

## **SEXUAL DESIRE, TESTICULAR MEASUREMENTS AND SEMEN QUALITY OF BUFFALO BULLS TREATED WITH COMBINATIONS OF TRACE ELEMENTS AND VITAMIN E.**

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### **ABSTRACT**

A total of twenty healthy Egyptian buffalo bulls with an average of 500 kg live weight and 2.5 - 4.0 years old was used to study the effect of selenium and vitamin E (Se+Vit. E), Se and zinc (Se+Z), Vit. E+Z and Se, Vit. E+Z) on sexual desire, testicular measurements, some physical semen characteristics and liver function of buffalo bulls. The experimental animals were divided into five similar groups (4 bulls each). Bulls in the 1<sup>st</sup> group were left without any treatment and served as a control group (G1). Bulls were i.m. injected with 10 mg Se as sodium selenite and oral dose (100 IU) of Vit. E as  $\alpha$ -tocopherol acetate per head (G2); i.m. injected with 10 mg Se and oral dose (5 g) of zinc oxide per head (G3); oral dose (100 IU) of Vit. E plus 5 g zinc oxide per head (G4); or i.m. injected with 10 mg Se and oral dose (100 IU) of Vit. E plus 5 g zinc oxide per head (G5). All treatments were twice/week for five months experimental period, two months as preliminary period and three as a main collection period. During the main collection period, sexual desire, testicular measurements, semen ejaculate volume (SEV), progressive sperm motility (PSM), sperm abnormality (SAB), sperm cell concentration (SCC) and total sperm output (TSO). Results showed that reaction time (RT) was shorter ( $P<0.05$ ) and serum testosterone concentration was higher in all treated groups than in the control group. The shortest RT (68.92 sec) and the highest testosterone concentration (0.77 ng/ml) were recorded in G4. Values of testicular volume, scrotal circumference and testis tone firmer were higher ( $P<0.05$ ) in all treated groups than in the control group, being the highest in G4 and the lowest in G1. Both SEV and percentage of PSM increased ( $P<0.05$ ) in G2, G4 and G5 as compared to G1. The highest values of SEV and PSM were obtained for G4; however, values of G3 treated with Se+Z did not differ significantly from those in G1. Percentage of SAB reduced ( $P<0.05$ ), while sperm cell concentration (SCC/ml) and total sperm output (TSO/ejaculate) increased ( $P<0.05$ ) in all treated groups as compared to the control one. In conclusion, the current study may indicate beneficial effects of *in vivo* treatment of buffalo bulls with oral dose of Vit. E (100 IU) as  $\alpha$ -tocopherol acetate plus 5 g zinc oxide per head twice/week for two preliminary months and three main semen collection months on sexual desire, testicular measurements and semen quality.

**Keywords:** Buffalo semen, Se, Vit. E, zinc, libido, testis, sperm motility, acrosome.

### **INTRODUCTION**

Selenium (Se) is an essential element because it is an integral component of the enzyme glutathione peroxidase (Erskine, 1993). Tissue concentrations of Se are highly correlated with glutathione peroxide activity and directly related to Se intake (Smith *et al.*, 1979). Vitamin E (Vit. E) is involved in the normal phosphorylation reaction, especially of high energy phosphate compound (ATP), which had an important role for bovine sperm motility and metabolic activity. The dl- $\alpha$ -tocopherol was detected to be involved in the formation of biological membranes including the mitochondrial

membranes (McDowell, 1989) which contain the main machinery for trapping, covering and supply sperm energy (Mann and Lutwak-Mann, 1981). The relationship between Se and Vit. E was focused on as the two nutrients has a synergistic relationship in protection of tissue from peroxide damage. Thus the presence of either nutrient may have sparing action on the other (Hoekstra, 1975). Selenium is incorporated into the mitochondrial capsule thus, affecting the structure development of spermatozoa and other functional aspects (Marin-Guzman *et al.*, 1997). The minimum Vit. E requirement of normal animals is approximately 30 ppm of diet (McDowell, 1989). Moreover, literature survey has failed to reveal sufficient specific recommendation in determination of the optimum allowances of artificial insemination bulls used for semen production.

The importance of zinc (Z) for human and animal appears from the fact that zinc acts as component and activator of over 200 metal enzymes and hormones (Riordon and Valle, 1976). Zinc is essential element for a multitude of body functions, including the acid base balance (Hahn and Baker, 1993), DNA and nutrients metabolism (Banerjee, 1988), immunity protection (Gross *et al.*, 1979), fertility (Apgar and Travis, 1979) and many of other physiological processes. Also, the importance of zinc as co-factors for superoxide dismutase activity (Keen and Graham, 1989) In spite of zinc is widely distributed in feeds (Miller, 1970), a field study indicated that the usual Egyptian rations were lacking in zinc (Attia *et al.*, 1987). The deficiencies of zinc in males have resulted in impaired spermatogenesis and testosterone production. Also, zinc supplementation has been showing its advantages in spermatoc production (Reeves and Odeel, 1988). On the other hand, zinc and tocopherol (Vit. E) have to be supplied via the feed as they can not be produced by the animal itself (Ullrey, 1980).

Therefore the current study aimed to investigate the effect of some combinations of trace elements and Vit. E (Se+Vit. E, Se+Z, Vit. E+Z and Se, Vit. E+Z) on sexual desire testicular measurements and some physical semen characteristics of buffalo bulls.

## **MATERIALS AND METHODS**

The present study was carried out at El-Gemmizah Experimental Research Station, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, in co-operation with Department of Animal Production, Faculty of Agriculture, Mansoura University, during the period from June to October, 2009.

### **Animals and experimental groups:**

A total of twenty healthy Egyptian buffalo bulls with an average of 500 kg body weight and 2.5 - 4.0 years old were used in the present study. The experimental animals were divided into five similar groups (4 bulls each) based on live body weight and age at the beginning of the experimental period. All bulls were clinically free of the external and internal parasites. The testicular tone was glandular, all epididymal regions were present and both

testes were in normal size and moved freely up and down within the scrotal pouches.

Bulls in the 1<sup>st</sup> group were left without any treatment and served as a control group (G1). Bulls were i.m. injected with 10 mg Se in form of sodium selenite (22 mg Na<sub>2</sub>SeO<sub>3</sub>) and oral dose (100 IU) of Vit. E as  $\alpha$ -tocopherol acetate per head (G2); i.m. injected with 10 mg Se and oral dose (5 g) of zinc oxide per head (G3), oral dose (100 IU) of Vit. E plus 5 g zinc oxide per head (G4), or i.m. injected with 10 mg Se and oral dose (100 IU) of Vit. E plus 5 g zinc oxide per head (G5). All treatments were twice/week for five months as an experimental period, two months as preliminary period and three as a main collection period.

**Feeding and management system:**

A constant feeding regime was applied according to the live body weight as recommended by Animal Production Research Institute for buffalo bulls. Every bull was fed daily ration compared of 8 kg concentrate feed mixture (CFM), 6 kg rice straw and 6 kg berseem hay (*Trifolium alexandrinum*). Chemical analysis of different feed stuffs used in the basal ration is presented in Table (1). The ration was given individually to all bulls at 8.0 a.m. and 3.0 p.m., while, fresh water and mineral blocks were available for all bulls at all day times. The bulls were housed individually under semi-open sheds.

**Table (1): Chemical composition of different feed stuffs used in the basal ration.**

Ingredient	Chemical composition (% on DM basis)						
	DM	OM	CP	EE	CF	NFE	ASH
Concentrate mixture	91.50	88.74	15.85	4.70	13.66	54.53	11.26
Rice straw	92.30	79.63	3.47	1.41	35.10	39.65	20.37
Berseem hay	89.00	85.96	15.96	2.92	28.20	38.88	14.04

**Experimental procedures:**

**Sexual desire:**

Sexual desire was determined in term of reaction time (RT) and blood testosterone concentration as described by Chenoweth (1981). The RT as a time elapsed between the exposure of a bull to a suitable stimulus and the first copulation was recorded using stop-watch.

**Testicular measurements:**

They included testicular volume (ml), scrotal circumference (cm) and testis tone firmer (score) and were regularly recorded monthly during the main collection period. Testicular volume was determined by water displacement technique. Scrotal circumference was measured with a flexible, cloth measuring tape around the largest diameter (medial portion) of the testes and scrotum placed after pushing the testes firmly into the scrotum Mickelsen *et al.* (1982). However, testis tone firmer was determined via manual palpation (scored from 1: very soft up to 9: very firm) as described by Wildeus and Hammond (1993).

**Semen collection:**

Semen was collected from each bull in all the experimental groups twice/week by means of an artificial vagina between 8 and 9 a.m. for three months (main collection period). One false mount had been always allowed

before collection of the first ejaculates. One successive ejaculate was obtained from each bull at each day of collection. Semen was collected in a graduated collecting tube containing a double walled glass fitted directly into the bottom of the artificial vagina. Immediately after collection, the ejaculates were transferred to the laboratory and were placed in a water bath at 37°C and care was taken to avoid exposure of the semen to any unfavorable condition during or after collection.

**Semen evaluation:**

Semen ejaculate volume (ml), percentages of progressive sperm motility and sperm abnormalities were determined according to Salisbury *et al.* (1978), while sperm-cell concentration ( $\times 10^9$  ml) was estimated using hemacytometer according to Khan (1994). The total sperm-output per ejaculate was estimated by multiplying the ejaculate volume in ml by the sperm cell concentration per ml.

**Blood samples:**

At the end of the experimental period, blood samples were taken from all animals in each group without anticoagulant and centrifuged after 2-3 h at 3000 rpm for 20 minutes for the separation of serum, which was kept at -20°C until determination of testosterone. Testosterone concentration was estimated by radioimmunoassay (RIA) according to the procedure described by Ekins (1984).

**Statistical analysis:**

Data were statistically analyzed using one way design (ANOVA) to establish the effect of treatment among experimental groups according to Snedecor and Cochran (1982). The differences among means were tested using Duncan's New Multiple Range Test (Duncan, 1955).

The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

## **RESULTS AND DISCUSION**

**Sexual desire:**

Results concerning sexual desire of buffalo bulls presented in Table (2) revealed that reaction time (RT) was significantly ( $P<0.05$ ) shorter and testosterone concentration in blood serum was significantly higher in all treated groups (G2, G3, G4 and G5) than in the control group (G1). The shortest RT (68.92 sec) and the highest testosterone concentration (0.77 ng/ml) were recorded in G4, reflecting the highest sexual desire of buffalo bulls treated with a combination of Vit. E+Z as compared to those treated with Se+E (G2), Se+Z (G3) Se+E+Z (G5) and the control bulls (G1).

Wide variation in reaction time of Egyptian buffalo bulls was recorded by several investigators, being 54.15 sec (El-Hariri, 1973), 109 sec (Mohamed, 1981), 88.1 sec (Osman, 1988), 53.4 sec (Abd El-Latif, 2001) and 58.4 sec (El-Siefy, 2004). In accordance with the present results regard to RT, El-Siefy (2004) found that treatment with Se, E or Se+E improved the RT of buffalo bulls. However, the best results were recorded for the treatment with a combination of Se and E, being significantly ( $P<0.01$ ) shorter (48.6

sec) than for those treated with Se (53.7 sec) and E (72.9 sec). Similar results were reported by Abd El-Latif (2001) who showed that RT significantly decreased with Se treatment of buffalo bulls as compared to controls.

**Table (2): Effect of treatment on reaction time (second) and testosterone concentration (ng/ml) in blood serum of buffalo bulls.**

Experimental group	Reaction time (second)	Testosterone (ng/ml)
G1(Control)	103.58±3.61 <sup>a</sup>	0.53±0.04 <sup>d</sup>
G2 (Se+ E)	82.04±2.60 <sup>b</sup>	0.67±0.03 <sup>b</sup>
G3 (Se + Z)	85.04±2.16 <sup>b</sup>	0.60±0.03 <sup>c</sup>
G4 (E + Z)	68.92±2.14 <sup>d</sup>	0.77±0.04 <sup>a</sup>
G5 (Se + E + Z)	75.02±2.30 <sup>c</sup>	0.65±0.03 <sup>bc</sup>

a, b, c and d: Means denoted within the same column with different superscripts are significant at P≤0.05. Se: Selenium, E: Vitamin E and Z: Zinc.

Also, Khalifa (1997) reported that the addition of dietary zinc led to greatly decrease in the RT of buffalo bulls. Moreover, Khalifa (1997) demonstrated that the RT of buffalo bulls significantly decreased after supplementation with Z or Z+E.

In Egyptian buffalo bulls, Gabr (2000) demonstrated that the overall mean of testosterone concentration significantly increased from 0.7 ng/ml before zinc addition to 0.9 ng/ml post zinc addition in treated animals. Also, Ibrahim *et al.* (1996) found that Vit. E supplementation led to increase of the testosterone concentration in Friesian blood serum from 1.39 to 2.21 ng / ml and found positive correlation between testosterone and Vit. E concentration in blood serum. Abd El-Latif (2001) found increase in testosterone concentration of buffalo bulls treated with Se, E or Se+E during the pretreatment period. Moreover, El-Siefy (2004) found that the overall means of testosterone concentration in blood serum of Egyptian buffalo bulls were 0.63, 0.58 and 0.71 (ng/ml) for Se, E and Se+E groups, respectively. The differences among treated groups were not significant.

The benefits of testosterone on sexual desire may attributed to that it helps to build protein and is essential for normal sexual behavior and producing erections (Hafez, 1987). The effect of the injected Se on the anterior pituitary hormone secretion in cattle was also confirmed by Youssef *et al.* (1990) and Se seems to be has a further biological function in steroidogenesis of the Leyding cells (Ibrahim *et al.*, 1996). This based on the fact that glandular tissues especially the pituitary gland have the greatest Se concentration which have several specific metabolic functions on the leyding cells of the testes in producing testosterone hormone (Shamberger, 1983).

**Testicular measurements:**

Results of testicular measurements shown in Table (3) cleared that values of testicular volume (TV), scrotal circumference (SC) and testis tone firmer (TF) were significantly (P<0.05) higher in all treated groups (G2, G3, G4 and G5) than in the control group (G1), being the highest in G4 and the lowest in the control group (G1).

**Table (3): Effect of treatment on testicular volume (ml), scrotal circumference (cm) and testis ton firmer (score) of buffalo bulls.**

Experimental group	Testicular volume (ml)	Scrotal circumference (cm)	Testis tone firmer (score 1-7)
G1 (Control)	441.9±17.92 <sup>c</sup>	30.45±0.43 <sup>c</sup>	6.65±0.20 <sup>c</sup>
G2 (Se + E)	510.0±12.06 <sup>b</sup>	31.13±0.45 <sup>bc</sup>	6.92±0.20 <sup>bc</sup>
G3 (Se + Z)	482.7±8.00 <sup>b</sup>	31.85±0.36 <sup>b</sup>	7.33±0.27 <sup>b</sup>
G4 (E + Z)	609.2±17.70 <sup>a</sup>	33.07±0.28 <sup>a</sup>	8.00±0.11 <sup>a</sup>
G5 (Se + E + Z)	501.5±5.33 <sup>b</sup>	31.33±0.47 <sup>bc</sup>	7.28±0.10 <sup>b</sup>

a, b and c: Means denoted within the same column with different superscripts are significant at  $P \leq 0.05$ . Se: Selenium, E: Vitamin E and Z: Zinc.

These results were associated with the highest sexual desire of buffalo bulls treated with a combination of E+Z as compared to those treated with Se+E (G2), Se+Z (G3) Se+E+Z (G5) and the control bulls (G1).

Oldham *et al.* (1978); David and Fels (1984) and Lindsay *et al.* (1984) reported that testicular volume in bulls injected with Se+E was lower than that of bulls treated with Vit. E or Se. Also, Surai and Ionov (1992) reported that supplementation of male geese with 20 – 40 IU Vit. E/kg ration resulted in a significant increase in the weight of testis. In Egyptian buffalo bulls, Abd El-Latif (2001) found that average of TV was higher in groups treated with E or Se (594 and 570 ml, respectively) than in group treated with Se+E (503 ml). However, SC increased in response to the injection of Se or E, while Se+E seemed to have an opposite effect. Average score of testis tone post-treatment increased in proportional to pretreatment period for Se, E or Se+E treated groups, respectively.

#### Physical semen characteristics:

Results concerning physical semen characteristics of buffalo bulls shown in Table (4) revealed that semen ejaculate volume (SEV) and percentage of progressive sperm motility (PSM) significantly ( $P < 0.05$ ) increased for bulls in G2, G4 and G5 as compared to the control (G1). The highest values of SEV and PSM were obtained for G4; however, values of G3 treated with Se+Z did not differ significantly from those in the control (G1).

On the other hand, percentage of sperm abnormalities (SAB) significantly ( $P < 0.05$ ) reduced, while sperm cell concentration (SCC/ml) and total sperm output (TSO/ejaculate) significantly ( $P < 0.05$ ) increased in all treated groups as compared to the control group. It is of interest to note that the highest TSO in G4 was associated with the highest SEV and SCC as compared to the other groups. Generally, bulls in G4 treated with E+Z showed the best physical semen characteristics as compared to other treated groups and the control group.

In this respect, similar results were reported for the improvement in SEV of Vit. E+Z addition. In buffalo bulls, El-Siefy (2004) showed that SEV of buffalo bulls was larger ( $P < 0.05$ ) in Vit. E (2.9 ml) than in Se (2.4 ml) or Se+E (2.75 ml) groups. Other authors noted that SEV increased ( $P < 0.05$ ) with dietary supplementation of zinc (Salantiu *et al.*, 1981 and Petryankin *et al.*, 1987). Also, Khalifa (1997) found that treatment with Z and/or Vit. E

increased ( $P<0.05$ ) the SEV for three months after treatment. Moreover, Gabr (2000) found that the SEV of zinc treated group was higher ( $P<0.05$ ) than of the control group (3.49 vs. 3.0 ml).

**Table (4): Effect of treatment on physical semen characteristics of buffalo bulls.**

Group	Ejaculate volume (ml)	Progressive sperm motility (%)	Abnormal sperm (%)	Sperm cell concentration ( $\times 10^9$ /ml)	Total sperm output ( $\times 10^9$ /ejac.)
G1 (Control)	2.19 $\pm 0.10^c$	60.52 $\pm 0.87^c$	19.27 $\pm 0.75^a$	0.95 $\pm 0.02^a$	2.00 $\pm 0.08^d$
G2 (Se + E)	2.57 $\pm 0.07^{ab}$	65.36 $\pm 0.91^a$	16.12 $\pm 0.67^b$	1.22 $\pm 0.03^d$	3.11 $\pm 0.09^c$
G3 (Se + Z)	2.37 $\pm 0.09^{bc}$	59.79 $\pm 1.00^c$	16.20 $\pm 0.64^b$	1.28 $\pm 0.02^c$	3.04 $\pm 0.15^c$
G4 (E + Z)	2.80 $\pm 0.08^a$	64.38 $\pm 0.94^{ab}$	14.74 $\pm 0.60^b$	1.50 $\pm 0.03^a$	4.21 $\pm 0.15^a$
G5 (Se + E + Z)	2.65 $\pm 0.07^a$	62.86 $\pm 0.88^b$	15.59 $\pm 0.34^b$	1.37 $\pm 0.03^b$	3.65 $\pm 0.12^b$

a, b, c, d and e: Means denoted within the same column with different superscripts are significant at  $P\leq 0.05$ . Se: Selenium, E: Vitamin E and Z: Zinc.

Progressive sperm motility is one of the most important semen quality tests because fertility is highly correlated with the percentage of motile spermatozoa (Kammeror *et al.*, 1972). In accordance with results of PSM, Khalifa (1997) found that PSM in buffalo semen was higher ( $P<0.01$ ) in Vit. E+Z supplemented group than in the control one (74.27 vs. 69.72%). Also, Gabr (2000) reported that PSM in buffalo semen was higher ( $P<0.05$ ) post- than pre- zinc supplementation (66.1 vs. 55.2%). In bull semen, Udala *et al.* (1995) observed that PSM in fresh bull semen improved ( $P<0.05$ ) in group received Vit. E as compared to Se+Vit. E. Similar results were reported by Al-Gindy (2001) in rams.

The presence of an excessive number of abnormal spermatozoa in the ejaculate will reduce the probable fertilizing capacity of sperm. The percentage of SAB has been found to be negatively correlated with fertility, which usually is not affected until the level of SAB exceeds 20-25% (Abd El-Ghaffar, 1992). The beneficial effect of Vit E on SAB in working on Egyptian buffalo bulls was reported by several authors (Al-Gindy, 2001; Abd El-Latif, 2001 and El-Siefy (2004). Also, Erdinc *et al.* (1986) demonstrated that the addition of 60 mg Vit. E to a basal ration decreased SAB of ram semen to 9.28% as compared to 15.9% in the control. On the other hand, addition of dietary zinc led to significantly improved in the abnormalities of Egyptian buffalo spermatozoa. In this respect, Misra *et al.* (1989) detected a significant positive correlation between percentage of SAB and zinc concentration in buffalo semen. Gabr (2000) demonstrated that the mean values of SAB for the control and buffalo bulls treated with zinc were 21.2 and 13.5%, respectively. Recently, Biomy (2008) reported that zinc oxide supplementation (75 or 150 ppm) led to marked decrease ( $P<0.05$ ) in SAB as compared to un-supplemented rabbits. In addition, the superior effect of

supplementation of buffalo bulls with Z+Vit. E on reducing the percentage of total SAB was reported by Khalifa (1997).

Also, SCC and TSO of Egyptian buffalo bulls were found to be improved by addition of Vit. E, Z or Vit. E+Z. In this line, Abd El-Latif (2001) found that Vit E led to improve of TSO per ejaculate. El-Siefy (2004) found that TSO was higher ( $P<0.05$ ) in buffalo bulls treated with Vit. E ( $3.8 \times 10^9$ ) than those treated with Se+E ( $3.43 \times 10^9$ ). Also, several authors showed that dietary zinc addition increased ( $P<0.05$ ) SCC and TSO per ejaculate of Egyptian buffalo bulls (Ibrahim and Yousri, 1992 and Gabr, 2000). Similar trend of SCC and TSO per ejaculate by zinc addition was reported in rabbits (Biomy, 2008). Furthermore, Khalifa (1997) reported that supplementation of Egyptian buffalo bulls with zinc and/or Vit. E led to a significant increase in SCC and TSO per ejaculate. The maximum rate of increase was found in Z+Vit. E treated bulls. Moreover, Misra *et al.* (1989) and El-Anwar and Badr (1996) detected a significant positive correlation between sperm concentration and zinc level in the buffalo. The impact of Vit. E on spermatogenesis was demonstrated by McDowell (1989), who indicate that Vit. E is thought to be exerted a pronounced stimulatory influence on biosynthesis and metabolism of prostaglandin including  $\text{PGF}_2\alpha$  in rabbit testis. Also,  $\text{PGF}_2\alpha$  was found to increase SCC in bull semen by acceleration of sperm passage from testes to the ejaculate El-Azab *et al.* (1996).

In conclusion, the current study may indicate beneficial effects of *in vivo* treatment of buffalo bulls with oral dose of Vit. E (100 IU) as  $\alpha$ -tocopherol acetate plus 5 g zinc oxide per head twice/week for two preliminary months and three main semen collection months on sexual desire, testicular measurements and semen quality.

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### الرغبة الجنسية و مقاييس الخصية وجودة السائل المنوي لطلاق الجاموس المعاملة بخليط من المعادن النادرة وفيتامين هـ .

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استخدم في هذه الدراسة عشرون طلوقة جاموسي تتراوح أعمارها من ٢,٥-٤ سنوات بمتوسط وزن حتى ٥٠٠ كجم بغرض دراسة تأثير معاملة تلك الطلاق بـ السيلينيوم+فيتامين هـ (٢ج)، السيلينيوم+زنك (٣ج)، فيتامين هـ+زنك (٤ج) والسيلينيوم+فيتامين هـ+زنك (٥ج) على الرغبة الجنسية و مقاييس الخصية وجودة السائل المنوي. قسمت الحيوانات المعاملة (٢ج-٥ج) إلى خمس مجموعات متشابهة بكل منها أربع حيوانات، وكانت المجموعة الأولى "مقارنة" بدون معاملة (١ج)، المجموعة الثانية تم حقن كل طلوقة بـ ١٠ ملجم سيلينيوم في العضل في صورة سليلات الصوديوم بالإضافة الي ١٠٠ وحدة دولية من فيتامين هـ في صورة الفاتوكوفيرول عن طريق التجريع، المجموعة الثالثة تم حقن كل طلوقة ١٠ ملجم سيلينيوم في العضل بالإضافة الي ٥ جم أكسيد زنك عن طريق التجريع، المجموعة الرابعة تم تجريع كل طلوقة ١٠٠ وحدة دولية من فيتامين هـ بالإضافة الي ٥ جم أكسيد زنك، كما تم إعطاء كل طلوقة في المجموعة الخامسة ١٠ ملجم سيلينيوم حقن عضلي بالإضافة الي ١٠٠ وحدة دولية من فيتامين هـ + ٥ جم أكسيد زنك عن طريق التجريع. وتم إعطاء كل المعاملات بمعدل مرتين فيسبوع لمدة خمسة أشهر طول فترة التجربة و التي اعتبرت الثلاثة أشهر الأخيرة منها هي الفترة الرئيسية و التي أخذت خلالها القياسات التالية: الرغبة الجنسية (وقت التجاوب الجنسي + تركيز التستوستيرون) ، مقاييس الخصية (حجم الخصية-محيط كيس الصفن وملمس الخصية) والاختبارات الطبيعية للسائل المنوي (حجم القنفة-الحيوية-نسبة الحيوانات المنوية الشاذة-تركيز الحيوانات المنوية/مل-إجمالي تركيز الحيوانات المنوية/قنفة). أظهرت النتائج وجود اختلافات معنوية علي مستوى ٥% لكل من قياسات الرغبة الجنسية في المجموعات المعاملة مقارنة بمجموعة الكنترول وأظهرت مجموعة السيلينيوم+فيتامين هـ أقل وقت للتجاوب الجنسي (٦٨,٩٢ ثانية) وأعلي تركيز لهرمون التستوستيرون (٠,٧٧ نانو جرام / مل). أظهرت الدراسة أيضا وجود اختلافات معنوية علي مستوى ٥% لحجم الخصية-محيط الصفن-ملمس الخصية لكل المجموعات المعاملة مقارنة بمجموعة الكنترول حيث سجلت أعلى القياسات في المجموعة الرابعة و أقلها في المجموعة الأولى (الكنترول). أشارت النتائج إلي وجود اختلافات معنوية علي مستوى ٥% في حجم القنفة و كذلك الحركة التقدمية للحيوانات المنوية، وسجل أكبر حجم للقنفة وأعلي نسبة للحركة التقدمية وأعلي انخفاض للنسبة المنوية للحيوانات المنوية الشاذة وزيادة إجمالي تركيز الحيوانات المنوية/قنفة في المجموعة الرابعة. و نستخلص من النتائج التأثير الإيجابي لتجريع طلاق الجاموس بـ ١٠٠ وحدة دولية من فيتامين هـ في صورة الفاتوكوفيرول أسيتات بالإضافة الي ٥ جم أكسيد زنك/طلوقة مرتين في الأسبوع لمدة ثلاثة أشهر علي تحسن الرغبة الجنسية و مقاييس الخصية وجودة السائل المنوي.

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