantly different at ($p \leq 0.05$)

A.B.C. means in the same column have the different superscript are significantly different at ($p \leq 0.05$)

Conclusion

This study has demonstrated a clear positive effect of vitamin E and Se injection on semen characteristics, testes measurements in Karadi rams. Thus this method of administration or injection could be employed in improving the reproductive performance of Karadi Rams in Iraq.

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