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Effect of Selenium and /or Vitamin E Administration on Semen Characteristics, Plasma Testosterone Level and some Immunogenetic Constituents in seminal plasma proteins of Baladi Bucks

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Abstract: Twenty two Baladi bucks were divided into 4 groups. The first group is a control group (injected I.M. with saline); the second group injected with sodium selenite; the third group injected with vitamin E, while the fourth group was injected with both selenium and vitamin E. Experimental animals have been injected twice / week for 2 months. Semen and blood samples were collected every week. Additional blood samples were collected every half an hour for 4 hours before and after the experiment. Recording the reaction time, complete semen picture, electrophoretic and monogenetic analysis of seminal plasma and radioimmunoassay of plasma testosterone levels were carried out. Results showed that administration of selenium and /or vitamin E improved the sexual desire as indicated by reduction of the reaction time. Semen characteristics were improved as monitored by the increase in both sperm cell concentration and percentage of alive sperms and in the decrease of sperm abnormalities and acrosomal damage. Moreover, plasma testosterone levels showed remarkable increase as compared to pre-injection levels. With the advance of time, the changes were obvious in bucks injected with selenium and vitamin E. Seminal plasma proteins contain 15 polymorphic genetic markers, which is controlled by $F\alpha 2^A$, $S\alpha 2^A$ and Mc^1 genes, they did not change through administration of selenium and /or vitamin E. In conclusion, administration of selenium and / or vitamin E markedly improved the sexual desire, semen characteristics and plasma testosterone levels in Baladi bucks without changing the genetic markers.

Key words: Baladi Bucks · Selenium · Vitamin E · Semen · Testosterone · Immunogenetic Constituents

INTRODUCTION

Reproductive performance of farm animals depends mainly on adequate balanced level of vitamins and essential minerals due to their vital roles in cellular metabolism, maintenance and growth. It was reported that administration of vitamins especially A, D, E and /or selenium improved reproductive trails of bovines in both female [1, 2] and male [3]. In small ruminants, reproductive performance was improved following administration of vitamin and / or selenium in rams [4-6]. Insufficiency of selenium has been associated with reproductive complications and decreased sperm quality of rats, mice, chickens, pigs, sheep and cattle [6, 7]. It has been reported that adding selenium in diet improves reproductive performance of mice, sheep and cattle [8].

Supplementation with selenium and / or vitamin E improved the libido in buffalo-bulls [9] and improved semen characteristics as indicated by increased sperm concentration, motility and alive sperm percent and decreased sperm abnormalities in bulls [3, 10] rams [5] and improved longevity and quality of cooled sperms of rams [11]. Moreover, vitamin was E found to decrease the sperm abnormalities in mice sperm head [12]. In addition, it has been reported that both vitamin E and selenium have an antioxidant and immune stimulatory effects [13 - 15]. Goats have a prominent situation in most of developing countries including Egypt [16, 17], so, the present study was carried out to investigate the impact of administration of selenium and / or vitamin E on semen characteristics, plasma testosterone level and immunogenetic constituents in Baladi bucks as a trail to improve fertility of such breeds.

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MATERIALS AND METHODS

In the present study, data were analyzed on an experiment carried out in the National Research Centre Experimental Farm, Abu-Rawash, Giza, Egypt during the breeding season (September-March).

Experimental Animals: Twenty two mature bucks aged 2-3 years; weighing 30-35 kg were kept freely away from does in covered shelter under the prevailed environmental condition.

Each animal was fed daily one kg. commercial concentrate mixture. Barseem (December-May), water and rice straw were provided *ad libitum*.

Experimental Design: Bucks were trained for semen collection for one month, before the start of the experiment. Animals were divided into 4 groups.

Control Group: includes 7 bucks, each is injected with 1 ml of sterile saline I.M.

Selenium Group: includes 5 bucks, each is injected with 0.10 mg/kg (live body weight) sodium selenite in 1 ml sterile distilled water [2].

Vitamin E Group: includes 5 bucks, each is injected with 1.35 IU/kg. Live body weight vitamin E acetate [18].

Vitamin E Group and Selenium: includes 5 bucks, each is simultaneously injected with the same doses as in groups 2 and 3; twice a week for two months.

Sampling

Semen Collection and Evaluation: A single semen ejaculate (from each buck) following a false mount was collected weekly using artificial vagina (44-45 °C) [19] and an estrous female as a teaser. Libido was expressed by estimating the reaction time/ minute as the interval of time between introducing the buck to the estrous female after the false mounting till ejaculation [20].

Complete semen evaluation including ejaculate volume, motility, concentration, alive sperm, abnormal sperm and acrosomal damage is performed [21] samples were centrifuged (X1500 g/15 minutes at 4 °C) and the separated plasma is kept at -20°C for chemical analysis.

Blood Samples: Jugular blood samples (5 ml) are collected before injection (0-time) as well as weekly throughout the experimental period. Additional blood samples were collected (0-time as well as after two months) every half an hour for four hours to follow up changes in testosterone level. Plasma samples were separated by centrifugation (X 3000 g/ 15 minutes at 4°C) and kept frozen at -20°C or biochemical analysis.

Analysis

Radioimmunoassay (RIA) of Plasma Testosterone: Testosterone level is assayed by RIA [22] in blood plasma using commercial kits from Diagnostic Product Corporation (Los Angles, USA). Assay has sensitivity of 0.04 ng/ml with inter- and intra-assay C.Vs. both being <13%.

Electrophoretic Analysis of Seminal Plasma Proteins: Seminal plasma is subjected to electrophoresis using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) [23]. The genotyping and gene frequency of seminal plasma proteins are estimated according to Hardi-Weinberg [24].

Statistical Analysis: Data are statistically computed and analyzed using two ways analysis of variance (ANOVA) and Chi Square test [25].

RESULTS

Different Effects of Selenium and / or Vitamin E were Recorded On

Libido: The reaction time is improved ($P<0.05$) in all supplemented groups than control. This improvement is obvious with the advancement of time. The more favorable reaction time is observed in bucks injected with both selenium and vitamin E simultaneously with time X treatment interaction ($P<0.05$) (Table 1).

Semen Characteristics: It was clear that selenium and / or vitamin E administration improve ($P< 0.05$) semen characteristics with advance of time as indicated by increased sperm cell concentration and percent of alive sperm and decreased incidence of sperm abnormalities and acrosomal damage. The improved characteristics are more obvious for those bucks injected simultaneously with selenium and vitamin E. However, the ejaculate volume revealed no significant changes due to the treatments (Table 2.1- 2.7).

Table 1: Effect of selenium and / or vitamin E administration on libido of Baladi Bucks (Mean ± SE)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	1.94 ± 0.18	2.93± 0.22	2.63 ±0.24	2.67± 0.33	2.17± 0.44	2.93± 0.23	Weeks 25.92*
Selenium	1.83 ± 0.28	2.10± 050	1.42± 0.08	1.17± 0.17	1.00± 0.29	1.48± 0.21	Groups 10.29*
Vitamin E	2.10 ± 0.24	2.50± 0.41	1.5± 0.20	1.50± 0.29	1.33±0.17	1.75± 0.19	Week Xgroup 3.30*
Sel.+Vit E	2.13 ± 0.14	2.40± 0.20	1.17± 0.19	1.17± 0.17	080± 0.09	1.50± 0.18	

Table 2: Effect of selenium and / or vitamin E administration on semen characteristics of Baladi Bucks (Mean ± SE)

2.1- Semen Volume (ml)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	0.80 ± 0.12	0.70± 0.08	0.63± 0.13	0.75± 0.20	0.68±0. 12	0.67± 0.05	Weeks 0.92
Selenium	0.78 ± 0.09	0.95± 0.05	0.57± 0.07	0.57± 0.07	0.65± 0.07	0.74± 0.06	Groups 0.25
Vitamin E	0.7 ± 0.17	0.63± 0.07	0.67± 0.17	0.67± 0.17	0.66± 0.05	0.66± 0.05	WeekXgroup0.39
Sel.+Vit E	0.70 ± 0.12	0.74± 0.09	0.75± 0.20	0.75± 0.20	0.72± 0.05	0.72± 0.05	

2.2- Sperm concentration ($X10^9$/ml)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	2.11± 0.07	2.30± 0.06	2.60± 0.07	2.70± 0.10	2.60± 0.09	2.50± 0.05	Weeks 67.73*
Selenium	2.23± 0.09	2.30± 0.01	2.80± 0.01	2.70± 0.10	2.90± 0.02	2.60± 0.09	Groups 10.31*
Vitamin E	2.20± 0.04	2.30± 0.09	2.80± 0.05	2.67± 0.08	3.00± 0.09	2.60± 0.08	WeekXgroup3.79*
Sel.+Vit E	2.90± 0.07	2.20± 0.05	2.90± 0.05	3.1± 0.09	3.30± 0.09	2.80± 0.10	

2.3- Mass activity (Score 1-5)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	1.88± 0.26	1.86± 0.40	1.75± 0.48	2.67± 0.33	2.25± 0.25	2.06± 0.21	Weeks 11.09*
Selenium	2.00± 0.58	2.00± 0.70	3.33± 0.33	3.67± 0.33	3.67± 0.67	3.08± 0.33	Groups 8.73*
Vitamin E	2.00± 0.44	3.00± 0.50	3.33± 0.33	3.67± 0.67	4.00± 0.58	3.38± 0.27	WeekXgroup1.42*
Sel.+Vit E	2.2± 0.48	2.00± 0.30	4.00± 0.31	4.00± 0.58	4.60± 0.24	3.50± 0.28	

2.4- Individual sperm motility (%)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	55.00±3.22	55.00±3.62	56.25±4.73	66.67±3.33	65.00±2.89	59.44±2.17	Weeks 29.58*
Selenium	51.67±6.00	65.00±6.45	81.67±1.67	81.70±1.67	83.33±1.67	76.92±2.97	Groups 14.92*
Vitamin E	53.33±4.01	60.00±5.77	78.33±1.67	76.70±1.68	81.67±1.67	74.17±2.88	Week X group 2.02*
Sel.+Vit E	57.00±3.74	63.57±4.72	83.33±1.66	83.33±1.68	87.00±1.22	77.62±2.75	

2.5- Alive sperm (%)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	55.19± 2.57	65.36± 2.69	66.25± 5.91	65.00±2.89	71.67± 6.00	66.62± 1.99	Weeks 42.87*
Selenium	57.97± 3.25	63.33± 4.41	81.67± 1.67	83.33±3.33	90.00± 2.89	79.58± 3.28	Groups 12.51*
Vitamin E	58.14± 3.33	64.33± 5.20	81.67± 1.67	78.33±1.67	83.30± 3.03	76.82± 2.67	Week X group 1.86*
Sel.+Vit E	56.56± 3.18	68.92± 3.36	85.83± 1.54	86.67±3.33	90.00± 1.58	80.83± 2.31	

2.6- Total sperm abnormalities (%)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	11.01±1.08	11.39±1.19	11.63±0.94	10.00±1.73	9.67±1.45	10.89±0.64	Weeks 7.77*
Selenium	10.26±1.25	10.93±0.41	8.73±0.93	7.67±0.88	7.00±1.15	8.76±0.57	Groups 3.89*
Vitamin E	11.10±121	12.14±0.44	9.33±1.76	7.33±1.45	6.67±1.20	9.43±08.0	Week X group 0.72*
Sel.+Vit E	10.42±1.41	11.08±1.18	6.50±0.50	6.67±0.88	6.40±0.50	8.02±0.64	

2.7- Acrosomal damage (%)

		Time post injection (weeks)					
Control	Before injection	2	4	6	8	Overall mean	ANOVA
Control	5.59±0.50	5.33±0.43	6.63±0.24	5.67±0.33	6.38±0.63	5.90±0.25	Weeks 1.27*
Selenium	5.30±0.35	4.96±016	4.50±6.65	4.67±0.33	4.67±0.89	4.96±0.30	Groups 10.84*
Vitamin E	5.00±0.67	4.90±0.43	4.25±0.63	4.67±0.33	4.33±0.33	4.40±0.24	Week X group 1.67*
Sel.+Vit E	5.53±0.35	5.07±0.07	4.58±0.37	4.90±0.07	4.60±0.10	4.73±0.14	

Table 3: Effect of selenium and / or vitamin E administration on plasma testosterone level (ng/ml) of Baladi Bucks (Mean ± SE)

		Time post injection (weeks)			
Control	Before injection	First month	Second month	Overall mean	ANOVA
Control	0.16±0.04	0.17±0.00	0.19±0.01	0.22±0.06	Weeks 0.05*
Selenium	0.17±0.04	0.18±0.009	0.54±0.02	0.36±0.13	Groups 0.26*
Vitamin E	0.44±0.14	0.68±0.06	0.89±0.08	0.79±0.13	Week X group 1.12*
Sel.+Vit E	0.59±0.12	0.65±0.13	0.82±0.08	0.78±0.05	

Table 4: Effect of selenium and / or vitamin E administration on fractionation and genotypes of seminal plasma protein of Baladi bucks

Plasma protein loci	Alleles	Genotype	Phenotype	Genes frequency
α- globulin (Fα2 and Sα2)	A - B	(AA-BB) (AA-Bb)(Aa-BB) (Aa-Bb)	A	$F\alpha_2^A$ = 0.75
		(aa-BB) (aa-Bb)	B	$F\alpha_2^B$ = 0.25
				$S\alpha_2^A$ = 0.25
				$S\alpha_2^B$ = 0.25
β-globulin	A-B-C	(AA-BB) (AA-Bb) Aa-BB) (Aa-Bb)	A	Tf^A = 0.60
		(aa-BB) (aa-BB) (BB-cc) Bb-cc)	B	Tf^B = 0.30
		(aa-CC) (aa-CC) (bb-CC)(bb-Cc)	C	Tf^C = 0.10
Major globulins	Mc^1	(Mc^1 Mc^1-Mc^0 Mc^0)(Mc^1 Mc^1-Mc^0 mc^0)	Mc^0	Mc^0 = 0.167
	Mc^0	(Mc^1 mc^1-Mc^0 Mc^0)(mc^1 mc^1-Mc^1 mc^0)		
		(mc^1 mc^1-Mc^0 Mc^0)(mc^1 mc^1- Mc^0 mc^0)		

Plasma Testosterone Level: The effect of treatments on plasma testosterone levels is shown in table 3. Testosterone levels were improved in all groups with a time X treatment interaction (P<0.05). Moreover, frequent blood sampling, 60 days post-treatment revealed marked increase in plasma testosterone levels especially in bucks which injected simultaneously with selenium and vitamin E followed by vitamin E group then selenium group as compared with pre-injection levels.

Electrophoretic Pattern and Genotyping of Seminal Plasma Proteins: Electrophoretic patterns of seminal plasma proteins revealed 15 bands representing the different genotypes. These bands ranged in their molecular weight from 30-200 KDa. The majority of these bands (12 bands) are situated in α and β-globulin loci. Among these bands, 3 major components migrated towards the anode while, a single component migrated towards the cathode. The most predominate genes in studied bucks are $F\alpha_2^A$, $S\alpha_2^A$, Tf^A and Mc^1 gene markers. Administration of selenium and /or vitamin E did not induce characteristic variations in either the electrophoretic pattern or gene frequency in the seminal plasma of Baladi bucks (Table 4).

DISCUSSION

Adequate balanced nutrition could encourage farm animals to express their biological genetic potentials, especially when bred under harsh prevailing environmental conditions [2].

In the current study, administration of selenium and / or vitamin E improved the sexual desire, semen characteristics and plasma testosterone levels of Baladi bucks as compared to the control as well as to the pre-treatment data. These results are in agreement with those of Baiomy *et al*. [6]. The reaction time became obviously shortened with production of good quality semen containing high concentration of normal, motile and alive sperm cell. Moreover, these changes are clearer with the advance of time; and in bucks which are injected simultaneously with both selenium and vitamin E followed by those groups which are injected with vitamin E then the selenium injected group. In this respect, it has been reported that the decreased reaction time in buffalo-bulls supplemented with vitamin E is attributed to the increased testosterone levels [9]. Also, similar improvements in semen characteristics had been recorded in rams [26], in bulls [27], in buffalo-bulls [9] and in rabbits [28]. In this study, the improvement in testosterone levels is in agreement with the results recorded by Kolb and Seehawer [14] who attributed these changes to the direct effect of these elements on the testicular tissue.

Selenium and / or vitamin E have a complementary effect as biological antioxidants, protecting the body against the damage done by the production of free radicals and consequently enhance the general health condition and fertility [14, 29]. Moreover, selenium and vitamin E are involved in the synthesis of prostaglandins [2], improved the performance of immune system and synthesis of testosterone from the testis [14]. In this respect AL-Azab *et al*. [30] concluded that, following administration of $PGF_{2\alpha}$, the sexual desire, semen characteristics and plasma testosterone levels of Frisian bulls get improved, they attributed the condition to stimulation of spermatogenic activity via direct effect on pituitary tissue or to its acceleration of the sperm passage into the ejaculation.

From the immunogenic point of view, the present study showed that seminal plasma proteins of Baladi bucks are polymorphic, whereas 15 genotypes are obtained which are α, β and major globulins. Moreover, these genotypes are distributed according to their molecular weight from 30-200 KDa. No available literature could be traced in this respect in bucks. However, in rams [31], bulls [23] and in Buffalo-bulls [32] 11, 25-30 and 8 fractions have been obtained; in the seminal plasma; respectively. All these authors agree that these fractions are polymorphic in nature and some of genotypes of major globulins are associated with fertility.

Concerning gene frequency of seminal protein loci, it is evident that $F\alpha^A$, $S\alpha 2^A$ and Mc^1 genes are the most predominant genes which may act through controlling the secretory function of the accessory glands. Also, tight relationships are reported between gonadotrophin, fertility and major protein genotypes of seminal plasma in bovine [33]. Such relationships indicated the possibilities of genetic control of reproduction in farm animals.

In the present study, administration of selenium and / or vitamin E induced non-detectable variations in either the electrophoretic patterns or gene frequency of seminal plasm of Baladi bucks and their condition could be attributed to the inter-action of multiple environmental and genetic factors to induce gene expression.

In conclusion, selenium and / or vitamin E administration found to improve the sexual desire and semen characteristics of Baladi bucks; especially if they are given in combination and in animals raised under harsh management conditions. These treatments alert the immune system, improved health status and fertility without affecting the genetic constitution. However, further investigations on a large number of animals, using different doses and routes still needed.

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