ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

The effect of selenium and vitamin E addition on semen quality of Awassi rams

Ahmed Ghafor Baker [1] Sarmad Abdul Razak Abood Alssadi [2] Ayhan Kamal Mohammad[3]

[1]Dept. of Animal Production, Faculty of Agriculture, University of Kirkuk
[2] Animal Physiology, Faculty of Agriculture, University of Kirkuk
[3]Animal Breeding, Faculty of Agriculture, University of Kirkuk

A B S T R A C T:

This study conducted in sheep farm/faculty of Agriculture/Kirkuk University on 16 young Awassi rams at age 15-16 months to determine the effect of Selenium and Vitamin E oral administration in some semen characteristics. Rams were divided randomly into four groups, the 1st group (T1) without treatment as control while the 2nd and 3rd groups (T2 and T3) were orally administered with 0.1 or o.2 mg mixed with 1000 IU vit. E respectively, while T4 was administered with only 1000 IU of vitamin E for 3 months. Ejaculate volume, pH, mass mobility and individual motility, precentage of normal, dead and abnormal sperm, sperm concentration were studied at end of each of 3rd month of the study. Selenium and vitamin E treatments had significantly increased pH in the treatments T2 and T3 compared with the control and T4 during the 1st month. In 2nd month T4 surpass significantly T3 and in 3rd month both T4 and T2 surpass significantly T3. Sperm concentration showed significant increase in T3 in comparison to other treatments in 2nd month, in 3rd month T3 and T4 were significantly higher than T2. Mass mobility showed significant incease in T4 during 2nd month while T2 increased significantly during 3rd month in comparison to control, T3 and T4. Individual mobility showed significant increase in T4 in comparison to control, T2 and T3 during 2nd month and significant incease in comparison to T3 during 3rd month. Dead sperm percentage recorded significant decrease in T4 in comparison to T2 and T3 in both 2nd and 3rd months. Normal sperm percentage showed significant increase in T4 upon other groups in 2nd month while T2, T3 and T4 showed significant increase upon control in 3rd month. Abnormal sperm percantage recorded significant decrease in T4 in comparison to other groups in 2nd and 3rd months.

KEY WORDS: Selenium, Vitamin E, Semen characteristics, Awassi Rams
DOI: http://dx.doi.org/10.21271/ZJPAS.33.s1.14
ZJPAS (2021) , 33(s1);127-134 .

INTRODUCTION:

The reproductive system improving is very important impact in sheep breeding and closely related with increasing of productive qualification (AL-Haboby, *et al*. 2003). There are many vitamins and minerals, which are necessary to maintain and improve of reproductive quality and quantity (Brown and Arthur, 2001). Vitamin E and Selenium are two of the important nutrients that can affect several biological processes including spermatogenesis and semen quality (Ahmed, *et al.* 2012).

There is physiological synergism between selenium and vitamin E. Previous reports Netto, *et al*. (2014); Yousef, *et al*. (2003) has suggested that vitamin E and selenium are important nutrients that act synergistically and can affect many biological processes including spermatogenesis and semen quality. 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". Study of AL-Haboby, *et al*. (2004) shows the critical role of Vit. E and Selenium in maintains of lipido and improvement semen quality and increasing of spermatogenesis .

* **Corresponding Author:**
Ahmed Ghafor Baker
E-mail:
Article History:
Published: 15/03 /2021

Vitamin E is believed to be the primary components of the antioxidant system of the spermatozoa (Zubair, et. al. 2015). Supplemental vitamin E has been shown to increase total sperm output and sperm concentration in rams (Azawi and Hussein, 2013). Deficiency of vitamin E may lead to reproductive organ damage, such as degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules (Yue, et. al. 2010). Selenium is an essential dietary trace element and is always of research interest required for the maintenance of male fertility by way of testosterone biosynthesis, formation and normal development of spermatozoa (Brown and Arthur, 2001). In males bred on a low selenium diet, male hypogonadism was found as well as reduced production and deteriorated semen quality (Koyuncu and Yerlikaya, 2007).

Therefore, the aim of the present study is to evaluate the influence of the combination of vitamin E and Se on reproductive performance in rams.

Materials and Methods

This study conducted on 16 young Awassi rams (aged 15-16 months and average wright of 62 kg) during the period February - March 2018, all rams were subjected to normal farm feeding and health care. The rams were divided randomly into 4 groups with equally average body weights, the 1st treatment as control while the 2nd group was administered with only 1000 IU of vitamin E, writer while the 2nd and 3rd groups (T2 and T3) were orally administered with 0.1 or 0.2 mg mixed with 1000 IU Vit. E respectively, while 4th group was administered with only 1000 IU of vitamin E for 3 months. Semen collected using electro ejaculator at the end of each of three months of the study to conduct some physical and microscopic tests: ejaculate volume (David *et al*. 2007), pH, color (Evan and Maxwell 1987) and viscosity (WHO 2010), mass activity (Evan and Maxwell 1987) and individual motility (Walton, 1933), precentage of normal and dead sperm (Campbell *et al*. 1956) and abnormal sperm (Milovanov, 1960), sperm concentration (Salisbury, *et al*. 1943). All data were analyzed by using SAS software (SAS, 2005), also the means were compared by using Duncan Multiple Range Test (Duncan, 1955).

Results and Discussion

The ejaculate volume was not affected by Selenium with/without vitamin E supplementation in all treated groups. As shown in table (1), not all treated groups showed any significant improvement in semen volume. These result agreed with Ghorbani, *et al*. (2018) who reported no change had been happen to semen volume to adult rams fed dietary capsules contain 0.11 mg/ kg Selenium for 4 months. Another study El-Sheshtawy, *et al*. (2014) showed that treated of local Fawn with injectable 1.35 I.U./ kg Vit. E did not lead to any increasing in ejaculate volume. The results of Table (1) indicate significant differences ($p< 0.05$) in pH values between all experimental treatments. In the 1st month, (T2) and (T3) significantly ($p< 0.05$) exceed (T4) and control, while (T4) significantly ($P\leq 0.05$) outperformed (T3) in the 2nd month, while in the 3rd month of the study, (T2) and (T4) outweighed Significantly ($P\leq 0.05$) on the (T3).

The results in Table (1) agree with Karakuş, *et al*. (2016) that the significant increase in the pH concentration in adult rams treated with Vit. E coincided with significant improvement in sperm concentration, individual, and group movement rates. The reason for this is that the basophilic environment (represented by in hydrogen ion decreasing in the semen in the current study) led to an improvement in the microscopic parameters of the sperm (Ghorbani, *et al*. 2018). The decrease in the percentage of dead and distorted ones is because the basal slanting environment (represented by the low hydrogen ion in the semen in the present study) has led to an improvement in the microscopic parameters of sperm, as for Vit. E; Ghorbani, *et al*. (2018) demonstrated that there was no significant increase in the value of (pH) in selenium-treated groups, and the researcher considered these results positive and consistent with the significant increase in the microscopic criteria of sperm specifically in selenium-treated groups.

The results of Table (2) showed a significant superiority ($p\leq 0.05$) for sperm concentration values of the third group at the expense of the other groups in the 2nd month; and also the (T3) and (T4) gave a significant superiority ($p\leq 0.05$) in the 3rd month over (T2) and exceeded not significant on the control group. The findings in the study concur with Anita and Jacyno (2005) that increased sperm concentration in the (T3) and (T4) treated with Vit. E and

Selenium may be due to the role of Vit. E and Selenium directly as antioxidants to get rid of free radicals that cause damage for spermatozoa (Brzezinsks-slebodzinska, *et al*. 1995). Selenium has a key role in the normal development of testicular tissue and improves spermatogenesis. Therefore, deficiency in Selenium leads to impaired function of testicular tissue and thus affects the shape and concentration of sperm (Calvin, *et al*. 1987), where Vit. E protects leydig cells from oxidation compounds, which increases the synthesis and secretion of testosterone by the Leydig cells, thereby developing and improving testicular tissue, which in turn causes sperm synthesis (Flohe, 2007). Lack of Vit. E has led to a decrease in the number of sex cells germ cells, resulting in a decrease in Sperm count. The results were consistent with Yousef, *et al*. (2003) and Hedayati, *et al*. (2009) on the role of Vit. E and Selenium in increasing sperm concentration when Erdinc, *et al*. (1987) found that rams injected at 2-2.5 years of age were 10,000 I.U. of vitamin E daily for two and a half months were good at increasing sperm concentration in rams.

The results of the study in table (2) showed a significant improvement ($p \leq 0.05$) in the mass movement of the sperm of Awassi rams for the (T4) in the 2nd month (84.00 ± 2.94) compared to control (68.75 ± 3.49). The (T2) showed a significant difference ($p \leq 0.05$) in their rates in the third month compared to the (T3) and (T4) and control groups. Increased sperm motility may be attributed to the ability of Vit. E to penetrate the plasma membrane of sperm minimizes damage from free radicals and works to remove the effective types of oxygen (Oxygen Specie Reactive OSR) (Aitken and Clarkson, 1988). Vit. E played an important role in supplying metabolic energy for sperm motility by improving of mitochondrial respiratory efficiency in relation with morphologic alterations of the sperm midpiece, where mitochondria are found, were associated with high mitochondrial functionality and increasing sperm motility (Ener *et al*. 2016). High percentage of live sperm in Vit. E and Selenium treatments compared to the control treatment may be due to their vital role in protecting sperm fat from the formation of peroxides as effective antioxidants within sperm membranes by breaking the chain of peroxides and maintain the integrity of the plasma membrane of the sperm through its presence within Phospholipids membranes spermatozoa also maintains the composition of fatty acids in it by increase its resistance to oxidation (Shiro, 1993).

The results of the statistical analysis in Table (3) recorded the highest percentage of individual movement of sperm ($p < 0.05$) in the rams of the (T4) at the expense of the rest of the experiment treatments in the 3rd month and on the control groups and the (T2) in the 2nd month. (T3) also recorded a significant difference in their rates ($p < 0.05$) compared to the control group rates, specifically in the 2nd month of the experiment.

The results of the study were consistent with Ali, *et al*. (2009) which indicated the role of Vit. E and Selenium in increasing the individual sperm motility of the Awassi rams (Mahmoud, *et al*. 2013). This improvement in individual sperm motility may be attributed to the distinct efficacy of Vit. E in protecting the spermatic plasma membrane from unsaturated acid peroxides while Burk, *et al*. (2007) demonstrated the role of Selenium in reducing the rate of sperm deformations and thus maintaining sperm straightness and leveling. Its regularity in its individual movement and swimming ensures that it reaches the target cell (egg) in the female reproductive system.

The percentage of dead sperm in this study showed a significant decrease ($p < 0.05$) in the percentage of dead sperm in semen treated with the dose as the experiment progressed. At 2nd month, (T4) group recorded significant decreasing in dead sperms ratio in compare with 2nd and control group. This decrease in the dead sperm rates continued to be the lowest in the 3rd month of the experiment, reaching (6.25 ± 0.75) compared to the rest of the experimental groups. The results concur with Soleimani, *et al*. (2009) attributed this decrease to the vital role of Vit. E and Selenium in increasing sperm vitality and reducing dead sperm, this is due to the role of vitamin E in protecting the plasma membrane from unsaturated fatty acids peroxides, while Selenium prevents deformations in the sperm's tail and maintains the integrity of the sperm and its motor and metabolic function. Therefore, there is an important vital functional link between Vit. E in the plasma membrane structure and mitochondrial membrane and its role in preventing damage caused by oxidative agents and free radicals (Mc Dowell, 1989).

The results of Table (4) in the 3rd month specifically showed a significant increase ($p \leq 0.05$) in the normal sperm ratio of the Awassi rams of the three treatment groups compared with the control group. In the 2nd month of the

experiment, the (T4) group compared the control groups and the (T2) group had the highest ($p \leq 0.05$) rates of normal sperm in the whole months of the experiment. Our current study has shown that Vit. E and Selenium have a positive effect on improving normal sperm content with normal tissue structure and reducing dead and deformed sperm. These findings corroborate the findings of Correa and Zavos (1994) that showed a positive correlation with higher sperm motility and a negative correlation with dead and distorted sperm ratio, similar to Burk *et al*. (2007) pointed out that Selenium acts as a co-factor of the phospholipid hydroperoxide glutathione peroxidase (GSH-px), which directly destroys unsaturated fatty acid peroxides; the highest activity of GSH-px is during the differentiation process. Differentiation that occurs to Spermatogonia cells, therefore, Selenium deficiency leads to confusion in the work of the testicular tissue, which affects the shape, concentration and movement of sperm (Flohe, 2007) and helps Selenium to develop normal testicular tissue and improve the process of spermatogenesis (Behne, *et al*. 1982).

The results of the statistical analysis in Table (4) showed that there was a significant decrease ($p \leq 0.05$) in the distorted sperm ratios of the rates of the (T4) group compared with the rest of the groups in the 3rd month and with the control groups and the (T2) in the 2nd month. Our current study shows that vitamin E and Selenium have a positive effect in improving sperm motility and reducing dead and distorted sperm ratio. These findings are consistent with Kupfer, *et al*. (1986) and Mahmoud, *et al*. (2013) which demonstrated the role of Vit. E in reducing the rate of abnormalities in the acrosome by Vit. E on two axes: firstly, increasing the effectiveness of the Superoxide Dismutase (SOD) and the elimination of the root of the superoxide anions, which promotes the production of peroxides that cause deformation of the plasma membrane and the acrosome in the sperm (Khalifa, 1997). Selenium prevents deformations in the tail sperm and maintain the integrity of the sperm and it has motor and metabolic function Mahmoud, *et al*. (2013). This superiority may also be due to the antioxidant efficacy of Vit. E and Selenium, which act as effective free radicals that in turn distort the central sperm. The study of Brzezinsks-slebodzinska, *et al*. (1995) and Marin-Guzman, *et al*. (1997) also indicated that Selenium deficiency causes an increase in sperm count containing Cytoplasmic droplets. Vit. E and Selenium work directly as antioxidants from free radicals that cause sperm damage.

Table 1 – Semen ejaculate volume and pH according to vitamin E and Selenium supplementation (mean ± S.E)

Traits	**Ejaculate Volume / ml**			**Hydrogen Ions Concentration (pH)**		
Treat/months	**1**	**2**	**3**	**1**	**2**	**3**
General mean	**1.48 ± 0.18**	**2.10 ± 0.13 a**	**2.07 ± 0.17 a**	**7.74 ± 0.16**	**7.37 ± 1.17 a**	**7.57 ± 0.15 a**
T1	**1.42 ± 0.21 a**	**2.35 ± 0.29 a**	**2.10 ± 0.24 a**	**7.25 ± 0.25 b**	**7.50 ± 0.28 ab**	**7.25 ± 0.25 ab**
T2	**1.00 ± 0.14 a**	**1.92 ± 0.21 a**	**2.22 ± 0.39 a**	**8.15 ± 0.29 a**	**7.25 ± 0.25 ab**	**7.90 ± 0.10 a**
T3	**1.55 ± 0.49 a**	**1.92 ± 0.24 a**	**1.75 ± 0.34 a**	**8.25 ± 0.25 a**	**6.75 ± 0.28 b**	**7.00 ± 0.40 b**

T4	**1.95 ± 0.49 a**	**2.20 ± 0.33 a**	**2.22 ± 0.35 a**	**7.32 ± 0.23 b**	**8.00 ± 0.40 a**	**8.00 ± 0.00 a**

Means with different superscript in the same column differ significantly ($p < 0.05$)

T1: control , T2 0.1 mg selenium+ 1000 IU vit. E, T3 0.2 mg selenium+ 1000 IU vit. E, T4 1000IU vit. E

Table 2 – Semen sperm concentration and mass motility according to vitamin E and Selenium supplementation (mean ± S.E)

Traits	**sperm concentration ($\times 10^6/cm^3$)**			**Mass Motility Percentage (%)**		
Treat/months	**1**	**2**	**3**	**1**	**2**	**3**
General mean	**2.13 ± 0.27**	**2.93 ± 0.39**	**2.27 ± 0.90**	**46.93 ± 4.89**	**73.43 ± 2.73**	**69.62 ± 1.84**
T1	**2.20 ± 0.31 a**	**2.24 ± 0.45 b**	**2.37 ± 0.90 ab**	**48.00 ± 11.17 a**	**68.75 ± 3.49 b**	**66.25 ± 5.45 b**
T2	**1.32 ± 0.49 a**	**1.72 ± 0.11 b**	**4.23 ± 0.13 a**	**42.00 ± 9.73 a**	**64.75 ± 5.48 b**	**77.50 ± 1.55 a**
T3	**2.22 ± 0.51 a**	**4.83 ± 0.51 a**	**4.83 ± 0.51 a**	**50.29 ± 13.29 a**	**76.25 ± 5.80 ab**	**67.25 ± 2.42 b**
T4	**2.79 ± 0.75 a**	**2.94 ± 0.89 b**	**4.88 ± 1.19 a**	**47.50 ± 8.43 a**	**84.00 ± 2.94 a**	**67.50 ± 1.04 b**

Means with different superscript in the same column differ significantly ($p < 0.05$)

T1: control , T2 0.1 mg selenium+ 1000 IU vit. E, T3 0.2 mg selenium+ 1000 IU vit. E, T4 1000IU vit. E

Table 3 – Semen individual motility and dead sperm percentage according to vitamin E and Selenium supplementation (mean ± S.E)

Traits	**Individual motility percentage**			**Dead sperm percentage**		
Treat/months	**1**	**2**	**3**	**1**	**2**	**3**
General mean	**75.25 ± 2.03**	**76.37 ± 3.79**	**73.62 ± 3.26**	**23.28 ± 3.04**	**13.87 ± 1.32**	**9.12 ± 0.60**
T1	**70.75 ± 4.88 a**	**62.50 ± 7.50 c**	**63.25 ± 5.49 b**	**25.50 ± 5.92 a**	**20.00 ± 2.04 a**	**11.25 ± 1.25 a**

T2	**75.50 ± 4.50 a**	**70.00 ± 5.40 bc**	**66.25 ± 2.3**	**20.50 ± 4.85 a**	**14.75 ± 1.88 ab**	**9.50 ± 0.28 a**
T3	**81.75 ± 3.19 a**	**82.00 ± 6.41 ab**	**74.25 ± 2.24**	**25.15 ± 6.62**	**12.00 ± 2.00 bc**	**9.50 ± 0.86 a**
T4	**73.00 ± 2.48 a**	**91.00 ± 1.35 a**	**90.75 ± 1.04 a**	**22.00 ± 8.77 a**	**8.75 ± 0.75 c**	**6.25 ± 0.75 b**

Means with different superscript in the same column differ significantly ($p < 0.05$)

T1: control , T2 0.1 mg selenium+ 1000 IU vit. E, T3 0.2 mg selenium+ 1000 IU vit. E, T4 1000IU vit. E

Table 4 – Semen normal sperm percentage and abnormal sperm percentage according to vitamin E and Selenium supplementation (mean ± S.E)

Traits	**Normal sperm percentage**			**Abnormal sperm percentage**		
Treat/months	**1**	**2**	**3**	**1**	**2**	**3**
General mean	**72.3 ± 1.58**	**69.50 ± 3.47**	**72.25 ± 2.02**	**15.78 ± 1.75**	**16.37 ± 1.73**	**9.50 ± 1.04**
T1	**70.75 ± 4.88 a**	**57.50 ± 5.95 b**	**61.75 ± 3.11 b**	**17.25 ± 3.79 a**	**21.25 ± 5.15 a**	**9.50 ± 2.90 a**
T2	**71.75 ± 3.19 a**	**63.25 ± 5.13 b**	**77.75 ± 4.49 a**	**15.2 ± 1.84 a**	**20.00 ± 0.00 a**	**11.00 ± 3.10 a**
T3	**75.5 ± 1.25 a**	**71.75 ± 4.44ab**	**71.50 ± 2.59 a**	**17.92 ± 4.42 a**	**14.25 ± 2.52 ab**	**10.25 ± 0.25 a**
T4	**71.50 ± 3.12 a**	**85.50 ± 3.52 a**	**78.00 ± 1.47 a**	**12.7 ± 4.21 a**	**10.00 ± 0.00 b**	**7.25 ± 1.10 b**

Means with different superscript in the same column differ significantly ($p < 0.05$)

T1: control , T2 0.1 mg selenium+ 1000 IU vit. E, T3 0.2 mg selenium+ 1000 IU vit. E, T4 1000IU vit. E

References

Ahmed, W.M., Hanafi, E.M. and Zaabal, M.M (2012). A Trial to Ameliorate the Reproductive Performance of Native Egyptian Cows Suffering from Reduced Fertility. Global Veterinaria. 8 (2): 174-178.

Aitken, R. J. and Clarkson, J. S. (1988). Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J. Andrology, 9: 367 – 376.

Al-Haboby. A., Hamra, A.H.A and Mabdi. A.K. (2003). Effect of Licorice Extract on semen Quality and Libido in Awassi Rams. Arab Authority for Agricultural Investment and Development .first number.

Al-Haboby, A.H., Hamra A.H., Al-Tamemmy, M.J. and Al-Rawi, T.S. (2004). Effect of vitamin E and selenium on semen quality, sexual activity, and some blood parameters of Awassi Rams. J. of Agric. 2: 58-62.

Ali, A.B., BomBoi, G. and Floris, B. (2009). Dose Vitamin E or Vitamin E pus Selenium improve reproductive performance of rams during hot weather. ITAL .J.Anim.Sci.Vol.8, 743-754.

Anita, K. and Jacyno, E. (2005). Effect of selenium and vitamin E supplementation on reproductive performance of young boars. Arch. Tierz. Dummerstorf. 1: 68-75.

Azawi, O.I. and Hussein, E.K. (2013). Effect of vitamins C or E supplementation to Tris diluent on the semen quality of Awassi rams preserved at 5 ˚C. Veterinary Research Forum, 4(3): 157–160.

Behne, D., Hofer, T., Berswordt – Wallrabe, R.V. and Elger, W. (1982). Selenium in the testis of the rat: studies on its regulation and its importance for the organism. J.Nutr., 1682 – 1687.

Brown, K.M. and Arthur, J.R. (2001). Selenium, selenoproteins and human health: a review. Public Health Nutr. 4: 593–599.

Brzezinsks-slebodzinska, E, Slebodzinsk, A.B, Retras, B. and Wieczorek, Q. (1995). Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biol. Trace Elemi. Res., 47: 69-74.

Burk, R.F., Olson, G.E. and Hill, K.E. (2007). Deletion of selenoprotein P gene in the mouse. In: D.L Hatfield, M.J. Berry and V.N. Gladyshev (eds Selenium. Its molecular biology and role in human health. Springer, New York, NY, USA, 111-122.

Calvin, H.I., Grosshans, K., Musicant – Shikora, S.R. and Turner, S.I.A. (1987). Developmental study of rat sperm and testis seleno proteins. J. Report. Fertile. 81: 1-11.

Campbell, R. C., Dott, H. M. and Glover, T. D. (1956). Nigrosin eosin as a stain for differentiating live and dead spermatozoa. J. Agri. Sci., Camb., 48: 1-8.

Correa, J.R. and Zavos, P.M. (1994).The hypo osmotic swelling test: Its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. Theriogenology .42,351-360.

David, I., Bodin, L., Lagriffoul, G., Manfredi, E. and Robert-Granie, C. (2007). Character process model for semen volume in AI rams: evaluation of correlation structures for long and short-term environmental effects. Genet. Sel. Evol. , 39(1):55-71.

Duncan, D. (1955). Multiple Ranges and Multiple F-test .Biometrics, 11:1- 24.

El-Sheshtawy, R.I., Ahmed, W.M., Zaabal, M.M. Ali, G.A. and Shalaby, S.I. (2014). Effect of Selenium and /or Vitamin E Administration on Semen Characteristics, Plasma Testosterone Level and some Immun;2ogenetic Constituents in seminal plasma proteins of Baladi Bucks. Global Veterinaria. 12(6): 878-884.

Ener, K., Aldemir, M., Isik, K., Okulu, E., Ozcan, M.F., Ugurlu, M., Tangal, S. and Ozayar, A. (2016). The impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocelectomy: a randomised controlled study. First International Journal of Andrology Andrologia. 48(7):829-834.

Evans, G. and Maxwell, W.M. C. (1987). Salamones Artificial Insemination of Sheep and Goats. Buffer worth. Sydney. Australia, Pp: 1-194.

Flohe, L. (2007). Selenium in mammalian spermiogenesis. Biol. Chem. 338: 987- 995.

Ghorbani, A., Moeini, M.M., Souri, M. and Hajarian, H. (2018). Influences of dietary selenium, zinc and their combination on semen characteristics and testosterone concentration in mature rams during breeding season. Journal of Applied Animal Research, 46(1): 813–819.

Hedayati, M, Tahmasbi, A, Falah, M. Rad, A. and Vakili (2009). Influence of selenium, vitamin E and Zn on semen quality of Blochi rams. EAAP-60th. Annual Meeting, Barcelona. (Abstract).

Karakus, K., Mert, N., Mert, H., Yörük, I., Aygün, T. and Tariq, M.M. (2016). Relationship between Vitamin Composition and Spermatological Characteristics in Semen of Different Ram Breeds of Turkey. Pakistan J. Zool., 48(4): 969-975.

Khalifa, T.A.A. (1997). Effect of vitamin E and zinc supplementation on sexual behavior and some semen characteristics of buffalo bulls. M.Sc. Thesis, Fac. Vet. Med. Cairo Univ., Egypt.

Koyuncu, M. and Yerlikaya, H. (2007). Effect of selenium-vitamin E injections of ewes on reproduction and growth of their lambs. South African Journal of Animal Science, 37 (3): 233 – 236.

Kupfer, U., Kupfer, S., Bachmann, P., Gaillard, K., and Schwab, W. (1986). Importance of α-tocopherol for A.I. Bulls. Zucht hygiene .21:71-76. 54(1).

Mahmoud, G.B., Abdel-Raheem, S.M. and Hussein, H.A. (2013). Effect of combination of vitamin E and selenium injections on reproductive performance and blood parameters of Ossimi rams. Small Ruminant Research, 113(1):103-108.

Marin-Guzman, J., Mahan, D.C., Chung, Y.K, Pate, J. L. and Pope, W. F. (1997) .Effect of dietary selenium and vitamin E on Boar performance and tissue reportse, semen quality and subsequent fertilization rates in maturegilts. Journal. Animal.

Scinces. 75: 2994-3003.

McDowell, L.R (1989). Vitamin E in: vitamins in animal Nutrition (McDowel), L.R. ed. Academic press, New York: 93-131.

Milovanov, V. K. (1960). Artificial insemination of livestock in the U.S.S.R., a visual-aid album program for scientific translation Jerusalem, pp. 90-105.

Netto, A.S., Zanetti, M.A., Correa, L.B., Del Claro, G.R.,Vieira Salles, M.S. and Vilela, F.G. (2014). Effects of Dietary Selenium, Sulphur and Copper Levels on Selenium Concentration in the Serum and Liver of Lamb. Asian-Australia's J. of Anim. Sci. 27(8): 1082–1087.

SAS (2005). SAS Users Guide: Statistics Version 6th ed.; SAS Institute Inc.; Gry.

Salisbury, G.W., beak, G. H., Elliot. and Willett, E. L. (1943). Rapid method of estimating the number of spermatozoa in bull semen. J. Dairy Sci., 26: 483-486.

Shiro, U. (1993). In: Mino, Nakamura, N., Piplock, A., Kayden, and H., Eds. Vitamin E: its Usefulness in Health and Curing Diseases. Japan Scientific Societies press, pp: 41 – 50.

Soleimani, M.M., Noorafshan, A., Momeni, H.R., Abnosi, M.H., Mahmoodi, M., Anvari, M., Hoseini, S.M. (2009). Stereological study of the effects of Vitamin E on testis structure in rats treated with para-nonylphenol. Asian J. Androl, 11: 508-516.

Yousef, M.I., Abdallah, G.A. and Kamel, K.I., (2003). Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Animal Reproduction Science, 76, 99–111.

Walton, A. (1933). Technique of artificial insemination. Mp. Bur. Anim. Genet., 56, Iiius- Edinburgh.

WHO, (2010). Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization.

Yue, D., Yan, L., Luo, H., Xu, X. and Jin, X. (2010). Effect of vitamin E supplementation on semen quality and testicular cell membrane and mitochondrial antioxidant abilities in Aohan fine-wool sheep. Animal Reproduction Science. 118:217–222.

Zubair, M., Ali, M., Ahmad, M., Sajid, M.S., Ahmad, I. and Gul, S.T. (2015). Effect of Selenium and Vitamin E on cryopreservation of semen and reproductive performance of animals (a review). Journal of Entomology and Zoology Studies, 3 (1): 82-86.