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Effect of Withdrawal Time of Combined Vitamin E and Selenium Toxicity on The Reproductive Character of Barki Rams

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ABSTRACT

Vitamin E and Selenium have long been recognized for their antioxidant effect in animals. However, in large repeated doses some harmful effects may be recognized on the animal's health. This study was carried out to investigate the effects of withdrawal time of combined vitamin E and selenium toxicity on reproductive characters of ram. Sixteen mature Barki rams (50-70 Kg) were randomly divided into two groups, eight rams each. The first group was kept as control and injected IM with 3ml saline twice/week for 64 days. The second group was injected IM with a combination of vitamin E and selenium at a dose of 3 ml (4.5 mg sodium selenite and 204 mg vitamin E)/ head twice weekly for 64 days then serum and semen samples were collected till 134th day. The obtained results showed that injection of combined vitamin E and selenium decreased semen volume and semen concentration, activities of seminal plasma catalase, glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), alkaline phosphatase (ALP), acid phosphatase (ACP), Ascorbic acid levels and total antioxidant capacity (TAC) from day 36 till day 78. These parameters restored to the normal ranges from day 92 to day 134. Moreover, sperm abnormalities, and malondialdehyde concentration in seminal plasma were increased from day 36 to day 78 then it decreased to attain the normal ranges on day 134.

This study indicated that the withdrawal time of combined vitamin E and selenium toxicity from the ram semen to return to the normal range is 70 days from the last dose.

Key words: *vitamin E, selenium, semen, rams, antioxidant.*

INTRODUCTION

Vitamin E and selenium (Se) are important nutrients that act synergistically and can affect many biological processes including spermatogenesis, semen quality (Yousef et al., 2003) and protecting against oxidative stress (Bernabucci et al., 2002).

Vitamin E and selenium (Se) play complementary roles as antioxidants (Dimitrov et al., 2007). Glutathione peroxidase (GSH-Px) is an enzyme involved in detoxification of hydrogen peroxide and lipid hydroperoxides while Se, is an essential component of glutathione peroxidase (GSH-Px), acts to destroy peroxides before they attach cell membranes (Sánchez-Gutiérrez et al., 2007).

Meanwhile, vitamin E acts within the membrane to prevent the formation of fatty acid hydroperoxides (Milad et al., 2001).

Both vitamin E and Se have been recommended to be administered together because they act synergistically to protect the tissues against oxidative damage, and improve immune competence (Hamam and Abou-Zeina, 2007). Vitamin E and selenium are believed to be the primary component of the antioxidant defence mechanism of spermatozoa (Surai et al., 2000) and a major antioxidant against the free radicals (Khan, 2011). Supplementation of vitamin E has been reported to increase reproductive capacity in chicken (Khan et al., 2012), rabbit (Yousef et al., 2003) and ram (Luo et al., 2004).

Selenium toxicity can either be acute or chronic, depending on the dosage and the period of exposure. Most chronic forms of the disease are due to high levels of selenium in the diet (Aitken, 2001). In ewes affected by chronic selenium poisoning major lesions include degeneration in cardiac muscles, pulmonary oedema and congestion, and liver congestion (Glenn et al., 1964).

The withdrawal time of vitamin E and selenium from the meat for slaughtered animal is reported to be 30 days (Beale et al., 1990) however no studies reported the withdrawal time for the semen to be used in the breeding.

The aim of this study was to investigate the withdrawal time of combined vitamin E and selenium toxicity on reproductive characters of ram through monitoring of serum testosterone, seminal plasma antioxidants profile as well as semen quality.

MATERIALS AND METHODS

This research was carried out at Animal Reproduction Research Institute (ARRI), Agriculture Research Center (ARC) in cooperation with the Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, University of Sadat City and Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Menoufia University. The committee of the University of Sadat City for animal care and use has approved all experimental procedure.

Animals

Sixteen mature fertile Barki rams aged 2-3 years old and weighted 50-70 Kg were used in this study during breeding season (October to February). All rams were of good general health condition and clinically free from external and internal parasites. They maintained under standard environmental conditions and were fed a balanced ration to meet the NRC requirements of rams according to NRC (2007) and water was available ad libitum.

Experimental design:

Rams were randomly assigned into two groups, 8 rams each.

The control group was injected IM with 3 ml normal saline twice/week for 64 days.

The second group was injected with the combination of vitamin E and selenium (Vitesel, Norbrock Laboratories Limited Newry, Co. Down, Northern Ireland) at a dose of 3 ml/ head containing 4.5 mg sodium selenite and 204 mg vitamin E IM twice weekly for 64 days (Shokry et al., 2020). The treatments were stopped in both groups from day 64 till the end of the study on day 134.

Collection of Serum samples:

Blood samples were collected from jugular vein once weekly for 64 days (treatment period) then every two weeks to day 134th (withdrawal period). The samples were allowed to clot for 30 minutes at room temperature, centrifuged at 3000 rpm for 10 minutes and serum samples were separated and kept at -20 ℃ for testosterone hormone assay.

Reproductive Profile:

Determination of serum testosterone level:

Serum testosterone level was determined using total testosterone ELISA kit assay (Biocheck, Inc. Foster City CA, USA), with a sensitivity of 0.05 ng/ml, according to the manufacturer instructions. The absorbance was measured by using a microtiter plate reader (TECAN Spectrafluor plus, Switzerland) at 450 nm wavelength (Tietz, 1995).

Semen collection and quality evaluation:

Ejaculates were collected in the morning (7 am) once a week for 64 days (treatment period) then every two weeks until day 134th (withdrawal period) by pre-warmed artificial vagina (Neustadt/Aisch, Müller, Nürnberg, Germany). Semen was incubated at 37 °C for evaluation. Semen motility, semen volume, sperm concentration, live dead sperm percentage and sperm abnormality were measured by using standard procedures (Evans and Maxwell, 1987) while plasma membrane integrity (PMI) of ram spermatozoa was assessed by hypo-osmotic swelling test (HOST) (Revell and Mrode, 1994).

Separation of seminal plasma:

The seminal plasma was separated immediately after semen collection. Fresh semen was centrifuged at 1500 rpm for 15 min. The supernatants were collected and re-centrifuged at 14.000 rpm for 10 min at 5°C to eliminate the remaining sperm. The collected seminal plasma was stored at –20°C for biochemical analysis (Asadpour et al., 2012).

RESULTS

1. Effect of injection of vitamin E and Se on semen quality:

Injection of rams with the combination of vitamin E and Se significantly decreased (P< 0.05) semen volume and sperms concentration (table 1). In contrast, it increased sperm abnormalities from 36th day till 78th day and started to increase until almost reaching to normal ranges at day 134 as compared with those of the control group. Meanwhile it did not affect sperm motility, live dead sperm percentage (viability), and HOST (table 1 and 2) and serum testosterone concentration (Figure 1).

2. Effect of injection of vitamin E and Se on Biochemical analysis and antioxidant defense system in rams' seminal plasma:

Injection of rams with the combination of vitamin E and Se reduced significantly the activities of rams' seminal plasma GR, GPx, SOD, catalase, ACP and ALP and reduced the concentrations of total antioxidant capacity and

Biochemical analysis and seminal plasma antioxidants profile:

Seminal plasma acid phosphatase and alkaline phosphatase activities were measured by using commercial kits (Bio Diagnostic, Egypt) (Hillmann, 1971; Kind and King, 1954).

The activities of seminal plasma antioxidant enzymes (Catalase, super oxide dismutase (SOD) glutathione peroxidase (GPx), glutathione reductase (GR)) according to Casao et al. (2010), the concentrations of oxidant (malondialdehyde (MDA) by Kumar et al., **(2016)** and antioxidant biomarkers (Ascorbic Acid by Harris et al. (1942)) and total antioxidant capacity (TAC) by Kumar et al. (2016) were measured by using commercial kits (Bio Diagnostic, Egypt).

Statistical analysis

Values were expressed as means± standard error for all variables. Statistical analysis was carried out by using t-Test to determine the significant differences among groups and P value < 0.05 were considered significant. All statistical analysis tests were carried out by using SPSS analysis program (IBM SPSS, Version 25).

Ascorbic acid from 36th till the 78th day of the experiment then these parameters started to increase from day 92nd till the end of the experiment (table 3,4 and 5).. Meanwhile, it elevated significantly the concentration of MDA in rams' seminal plasma also from 36th day till 46th day then decreased to the normal range at day 134th as compared with those of the control rams (table 4).

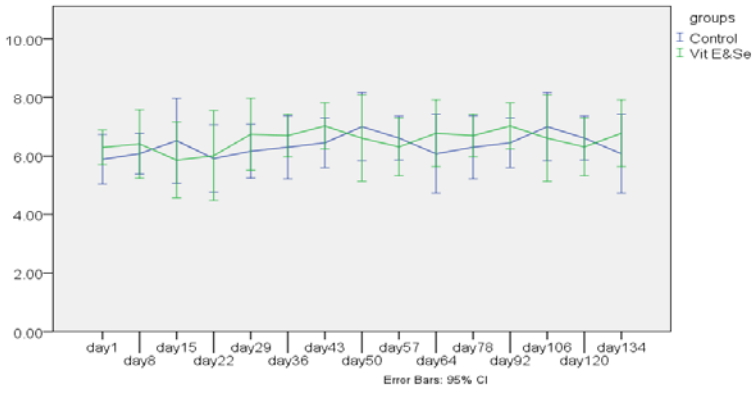

Fig. (1): Represent the effect of injection of vitamin E and selenium on ram serum testosterone (ng/ml)

Table (1): Represent the effect of injection of vitamin E and selenium on ram semen characters. semen volume, sperm motility, and sperm concentration.

Days	semen volume (ml)		sperm motility (%)		sperm concentration ($x10^9$)	
	Control	Vit E se	Control	Vit E se	Control	Vit E se
1	1.15 ± 0.04[a]	1.16 ± 0.03[a]	83.8 ± 1.3[a]	83.8 ± 1.3[a]	3.42 ± 0.18[a]	3.47 ± 0.12[a]
8	1.15 ± 0.03[a]	1.19 ± 0.03[a]	83.1 ± 1.6[a]	83.1 ± 1.6[a]	3.42 ± 0.08[a]	3.46 ± 0.16[a]
15	1.16 ± 0.04[a]	1.18 ± 0.04[a]	83.1 ± 0.9[a]	83.1 ± 1.6[a]	3.51 ± 0.17[a]	3.48 ± 0.09[a]
22	1.15 ± 0.04[a]	1.19 ± 0.05[a]	83.8 ± 1.8[a]	83.8 ± 1.3[a]	3.66 ± 0.10[a]	3.46 ± 0.16[a]
29	1.18 ± 0.03[a]	1.11 ± 0.01[a]	83.1 ± 0.9[a]	83.1 ± 2.1[a]	3.61 ± 0.22[a]	3.29 ± 0.11[a]
36	1.15 ± 0.02[a]	0.99 ± 0.04[b]	83.1 ± 1.6[a]	83.8 ± 0.8[a]	3.68 ± 0.15[a]	2.95 ± 0.07[b]
43	1.16 ± 0.04[a]	0.79 ± 0.03[b]	83.8 ± 1.3[a]	83.1 ± 1.6[a]	3.77 ± 0.08[a]	2.65 ± 0.06[b]
50	1.16 ± 0.03[a]	0.58 ± 0.03[b]	83.1 ± 1.3[a]	83.1 ± 1.3[a]	3.66 ± 0.15[a]	2.54 ± 0.07[b]
57	1.16 ± 0.02[a]	0.35 ± 0.03[b]	83.8 ± 1.6[a]	83.1 ± 1.6[a]	3.63 ± 0.14[a]	2.30 ± 0.09[b]
64	1.21 ± 0.02[a]	0.26 ± 0.03[b]	83.8 ± 1.3[a]	83.8 ± 1.3[a]	3.49 ± 0.15[a]	2.05 ± 0.08[b]
78	1.13 ± 0.04[a]	0.18 ± 0.03[b]	83.1 ± 1.6[a]	83.1 ± 2.1[a]	3.68 ± 0.15[a]	1.96 ± 0.09[b]
92	1.16 ± 0.03[a]	0.35 ± 0.02[b]	83.8 ± 1.3[a]	83.8 ± 0.8[a]	3.77 ± 0.08[a]	2.26 ± 0.10[b]
106	1.15 ± 0.04[a]	0.64 ± 0.03[b]	83.1 ± 1.3[a]	83.1 ± 1.6[a]	3.66 ± 0.15[a]	2.56 ± 0.12[b]
120	1.15 ± 0.03[a]	0.95 ± 0.03[b]	83.8 ± 1.6[a]	83.1 ± 1.3[a]	3.63 ± 0.14[a]	2.90 ± 0.13[b]
134	1.16 ± 0.04[a]	1.05 ± 0.04[a]	83.8 ± 1.3[a]	83.1 ± 1.6[a]	3.49 ± 0.15[a]	3.29 ± 0.13[a]

Mean ± S.E. Values carrying different superscripts in the same row are significantly different at (P _ 0.05).
Vitamin E and Selenium, Vit. E Se.

Table (2): Represent the effect of injection of vitamin E and selenium on ram semen characters. sperm viability (live dead %), sperm abnormality and. hypo-osmotic swelling test (HOST)

Days	Sperm viability (%)		Sperm abnormality (%)		HOST (%)	
	Control	Vit E se	Control	Vit E se	Control	Vit E se
1	85.6 ± 1.2[a]	85.0 ± 0.9[a]	6.6 ± 0.6[a]	6.6 ± 0.5[a]	80.5 ± 0.9[a]	80.8 ± 0.6[a]
8	85.5 ± 1.3[a]	85.5 ± 1.1[a]	6.4 ± 0.5[a]	6.8 ± 0.3[a]	80.3 ± 1.3[a]	80.5 ± 1.3[a]
15	85.4 ± 0.6[a]	85.8 ± 1.7[a]	6.3 ± 0.4[a]	6.9 ± 0.3[a]	80.5 ± 1.2[a]	80.1 ± 1.1[a]
22	85.3 ± 1.2[a]	85.5 ± 1.0[a]	6.1 ± 0.5[a]	6.8 ± 0.4[a]	80.6 ± 1.9[a]	80.1 ± 1.3[a]
29	85.8 ± 1.1[a]	85.1 ± 1.4[a]	6.4 ± 0.6[a]	7.0 ± 0.7[a]	80.0 ± 1.6[a]	80.3 ± 1.8[a]
36	85.5 ± 1.1[a]	85.6 ± 0.7[a]	6.3 ± 0.6[b]	8.8 ± 0.4[a]	80.4 ± 1.3[a]	80.1 ± 0.5[a]
43	85.1 ± 1.5[a]	85.9 ± 1.5[a]	6.3 ± 0.6[b]	9.8 ± 0.3[a]	80.0 ± 0.8[a]	80.0 ± 1.2[a]
50	85.3 ± 1.1[a]	85.8 ± 1.3[a]	6.5 ± 0.5[b]	10.4 ± 0.2[a]	80.1 ± 1.3[a]	80.3 ± 1.2[a]
57	85.1 ± 1.2[a]	85.3 ± 1.3[a]	6.4 ± 0.6[b]	11.0 ± 0.3[a]	80.5 ± 1.1[a]	80.5 ± 1.1[a]
64	85.8 ± 1.0[a]	85.0 ± 0.8[a]	6.8 ± 0.5[b]	11.5 ± 0.2[a]	80.6 ± 0.9[a]	80.8 ± 1.0[a]
78	85.5 ± 1.1[a]	85.6 ± 0.7	6.3 ± 0.6[b]	12.3 ± 0.3[a]	80.4 ± 1.3[a]	80.1 ± 0.5[a]
92	85.1 ± 1.5[a]	85.9 ± 1.5	6.3 ± 0.6[b]	10.9 ± 0.2[a]	80.0 ± 0.8[a]	80.0 ± 1.2[a]
106	85.3 ± 1.1[a]	85.8 ± 1.3	6.5 ± 0.5[b]	9.8 ± 0.3[a]	80.1 ± 1.3[a]	80.3 ± 1.2[a]
120	85.1 ± 1.2[a]	85.3 ± 1.3	6.4 ± 0.6[b]	8.4 ± 0.3[a]	80.5 ± 1.1[a]	80.5 ± 1.1[a]
134	85.8 ± 1.0[a]	85.0 ± 0.8	6.8 ± 0.5[a]	7.0 ± 0.3[a]	80.6 ± 0.9[a]	80.8 ± 1.0[a]

Mean ± S.E. Values carrying different superscripts in the same row are significantly different at (P _ 0.05).
Vitamin E and Selenium, Vit. E Se

Table (3): Represent the effect of injection of vitamin E and selenium on alkaline phosphatase activity (ALP), acid phosphatase activity (ACP) and Ascorbic acid.

Days	ALP (IU/L)		ACP (U/L)		Ascorbic acid (mg/dl)	
	Control	Vit E se	Control	Vit E se	Control	Vit E se
1	735.1 ± 4.98 [a]	734.5 ± 7.40 [a]	17.20 ± 0.42 [a]	17.09 ± 0.67 [a]	4.15 ± 0.18 [a]	4.18 ± 0.29 [a]
8	734.1 ± 6.93 [a]	738.0 ± 4.34 [a]	17.24 ± 0.27 [a]	17.23 ± 0.76 [a]	4.11 ± 0.11 [a]	4.16 ± 0.27 [a]
15	735.5 ± 8.68 [a]	735.1 ± 5.44 [a]	17.55 ± 0.62 [a]	17.54 ± 0.66 [a]	4.20 ± 0.28 [a]	4.05 ± 0.25 [a]
22	736.1 ± 5.5 [a]	734.1 ± 5.24 [a]	17.20 ± 0.73 [a]	17.01 ± 0.68 [a]	4.18 ± 0.29 [a]	4.05 ± 0.29 [a]
29	735.9 ± 6.08 [a]	718.1 ± 4.49 [a]	17.56 ± 0.41 [a]	16.52 ± 0.64 [a]	4.11 ± 0.20 [a]	3.75 ± 0.24 [a]
36	734.0 ± 6.87 [a]	706.4 ± 3.44 [b]	17.23 ± 0.56 [a]	15.05 ± 0.49 [b]	4.14 ± 0.21 [a]	3.08 ± 0.14 [b]
43	735.4 ± 8.47 [a]	694.4 ± 2.28 [b]	17.31 ± 0.65 [a]	13.77 ± 0.43 [b]	4.12 ± 0.20 [a]	2.98 ± 0.13 [b]
50	735.6 ± 9.64 [a]	684.3 ± 2.39 [b]	17.42 ± 0.49 [a]	12.56 ± 0.40 [b]	4.19 ± 0.23 [a]	2.90 ± 0.13 [b]
57	734.8 ± 5.42 [a]	675.5 ± 2.75 [b]	17.19 ± 0.37 [a]	11.23 ± 0.37 [b]	4.10 ± 0.28 [a]	2.60 ± 0.17 [b]
64	735.4 ± 4.77 [a]	667.4 ± 2.24 [b]	17.21 ± 0.35 [a]	9.92 ± 0.26 [b]	3.99 ± 0.25 [a]	2.37 ± 0.13 [b]
78	733.5 ± 6.98 [a]	664.0 ± 2.91 [b]	17.27 ± 0.38 [a]	9.85 ± 0.20 [b]	4.12 ± 0.13 [a]	2.27 ± 0.16 [b]
92	736.4 ± 2.74 [a]	677.0 ± 4.51 [b]	17.17 ± 0.40 [a]	10.59 ± 0.31 [b]	4.16 ± 0.15 [a]	2.56 ± 0.18 [b]
106	734.1 ± 5.72 [a]	694.8 ± 2.52 [b]	17.38 ± 0.45 [a]	11.89 ± 0.46 [b]	4.15 ± 0.12 [a]	3.04 ± 0.19 [b]
120	733.8 ± 4.79 [a]	710.3 ± 3.69 [a]	17.12 ± 0.32 [a]	13.33 ± 0.50 [b]	4.18 ± 0.18 [a]	3.51 ± 0.20 [b]
134	735.6 ± 5.00 [a]	715.3 ± 3.57 [a]	17.26 ± 0.39 [a]	14.41 ± 0.57 [b]	4.15 ± 0.15 [a]	3.88 ± 0.23 [a]

Mean ± S.E. Values carrying different superscripts in the same row are significantly different at (P _ 0.05).
Vitamin E and Selenium, Vit. E Se.

Table (4): Represent the effect of injection of vitamin E and selenium on catalase activity (CAT), malondialdehyde (MDA) and Total antioxidant capacity (TAC)

Days	CAT (U/L)		MDA (nmol/ml)		TAC (mM/L)	
	Control	Vit E se	Control	Vit E se	Control	Vit E se
1	1.69 ± 0.04 [a]	1.69 ± 0.03 [a]	3.70 ± 0.18 [a]	3.68 ± 0.14 [a]	2.56 ± 0.08 [a]	2.58 ± 0.11 [a]
8	1.69 ± 0.03 [a]	1.69 ± 0.04 [a]	3.65 ± 0.13 [a]	3.64 ± 0.14 [a]	2.57 ± 0.07 [a]	2.55 ± 0.05 [a]
15	1.70 ± 0.03 [a]	1.67 ± 0.03 [a]	3.75 ± 0.14 [a]	3.72 ± 0.15 [a]	2.63 ± 0.11 [a]	2.62 ± 0.11 [a]
22	1.69 ± 0.02 [a]	1.68 ± 0.04 [a]	3.68 ± 0.17 [a]	3.79 ± 0.15 [a]	2.68 ± 0.13 [a]	2.51 ± 0.09 [a]
29	1.70 ± 0.03 [a]	1.63 ± 0.03 [a]	3.70 ± 0.20 [a]	3.98 ± 0.15 [a]	2.57 ± 0.15 [a]	2.27 ± 0.06 [a]
36	1.70 ± 0.03 [a]	1.60 ± 0.03 [b]	3.69 ± 0.18 [b]	4.40 ± 0.14 [a]	2.55 ± 0.13 [a]	2.06 ± 0.05 [b]
43	1.69 ± 0.03 [a]	1.56 ± 0.03 [b]	3.72 ± 0.12 [b]	4.47 ± 0.13 [a]	2.64 ± 0.07 [a]	1.90 ± 0.05 [b]
50	1.68 ± 0.03 [a]	1.53 ± 0.03 [b]	3.62 ± 0.11 [b]	4.55 ± 0.12 [a]	2.56 ± 0.06 [a]	1.77 ± 0.06 [b]
57	1.70 ± 0.03 [a]	1.48 ± 0.03 [b]	3.75 ± 0.19 [b]	4.70 ± 0.11 [a]	2.52 ± 0.12 [a]	1.64 ± 0.05 [b]
64	1.69 ± 0.04 [a]	1.43 ± 0.03 [b]	3.70 ± 0.15 [b]	4.82 ± 0.11 [a]	2.55 ± 0.09 [a]	1.41 ± 0.04 [b]
78	1.68 ± 0.04 [a]	1.42 ± 0.03 [b]	3.69 ± 0.20 [b]	4.64 ± 0.14 [a]	2.64 ± 0.09 [a]	1.37 ± 0.05 [b]
92	1.68 ± 0.04 [a]	1.45 ± 0.02 [b]	3.74 ± 0.15 [b]	4.43 ± 0.13 [a]	2.61 ± 0.14 [a]	1.59 ± 0.07 [b]
106	1.68 ± 0.04 [a]	1.53 ± 0.02 [b]	3.68 ± 0.16 [b]	4.19 ± 0.14 [a]	2.61 ± 0.10 [a]	1.85 ± 0.09 [b]
120	1.69 ± 0.04 [a]	1.61 ± 0.03 [a]	3.68 ± 0.20 [a]	3.97 ± 0.13 [a]	2.53 ± 0.13 [a]	2.09 ± 0.08 [b]
134	1.69 ± 0.04 [a]	1.64 ± 0.03 [a]	3.71 ± 0.17 [a]	3.87 ± 0.14 [a]	2.61 ± 0.07 [a]	2.19 ± 0.06 [a]

Mean ± S.E. Values carrying different superscripts in the same row are significantly different at (P _ 0.05).
Vitamin E and Selenium, Vit. E Se.

Table (5): Represent the effect of injection of vitamin E and selenium on antioxidant defense system, glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities.

Days	GR (U/L)		GPx (U/ml)		SOD (U/ml)	
	Control	Vit E se	Control	Vit E se	Control	Vit E se
1	11.20 ± 0.29 [a]	11.70 ± 0.26 [a]	3.18 ± 0.16 [a]	3.15 ± 0.16 [a]	18.94 ± 0.53 [a]	18.75 ± 0.35 [a]
8	11.24 ± 0.31 [a]	11.51 ± 0.29 [a]	3.30 ± 0.13 [a]	3.24 ± 0.19 [a]	18.79 ± 0.42 [a]	18.64 ± 0.37 [a]
15	11.16 ± 0.29 [a]	11.55 ± 0.26 [a]	3.40 ± 0.13 [a]	3.14 ± 0.22 [a]	19.01 ± 0.61 [a]	18.80 ± 0.32 [a]
22	11.26 ± 0.25 [a]	11.35 ± 0.25 [a]	3.42 ± 0.21 [a]	3.16 ± 0.16 [a]	18.84 ± 0.37 [a]	18.47 ± 0.35 [a]
29	11.31 ± 0.30 [a]	10.89 ± 0.24 [a]	3.43 ± 0.21 [a]	3.02 ± 0.16 [a]	18.82 ± 0.44 [a]	17.92 ± 0.35 [a]
36	11.26 ± 0.27 [a]	10.33 ± 0.16 [b]	3.27 ± 0.19 [a]	2.71 ± 0.16 [b]	19.10 ± 0.54 [a]	16.86 ± 0.41 [b]
43	11.39 ± 0.20 [a]	9.98 ± 0.17 [b]	3.37 ± 0.19 [a]	2.59 ± 0.15 [b]	18.57 ± 0.52 [a]	16.73 ± 0.40 [b]
50	11.28 ± 0.25 [a]	9.70 ± 0.14 [b]	3.42 ± 0.17 [a]	2.46 ± 0.13 [b]	18.81 ± 0.45 [a]	16.62 ± 0.39 [b]
57	11.05 ± 0.26 [a]	9.43 ± 0.13 [b]	3.35 ± 0.19 [a]	2.34 ± 0.12 [b]	18.47 ± 0.56 [a]	16.27 ± 0.39 [b]
64	11.24 ± 0.26 [a]	9.23 ± 0.11 [b]	3.24 ± 0.12 [a]	2.25 ± 0.12 [b]	19.09 ± 0.55 [a]	15.86 ± 0.34 [b]
78	11.21 ± 0.28 [a]	10.20 ± 0.22 [b]	3.21 ± 0.18 [a]	2.22 ± 0.11 [b]	18.71 ± 0.48 [a]	15.62 ± 0.45 [b]
92	11.11 ± 0.23 [a]	10.44 ± 0.24 [b]	3.35 ± 0.15 [a]	2.46 ± 0.11 [b]	18.72 ± 0.44 [a]	16.11 ± 0.41 [b]
106	11.28 ± 0.25 [a]	10.70 ± 0.26 [a]	3.15 ± 0.22 [a]	2.66 ± 0.11 [b]	18.71 ± 0.50 [a]	16.58 ± 0.35 [b]
120	11.40 ± 0.19 [a]	10.94 ± 0.28 [a]	3.25 ± 0.20 [a]	2.88 ± 0.11 [b]	18.97 ± 0.58 [a]	17.09 ± 0.36 [b]
134	11.38 ± 0.23 [a]	11.19 ± 0.25 [a]	3.33 ± 0.21 [a]	3.01 ± 0.14 [a]	18.87 ± 0.56 [a]	17.51 ± 0.30 [a]

Mean ± S.E. Values carrying different superscripts in the same row are significantly different at (P _ 0.05).
Vitamin E and Selenium, Vit. E Se.

DISCUSSION

Animal studies have shown that selenium toxicity is accompanied by indicators of oxidative injury. However, the mode of action of selenium at both the cellular and molecular level is not yet fully understood. It has been suggested that selenium toxicity may be due to the interaction of selenite with glutathione to form reactive selenotrisulfides to produce toxic superoxide and hydrogen peroxide (Spallholz, 1994, 1997; Tinggi, 2003).

A previous study in vitamin E showed a general increasing trend in improving semen quality at oral 400 IU/buck/ day for 2 months. However, the dose of 800 IU/kg had no useful effect in further improving the semen quality (Majid et al., 2015).

In chronic selenium toxicity, the animal should take a period of time to recover and return to normal production and reproduction status. Few studies reported the withdrawal time of the vitamin E selenium toxicity.

In the present study the rams were injected with the combination of vitamin E and selenium at a dose of 3 ml / head (4.5 mg sodium selenite and 204 mg vitamin E two times / week for 64 day) resulted in a drastic effect on the semen characters then the rams allowed to recover till the day 134 as the semen character nearly back to normal status.

The results of the current study showed that injection of rams with the combination of vitamin E and selenium decreased semen volume and sperm concentration from 36th day to 78th day then return to normal ranges on day 134th. While its increased sperm abnormality from 36th day till the 78th day then it decreased from 92nd to the end of the experiment on day 134th which almost returns to normal range. These findings were accompanied with increasing of lipid peroxidation and reduction of antioxidant enzyme system activities and total antioxidant capacity of the seminal plasma. Otherwise withdrawn of the toxicity appeared at the day 134th by decreasing of lipid peroxidation and increasing of the antioxidant enzyme system activities and total antioxidant capacity of the seminal plasma from day 92th to the end of the experiment.

Our findings were supported by previous results indicating that supplementation of high doses of vitamin E for 12 months negatively influenced cocks semen quality as it decreased sperms concentration per ejaculate and total numbers of spermatozoa while it increased sperms abnormality (Danikowski et al., 2002). In addition, high selenium intake in men for 120 days decreased the percentage of motile sperm by 32% at day 91 of treatment and by 18% at

day 120 (Hawkes and Turek, 2001). On the other hand, previous studies reported that injection of bulls with selenium at a dose of 5, 10, 20, or 40 mg per 90 kg BW at 6, 16, 22, and 28 weeks, respectively, did not improve semen production or quality (Bartle et al., 1980), and supplementation of boars either with Se (0.5 ppm) or vitamin E (220 IU/kg) had no effects on semen measurements (Marin-Guzman et al., 1997). Also, Se supplementation at a dose of 0.3 mg/kg has no effects on semen quality, while low-Se intake increases sperm abnormality rate (Shi et al., 2010). Selenium is incorporated into sperm seleno-protein, GPx, and is believed to have both enzymatic and structural functions (Ursini et al., 1999) as GPx-deficient human spermatozoa display sperm alterations, such as impaired motility, structural abnormalities of the midpiece, and loss of tails (Imai et al., 2001). Thus, there is a contradiction in the effect of vitamin E and Se supplementation on sperm quality and quantity among different studies as (Mahmoud et al., 2013) demonstrated that treatment of rams with a combination of 5 mg sodium selenite and 450 mg Vit E twice weekly for 1-month improved semen quality and quantity.

These contradictory effects of the combination of vitamin E and selenium may attribute to the length of the administration because the inferior effects of supplementation of rams with the combination did not appear until 36th day of the experiment and selenium has been indicated to be toxic to mammals depending on the dose and duration of administration (ATSDR, 2003).

The adverse effects of the combination of vitamin E and Se on rams' semen character from 36th till the end of the experiment may be due to the induction of oxidative stress through increasing the lipid peroxidation and reducing the antioxidant enzymes system activities in seminal plasma during this period. This explanation was based on the results of the previous study indicating that selenite reacts with glutathione endogenously in cells or extracellularly causing toxicity by the formation of superoxide free radical and elemental selenium (Mézes and Balogh, 2009).

CONCLUSION

Injection of combined Vit E and selenium should be restricted to the recommended doses to avoid the harmful effect. The withdrawal time of the Vit E and selenium toxicity on rams, to be returned to the breeding, is about 70 days.

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