

## EFFECT OF INJECTABLE SELENIUM ON QUALITY AND FREEZABILITY OF EGYPTIAN BUFFALO SEMEN

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### ABSTRACT

Three sexually mature and clinically normal buffalo bulls were used in this experiment to study the effect of injectable selenium (Se) on quality, and freezability of Egyptian buffalo semen and on plasma testosterone concentration. The bulls were almost at the same age (3.5 years) and body weight (550 kg) at the beginning of the experiment. The data were collected throughout a 12 weeks period. This time interval comprised a pre-treatment period of four weeks (control) and a supplementary period of 8 weeks. During the supplementary period, each bull was injected intramuscularly with 10 mg Se twice weekly. The semen was collected by means of artificial vagina twice weekly. Twenty four ejaculates collected during pre-treatment period and 48 ejaculates collected during Se supplementation period were extended in Tris-egg yolk-glycerol extender and packed into mini straws (0.25 ml). After 4 h equilibration at 5°C, these straws were frozen in vapour of LN<sub>2</sub> and stored for 24 h at -196°C before thawing and evaluation. The supplementary Se significantly ( $P < 0.05$ ) increased ejaculate volume (2.8 vs. 2.06 ml), live sperm (69.8 vs. 61.3%), sperm concentration ( $1.41$  vs.  $1.03 \times 10^6$ /ml) and sperm output ( $4.0$  vs.  $2.11 \times 10^6$ ) per ejaculate and significantly ( $P < 0.05$ ) decreased sperm abnormalities (10.4 vs. 15.0%) as compared to the pre-treatment period. In addition, treatment with injectable Se increased fructose and Se concentrations in semen. Also, supplementary Se significantly ( $P < 0.05$ ) increased blood serum testosterone (0.695 vs. 0.26 ng/ml). The treatment with Se resulted in higher ( $P < 0.05$ ) frozen-thawed motility and live spermatozoa compared to the pre-treatment period. It is concluded that injection of 10 mg Se twice weekly should be considered adequate for improvement of quality and freezability of Egyptian buffalo semen.

**Keywords:** Buffalo, selenium, semen, freezability.

### INTRODUCTION

Selenium (Se) is an essential trace element. Alvarez and Storey (1992) reported that spermatozoa have the capability to generate high levels of reactive oxygen species (ROS) which can reduce the viability and fertility. However, small amount of ROS are necessary for the initiation of critical functions, such as capacitation and acrosome reaction induction (Lamirande and Gagnon, 1993). Therefore, a balance between ROS production and antioxidant protection is necessary to assure normal sperm function. The antioxidant protection of semen is provided by enzymes such as superoxidase dismutase, glutathione peroxidase (GPX) and catalase and other substances (albumin, glutathione, taurine and hypotaurine) contained within the sperm cells or in the seminal plasma (Lewis *et al.*, 1997). Selenium has an important metabolic role as a co-factor of the enzyme glutathione peroxidase which is considered one of the antioxidant defense system in the body. Selenium is also incorporated into the mitochondrial capsule thus, affecting the structural development of spermatozoa (Marin-Guzman *et al.*, 1997) and other functional aspects. Little conflicting informations are available concerning the effect of Se on male fertility, Hassan Omaira (1994) reported

that injectable Se led to increase individual sperm motility, sperm cell concentration, live sperm percentage and reduce sperm abnormalities, while it has no effect on ejaculate volume of Egyptian buffalo semen. In rams, Al-Gindy (2001) found that supplementation of Se did not affect sperm concentration, percentage of viable sperm and sperm abnormalities, while it increased ejaculate volume. In Egyptian buffalo bulls, El-Siefy (2004) found that injection of Se improved all semen physical characteristics, freezability and fertility of buffalo spermatozoa. The aim of the present study was to investigate the effect of injectable Se on semen quality and freezability of buffalo semen.

## **MATERIALS AND METHODS**

### **Experimental animals and management:**

The current work was conducted at Mehallet Moussa Buffalo Experimental Station, Animal Production Research Institute, Ministry of Agriculture. Three healthy and sexually mature buffalo bulls were used in this study. The average age and body weight of the bulls were 3.5 years and 550 kg, respectively. The bulls were individually penned in 4 x 5 meters adjacent boxes with counter-asbestos sheds of 4 meters height. Throughout the experimental period, the animals were kept under the normal feeding and management conditions applied on the farm for dry feeding season. During the dry feeding season (from June 1<sup>st</sup> to August 31<sup>st</sup>, 2004) each bull received a daily ration of 5.5 kg concentrated mixture cubes, 6 kg rice straw and 2 kg berseem hay. The concentrate cubes contained 48% decorticated cotton-seed cake, 21.5% wheat bran, 20% maize, 4.5% rice polish, 3% molasses, 2% lime stone and 1% sodium chloride. The bulls were allowed to drink water twice daily. In addition, they also had regular exercise and daily washing under the running water.

### **Experimental design:**

Animals were fed their dietary requirements for a preliminary period of 4 weeks during June month. This preliminary period served as the control for the subsequent treatment. Starting from July, all buffalo bulls were intramuscularly injected with 10 mg selenium (Se, as sodium selenite, ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) per head twice weekly and continued until the end of the study (8 weeks). Semen was collected twice weekly with an artificial vagina. Ejaculate volume was measured to the nearest 0.1 ml using a graduated collection tube. Percent sperm individual motility was estimated to the nearest 5% on a bright field stage microscope (at 38°C) and a magnification of 450 x. Percent live sperm was estimated using the eosin-nigrosin staining technique (Barth and Oko, 1989). The percentage of eosinophilic (unstained) cells was calculated from a total number of 200 spermatozoa using a magnification of 650x. Abnormal sperm percentage was estimated on the same smears prepared for live/dead counts. Sperm cell concentration per ml was determined according to the conventional procedure described by Sorensen (1979) using the improved type of Neubour haemocytometer. The sperm concentration per ejaculate was calculated by multiplying the ejaculate volume (ml) by sperm

concentration/ml. Acrosome integrity percentage was determined by using a Gimsa stain procedure as described by Watson (1975). Some chemical components of buffalo seminal plasma were measured for the first three weeks of pre-treatment period and for the 2<sup>nd</sup>, 5<sup>th</sup> and 8<sup>th</sup> weeks of treatment period. Initial fructose was measured according to technique adopted by Ashwall (1957), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured using the method described by Schmidt and Schmidt (1963), total protein(g/100 ml) and total cholesterol (mg/dl) were evaluated according to Gornall *et al.* (1968) and Allain (1974). Selenium concentrations ( $\mu\text{g/ml}$ ) in the whole semen as well as in blood plasma were assessed by atomic absorption spectrophotometer. Plasma testosterone concentration (ng/ml) was determined using testosterone ( $^{125}\text{I}$ ) coated tube RIA kits (Orion Diagnostic, Finland).

**Freezing procedure:**

Only samples with 65% of spermatozoa exhibiting progressive motility were diluted with Tris-egg yolk extender at 34°C to yield a concentration of  $20 \times 10^6$  sperm/ml. The chemical components of the extender were: 3.61 g Tris [(hydroxymethyl) amino-methane], 1.89 g citric acid, 20 ml egg yolk, 5 ml glycerol, 0.25 g lincomycin, 0.005 g streptomycin and completed with distilled water to 100 ml. Only 15 out of 24 ejaculates collected during preliminary period and 48 out of 48 ejaculates collected during supplementary. Selenium were extended with Tris extender and placed into a refrigerator at 5°C for 4 hrs, for equilibration. 0.25 ml straws filled with equilibrated semen and then frozen in the vapours of LN<sub>2</sub>. These straws were stored in the LN<sub>2</sub> at -196°C for 24 h before thawing and evaluation. After 24 h the frozen semen was thawed by dipping the frozen straws into a water bath at 37°C for 30 sec., then the percentage of progressive motile spermatozoa and live spermatozoa were estimated.

**Statistical analysis:**

Data obtained were statistically analysed using General Linear Models procedure adapted by SPSS (1997) for User's Guide.

## **RESULTS AND DISCUSSION**

### **1. Physical characteristics of buffalo semen:**

Results presented in Table (1) show that injection of Se (10 mg) had a beneficial effect on semen physical quality by increasing ejaculate volume (35.9%), live sperm percentage (13.5%), sperm concentration (36.9%) and total sperm number/ejaculate (89.6%) and decreasing the percentage of abnormal spermatozoa (30.7%). Advanced sperm motility, and acrosome integrity percentage were not affected significantly by Se treatment. El-Siefy (2004) found that the supplementation of 10 mg Se per head twice weekly in summer and winter season significantly increased semen volume, sperm mass motility and advanced motility, sperm live percentage, sperm concentration, total sperm output, acrosome integrity and decreased sperm abnormalities. The effect of Se supplementation on quality of semen may be attributed to the fact that sufficient Se is required for the normal spermatogenesis and sperm motility (Wu *et al.*, 1973) which may explain the

increase in sperm motility, sperm cell concentration and sperm output per ejaculate. Wallace *et al.* (1989) recorded a pronounced reduction in sperm count of severely Se-deficient mice. In agreement with our results, Hassan Omaira (1994) found that individual buffalo sperm motility increased significantly from 40% at the 3<sup>rd</sup> week up to 60% at the 7<sup>th</sup> week and remained high until the end of experiment. In addition, sperm cell concentration was significantly increased by the repeated doses of Se from 524 at the 2<sup>nd</sup> week up to  $940 \times 10^6$ /ml spermatozoa at the 8<sup>th</sup> wk of the experiment. Abd El-Latif (2001) found that treatment with Se was superior in sperm concentration in buffalo bulls. Also, Marin-Guzman *et al.* (1997) found that boars fed diets low in Se had a greater detrimental effect on the percentage of sperm motility than diets inadequate in vitamin E.

## **2. Chemical semen characteristics:**

The effect of injectable Se on some chemical components of buffalo seminal plasma is presented in Table 2. Treatment with Se tend to decrease the level of GOT and GPT in buffalo seminal plasma ( $48.3 \pm 2.05$  and  $23.3 \pm 1.4$  (U/L), respectively than those in pre-treatment period ( $54.8 \pm 2.03$  and  $26.91 \pm 1.56$  (U/l), respectively. Also, the present results clearly showed that the GOT activity in buffalo seminal plasma was greatly higher than that of GPT. Such results are in agreement with the finding of El-Shamãa (2002). The lower release of GOT and GPT enzymes in the seminal plasma during supplementary Se period may be due to the fact that Se is able to maintain cell membrane of buffalo spermatozoa. However, Hassan Omaira (1994), Abd El-Latif (2001) and El-Siefy (2004), they found that treatment with Se led to increase GOT and GPT in Egyptian buffalo seminal plasma.

Data presented in table 2 indicated that semen from injectable bulls had greater ( $P < 0.05$ ) fructose concentration (359.4 mg/100 ml) in the semen collected during the treatment period than that collected in pre-treatment period (314.5 mg/100 ml). This finding may be attributed to a higher activity of accessory sexual glands as a result of effect of treatment. The present increase of fructose concentration due to Se treatment is consistent with the finding of El-Siefy (2004). Selenium treatment increased cholesterol and total protein concentration during treatment period compared to the pre-treatment period, but differences were not significant. Selenium concentration in the seminal plasma during the supplementary period ( $14.5 \pm 2.3$  µg/ml) was two folds (226.6%) greater than its level during the pre-treatment period ( $6.4 \pm 1.3$  µg/ml). This finding is in complete agreement with the results reported by El-Siefy (2004). Selenium concentration in the ejaculated semen exceeded its blood concentration during pre-and supplementary Se period by 21.1 and 33.4 folds, respectively. However, the overall mean of Se level in the blood serum during supplementary Se period (0.434 µg/ml) was 1.43 times its level during the pre-treatment period (0.303 µg/ml). The tremendously higher levels of Se in the ejaculated semen as compared with its levels in the blood may suggest that Se circulating in the blood is continually trapped by the target organs of male reproductive tract, mainly tissues of testis and epididymis and seminal vesicle secretion (Kantola *et al.*, 1988 and Saaranen *et al.*, 1989). The present values completely agree with those of El-Siefy (2004) but, they were higher than the finding of Al-Gindy (2001) in rams.

Table (1): Effect of injectable Se on buffalo semen physical characteristics.

Item	Pre-treatment period (weeks)				X ± SE	Treatment period (weeks)								X ± SE
	Wk1	Wk2	Wk3	Wk4		Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	
Ej. volume (ml)	2.0± 0.29	2.1± 0.33	2.3± 0.21	1.83± 0.11	2.06± 0.12	2.8± 0.58	2.9± 0.55	3.2± 0.94	3.1± 0.54	2.0± 0.18	2.9± 0.41	2.7± 0.33	3.0± 0.63	2.8± 0.19
Sperm conc. x 10 <sup>6</sup> /ml	1.12± 0.23	1.1± 0.3	0.91± 0.15	0.98± 0.4	1.03± 0.3	1.49± 0.11	1.46± 0.13	1.55± 0.18	1.5± 0.16	1.3± 0.17	1.3± 0.13	1.31± 0.24	1.37± 0.11	1.41± 0.3
Total sperm output x 10 <sup>6</sup>	2.22± 0.33	2.35± 0.48	2.09± 0.29	1.70± 0.23	2.11± 0.17	4.34± 1.0	4.23± 1.2	4.77± 1.3	4.65± 0.96	2.6± 0.47	3.77± 0.61	3.54± 0.80	4.11± 0.10	4.00± 0.33
Advanced motility (%)	40± 3.4	70.8± 3.9	69.6± 6.2	68.3± 4.5	69.7± 2.3	62.5± 5.2	69.2± 7.2	76.7± 2.4	72.5± 3.8	69.2± 2.3	70.9± 3.9	70.0± 1.2	71.7± 2.7	71.2± 1.4
Live sperm %	58.8± 6.1	64.3± 5.1	56.8± 8.4	65.3± 10.06	61.3± 3.6	62.7± 7.1	70.7± 6.1	73.0± 3.8	72.7± 5.1	70.0± 4.4	69.2± 5.3	68.5± 3.2	70.0± 4.5	69.6± 1.7
Sperm abnormalities %	15.3± 1.7	17.2± 1.2	13.5± 1.3	14.0± 0.63	15.0± 0.66	9.5± 0.42	10.5± 0.42	10.5± 0.67	10.7± 0.67	10.8± 0.70	10.2± 0.47	10.5± 0.61	10.7± 0.91	10.4± 0.21
Acrosome integrity %	90.8± 0.9	92.3± 1.08	88.33± 1.7	90.7± 2.4	90.5± 0.82	90.8± 5.7	91.5± 5.1	95.3± 3.4	94.8± 2.0	92.7± 1.5	94.0± 1.03	95.67± 0.76	94.7± 0.92	93.6± 0.51

Means with different small letters within each row are statistically different at 0.05 level  
 Means with different capital letters within each row are statistically different at 0.05 level

Table (2): Effect of injectable Se on some chemical components of buffalo seminal plasma.

Item	Pre-treatment period (weeks)			Treatment period (weeks)								Overall mean ± SE	
	1 <sup>st</sup> wk	2 <sup>nd</sup> wk	3 <sup>rd</sup> wk	1 <sup>st</sup> wk	2 <sup>nd</sup> wk	3 <sup>rd</sup> wk	4 <sup>th</sup> wk	5 <sup>th</sup> wk	6 <sup>th</sup> wk	7 <sup>th</sup> wk	8 <sup>th</sup> wk		
GOT (U/L)	60.0±5.23	52.0±5.55	54±5.34	48.7±1.7 ab	53±3.5 a	43±2.6 b	48.3±2.05	48.3±2.05	48.3±2.05	48.3±2.05	48.3±2.05	48.3±2.05	48.3±2.05
GPT (U/L)	31.7±1.45 a	28.3±1.1 a	22.0±2.38 b	26±3.2	21±1.6	23±2.6	23.3±1.4	23.3±1.4	23.3±1.4	23.3±1.4	23.3±1.4	23.3±1.4	23.3±1.4
Fructose (mg/100 ml)	307.8±35.1	300.5±9.5	333.8±6.9	337.7±11.3	374.3±10.6	366±15.9	359.4±8.3 A	359.4±8.3 A	359.4±8.3 A	359.4±8.3 A	359.4±8.3 A	359.4±8.3 A	359.4±8.3 A
Cholesterol (mg/dl)	54.0±6.7	59.9±10.2	69.8±5.9	44.7±8.2 a	85.5±3.9 b	82.5±14.7 b	70.9±7.7	70.9±7.7	70.9±7.7	70.9±7.7	70.9±7.7	70.9±7.7	70.9±7.7
Total protein (gm/100 ml)	3.31±0.92	3.63±0.24	3.25±0.32	3.63±0.5	4.2±0.65	3.95±0.31	3.67±3.13	3.67±3.13	3.67±3.13	3.67±3.13	3.67±3.13	3.67±3.13	3.67±3.13
Se concentration (µg/ml)	7.2±2.6	6.2±1.9	6.2±2.6	14.2±2.6	14.6±2.4	14.7±1.8	14.5±2.3 A	14.5±2.3 A	14.5±2.3 A	14.5±2.3 A	14.5±2.3 A	14.5±2.3 A	14.5±2.3 A

Means with different small letters within each row are statistically different at 0.05 level  
 Means with different capital letters within each row are statistically different at 0.05 level

The level of testosterone hormone in buffalo blood serum as well as the fructose concentration in the whole semen increased significantly ( $P < 0.05$ ) due to Se treatment (Table 2 and 3). This finding agree with the results obtained by Hassan Omaima (1994), Abd El-Latif (2001) and El-Siefy (2004). The former authors suggested that Se seems to be have a further biological function in steriodogenesis of the leydig cells. In the same trend, the results obtained by Youssef *et al.* (1990) confirmed that the injected Se affect the anterior pituitary hormones secretion in cattle. Such effect based on the fact that glandular tissues especially the pituitary gland and liver have the greatest Se concentration which have several specific metabolic functions (Shamberger, 1983) and this reflect the effect on the interstitial cells of testes (leydig cells) in producing androgen hormone.

**Table (3): Effect of injectable Se on testosterone and Se concentration in buffalo blood serum.**

Item	Pre-treatment period (weeks)				Treatment period (weeks)			
	1 <sup>st</sup> wk	2 <sup>nd</sup> wk	3 <sup>rd</sup> wk	Overall mean ± SE	2 <sup>nd</sup> wk	5 <sup>th</sup> wk	8 <sup>th</sup> wk	Overall mean ± SE
Testosterone conc. (ng/ml)	0.28± 0.01	0.25± 0.02	0.25± 0.03	0.26± 0.02 b	0.64± 0.2	0.58± 0.2	0.81± 0.1	0.695± 0.2 a
Se concentration (µg/ml)	0.3± 0.01	0.3± 0.02	0.31± 0.03	0.3± 0.01	0.48± 0.1	0.44± 0.2	0.40± 0.3	0.43± 0.2

Means with different small litters within each row are statistically different at 0.05 level

### 3. Freezability of buffalo semen:

Post-thaw progressive sperm motility and percentage of live spermatozoa after one day of deep freezing in liquid nitrogen tended to be higher ( $P < 0.05$ ) during treatment period compared to the pre-treatment period (Table 4). The corresponding percentages of increase were 14.7 and 8.9%, respectively. These findings are consistent with the finding of El-Siefy (2004, 42.5%) for the post-thaw progressive motility and lowest for the live spermatozoa (53.1%). Slightly increase in sperm abnormalities after frozen-thawing of semen was obtained during treatment period compared to the pre-treatment frozen semen ( $P > 0.05$ ). Perusal of literature revealed lower percentage of live spermatozoa (Sahu and Pandit, 1997 and Gupta *et al.*, 1998), lower post-thaw motility (36.6 to 40.52%) of frozen semen were reported by Tuli *et al.* (1985), higher incidence of abnormalities (20.8% or more, Nath *et al.*, 1991) and higher value of acrosome integrity (64.7%, Taraphder, 2002).

The findings of the present study shed some light on the importance of Se element in regulating the reproductive functions in Egyptian buffalo bulls. Also, it helps in improving semen physical parameters, minimizing release of both GOT and GPT from spermatozoa into seminal plasma and it helps in increasing post thaw progressive motility and live spermatozoa. The use of supplementary Se at the rate of 10 mg Se as sodium selenite per head twice weekly could be suggested.

**Table (4): Effect of Se injectable on buffalo semen freezability.**

Item	Pre-treatment period (weeks)				X ± SE	Treatment period (weeks)								X ± SE
	Wk1	Wk2	Wk3	Wk4		Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	
Motility (%)	36.7± .44	35± 2.88	40± 2.89	35± 2.89	36.7 <sup>a</sup> ± 1.54	39.2± 36.0	40.8± 0.83	42.5± 2.14	44.2± 1.53	40.9± 0.83	44.1± 1.53	43.3± 1.05	41.7± 0.83	42.1 <sup>a</sup> ± 0.61
Live (%)	47.7± 2.1	42.7± 0.67	41.7± 4.1	44.7± 1.45	44.16 <sup>b</sup> ± 1.24	49.4± 2.9	49.7± 1.73	45.0± 1.03	30.0± 2.67	48.2± 1.88	45± 1.03	48.5± 1.52	48.7± 2.06	48.1 <sup>b</sup> ± 0.89
Abnormal (%)	10.7± 0.88	11.7± 0.88	11.7± 0.66	12.7± 0.88	11.7± 0.41	12.7± 0.71	12.0± 0.37	13.2± 1.25	13.4± 0.49	12.3± 0.48	13.2± 0.47	14.4± 0.98	13± 0.68	13.03± 0.20
Acrosome integrity (%)	83.7± 0.86	85± 1.52	84.7± 0.33	80± 1.1	83.4± 0.75	83.4± 0.71	83.9± 0.6	82.7± 0.61	83.7± 0.42	82.9± 0.94	83.3± 0.88	83.3± 0.6	83.2± 0.5	83.3± 0.23

Means with different small letters within each row are statistically different at 0.05 level

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## تأثير حقن طلائق الجاموس المصرى بالسيلينيوم على جودة السائل المنوى وقابليته للتجميد

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أستخدم فى هذه التجربة ثلاث طلائق ناضجة جنسيا لدراسة تأثير حقن طلائق الجاموس المصرى بالسيلينيوم على جودة وقابلية السائل المنوى للتجميد وعلى تركيز هرمون التستستيرون بالبلازما ، وكان متوسط أعمار الطلائق ٣,٥ سنة ومتوسط وزنها ٥٥٠ كجم عند بدايه تجربته. وقد تم تجميع البيانات على مدار ١٢ اسبوع مقسمه الى فترة مقارنة أو ما قبل المعاملة (٤ اسابيع) وفترة المعاملة بالسيلينيوم (٨ أسابيع). أثناء فترة المعاملة تم حقن كل طلوقة عضليا بـ ١٠ ملجم سيلينيوم (سيلينيات صوديوم) مرتين اسبوعيا وتم جمع السائل المنوى بواسطة المهبل الصناعى مرتين اسبوعيا. تم تخفيف ٢٤ قنفة فى فترة المقارنة أو ما قبل المعاملة و٨ قنفة أثناء فترة المعاملة فى مخفف من التريس وصفار البيض والجليسرول بعدها عبات فى قصبيات (٠,٢٥ مل). وبعد ٤ ساعات من المولزته على درجة ٥°م تم تجميد تلك القصبيات على بخار من النتروجين السائل وحفظت بعدها فى النتروجين السائل على درجة حرارة -١٩٦°م لمدة ٢٤ ساعة قبل الإسالة والتقييم . أظهرت النتائج أن الحقن بالسيلينيوم أدى الى زيادة معنوية (احتمال أقل من ٠,٠٥) مقارنة بفترة ما قبل المعاملة فى حجم القنفة (٢,٨ مقابل ٢,٠٦ مل) ، نسبة الحيوانات المنوية الحية (٦٩,٦ مقابل ٦١,٣%) والتركيز (١,٤١ مقابل ١,٠٣ × ١٠<sup>٦</sup>/مل) وعدد الحيوانات المنوية الكلية (٤,٠ مقابل ٢,١١ × ١٠<sup>٦</sup>/قنفة) كما أدت المعاملة الى نقص نسبة الحيوانات المنوية الغير طبيعيه (١٠,٤ مقابل ١٥,٠%). وأظهرت المعاملة بالسيلينيوم زيادة معنوية فى تركيز الفركتوز وتركيز السيلينيوم فى السائل المنوى. كما أدت أيضا الى زيادة معنوية (احتمال أقل من ٠,٠٥) فى تركيز هرمون التستستيرون فى الدم. بعد التجميد والإسالة أظهرت المعاملة زيادة معنوية (احتمال أقل ٠,٠٥) فى الحيويه ونسبة الحى للحيوانات المنوية مقارنة بفترة ما قبل المعاملة. الخلاصة أن حقن طلائق الجاموس المصرى بـ ١٠ ملجم سيلينيوم مرتين اسبوعيا يمكن إعتباره كافيا لتحسين جوده وقابليه السائل المنوى للتجميد.