

EFFECT OF SELENIUM ON SEMINAL PLASMA ENZYMES AND LIPIDS IN LOW FERTILE BUFFALO-BULLS

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Abstract

Five sexually mature buffalo-bulls with poor semen quality, were injected intramuscularly with selenium. Each animal received 90 mg of selenium as sodium selenite, divided into 3 doses with one week interval in-between. Semen samples were collected weekly from Se-treated bulls during the period of experiment (12 weeks) and from the controls. The enzymatic constituents of the seminal plasma (GOT, ACP, AKP and LDH) after injection were significantly increased in parallel with the improvement of the physical characters of the semen. The significant variations in lipidogram (total lipids, cholesterol and plasma free fatty acids) in seminal of low fertile bulls were restored after Se-injection, to be nearly similar with the results of fertile bulls.

INTRODUCTION

Several workers proved that seminal plasma contained a number of enzyme activities in buffalo (Abdou *et al.*, 1974 & 1978). These enzymes play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism, in fertilization process and in the maintenance of constant osmotic pressure during preservation (Dhami and Kodagali, 1987). It is suggested that certain enzymes activities in semen are being widely used as a valuable parameter to evaluate semen quality (Abdou *et al.*, 1979). In addition, lipids of mammalian semen appear to serve as an important function. They play a significant role in maintaining the structural integrity of the highly organized membrane system. Poules *et al.* (1974) suggested that lipids of spermatozoa act as endogenous source of energy particularly during their transit from epididymis.

Recently, it had been detected that selenium injection improved the physical characters of buffalo-bulls (Omaira-Mohamed *et al.*, 1996). The aim of the present investigation is to illustrate the effect of this treatment in buffalo semen characters via their enzymatic and/or lipids constituents.

MATERIALS AND METHODS

The experiment was conducted on 27 sexually mature buffalo-bulls in Freezing Center of Buffalo Semen, Abbasia, Cairo. A preliminary examination of semen characteristics of these bulls for four weeks before Se-injection (1st - 4th week of the experiment) revealed that 5 bulls had poor semen quality. These animals were injected i.m. with selenium as sodium selenite, each received 90 mg Se, divided into three doses with one week interval in-between (4th, 5th & 6th weeks). They were checked by weekly collection of semen samples for six weeks after injection (4th-12th week). Five fertile bulls were sampled at 1st, 6th and 12th weeks of the experiment, as a control. Physical characters of the whole semen (volume, concentration, pH, individual motility, alive sperms and total abnormalities) were recorded in Table 1, which was previously mentioned by Omais Mohamed *et al.* (1996). Seminal plasma was separated by centrifugation at 3000 rpm and stored at -20°C for biochemical assay. Enzyme activities were determined according to the following glutamic oxaloacetic transaminase (Henry *et al.*, 1974), acid phosphatase (Moss, 1984); alkaline phosphatase (Ratliff and Hall, 1973) and lactate dehydrogenase (Kachmar and Moss, 1976), while, total lipids, cholesterol and free fatty acids were estimated after Zollener and Krisch (1962), Watson (1960) and Schuster 1979), respectively.

Statistical analysis of data was carried out according to Snedecor and Cochran (1973).

Table 1. Overall means (\pm SE) of semen characteristics before and after Se-injection of low-fertile bulls and in fertile animals.

	Low-fertile bulls		Fertile bulls (15)	F-value
	Before Se-injection (20)	After Se-injection (30)		
Volume (ml)	2.15 \pm 0.17	2.12 \pm 0.08	2.14 \pm 0.07	0.415
Concentration ($\times 10^6$ spz/ml)	495.50 \pm 31.74	816.87 \pm 31.69	792.24 \pm 65.18	7.605**
pH	6.78 \pm 0.02	6.76 \pm 0.0009	6.78 \pm 0.02	1.057
Individual motility (%)	33.75 \pm 1.72	59.57 \pm 1.38	60.2 \pm 2.46	12.019**
Alive sperms (%)	44.70 \pm 2.48	66.20 \pm 1.26	66.16 \pm 2.74	9.441**
Tablal Abnormalities (%)	4.20 \pm 0.34	2.90 \pm 0.18	2.88 \pm 0.41	2.948**

*Mean of post-treatment inspections.

By Duncan's multiple range test, means followed by the same letter are not significantly different at 5% level.

Table 2. Overall means (\pm SE) of seminal plasma enzymes and lipid levels before and after Se-injection of low-ferile bulls and in fertile animals.

	Low-ferile bulls		Fertile bulls (15)	F-value
	Before Se-injection (20)	After Se-injection (30)		
Got (U/L)	61.30 \pm 5.355	114.26 \pm 3.279	116.57 \pm 8.637	8.216
ACP (U/L)	72.67 \pm 1.3898	82.38 \pm 1.092	83.21 \pm 2.313	4.177**
AKP (U/L)	0.94 \pm 2.393	89.57 \pm 2.890	89.96 \pm 6.540	2.171
LDH (U/L)	148 \pm 9.434	257.04 \pm 12.280	255.47 \pm 27.867	8.366**
Total lipids (mg/100ml)	516.43 \pm 8.845	470.00 \pm 6.704	484.15 \pm 9.500	
Total cholesterol (mg/100ml)	139.35 \pm 4.799	162.81 \pm 4.525	160.13 \pm 8.610	3.744**
%out of T.lipids	26.97 \pm 0.790	34.87 \pm 1.090	334.31 \pm 1.723	3.904**
Free fatty acid (mmol/L)	2.67 \pm 0.102	1.48 \pm 0.112	1.69 \pm 0.180	10.583**

() Number of samples.

*P < 0.05.

** P < 0.01.

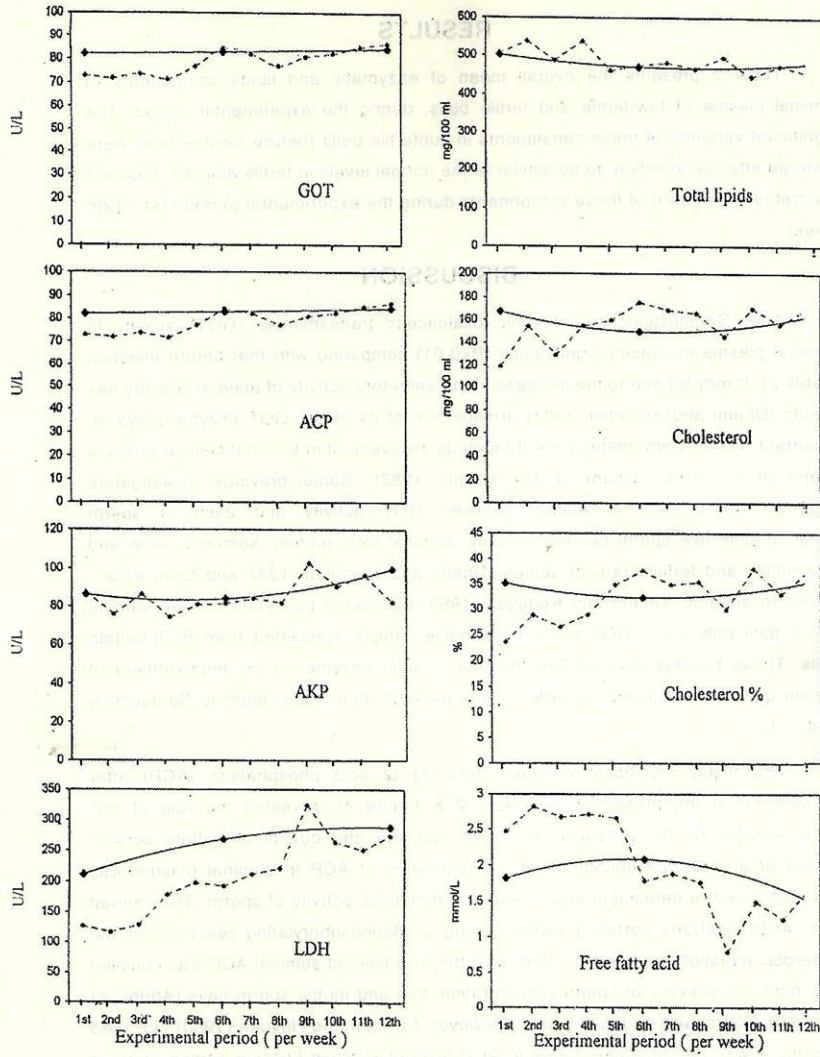


Fig. 1. Seminal plasma enzymes and total lipids and their fractions of low-fertile bulls (—■) before and after Se- injection and in fertile bulls (---■) during the experimental period.

RESULTS

Table 2 presents the overall mean of enzymatic and lipids components of seminal plasma of low-fertile and fertile bulls, during the experimental period. The significant variation of these constituents in subfertile bulls (before Se-injection) were restored after Se-injection, to be similar to the normal levels in fertile animals. Figure 1 illustrates the pattern of these components during the experimental period (1st - 12th week).

DISCUSSION

After Se-injection, the glutamic oxaloacetic transaminase (GOT) activity in seminal plasma increased significantly ($P < 0.01$) comparing with that before injection (Table 2). It may be due to the increase in the secretory activity of male accessory sex glands (Dhami and Kodagali, 1987). Irrespective of its origin, GOT enzyme plays an important role in sperm metabolism through its involvement in the vital cellular process (Dube *et al.*, 1982; Dhami and Kodagali, 1987). Some previous investigators suggested a positive correlation between GOT activity and each of sperm concentration, live sperm percent, motility, seminal total protein, semen volume and freezability and fertility rate of semen (Dhami and Kodagali, 1987 and Iqbal *et al.*, 1988). In addition, Dhami and Kodagali (1989) concluded that static semen sample had significantly lower GOT value than motile sample ejaculated from Surti-buffalo bulls. These findings may confirm the role of such enzyme on the improvement of semen quantity and quality recorded in the present study subsequent to Se-injection (Table 1).

The highly significant increase ($P < 0.01$) of acid phosphatase (ACP) after Se-injection in the present work (Table 2 & Figure 1), revealed the role of this micro-element on the improvement of the quantity and quality of buffalo semen. Abdou *et al.* (1978) mentioned that the high level of ACP in seminal plasma was associated with a detectable improvement of metabolic activity of sperm. They added that, ACP catalyzes certain phosphorylating or dephosphorylating reactions in the anaerobic metabolism of semen. Moreover, the high level of seminal ACP was coupled with high percentages of sperm concentration, live and motile sperm cells (Abdou *et al.*, 1978; Dhami and Kodagali, 1989). However, Stallcup and Hayden (1960) indicated that there was no correlation between ACP level of Holstein-Friesian semen and live sperm percent.

Concerning alkaline phosphatase (AKP), it was significantly increased after selenium injection (Table 2 & Figure 1). Little work was undertaken by earlier

investigators to investigate with the significance of AKP in the biological activity of semen. Meanwhile, there is an evidence that, it has certain role in other areas of the male reproductive tract. AKP is involved in the synthesis of fructose (Mann and Lutwak-Mann, 1951), and associated with the transport of sugar across membranes (Mann, 1964). These findings may interpret the significant increase of this enzyme during the present experiment to substitute the depleted fructose. However, Dhami and Kodagli (1989) found no significance in level of AKP in the buffalo seminal plasma of static and motile ejaculates.

Lactic dehydrogenase enzyme (LDH) is one of the metabolic requirements of tissue. It is found in non-cellular component of semen (Stallcup and Hayden, 1960; Donald *et al.*, 1979), and it is available to spermatozoa for oxidation and energy production (Blanco *et al.* 1976). LDH participates in catalyzed phase of fructolysis, in oxido-reduction between reduced nicotinamideadenine dinucleotide phosphatase (NADH) and pyruvic acid. The positive significant effect of Se-injection on seminal LDH level suggested in the present study, demonstrated its effect on the secretory activity of the male accessory sex glands. The positive parallels between this enzyme and sperm cell concentration, spermatozoal motility, live sperm and total abnormalities percent (Table 1), implies that seminal plasma LDH activity may be directly associated with the metabolic activity of spermatozoa in buffaloes. This has been previously confirmed in bulls (Roussel and Stallcup, 1964; Leffel *et al.*, 1979) and in buffaloes (Dube *et al.*, 1982; Dhami and Kodagali, 1989). However, Khilo (1990) recorded a significant correlation between the total LDH enzyme activity and ejaculate volume, but, it may not be directly associated with the metabolic activity of spermatozoa in buffaloes.

Total lipids and free fatty acid levels (Table 2 & Figure 1) were significantly decreased after Se-injection. Sidhu and Guraya (1978) recorded that the best semen quality had a lower total lipid value than the average level (2.34 vs. 3.5 mg/ml), which is consistent with the present study. It is known that buffalo spermatozoa contain comparatively more unsaturated fatty acids than other species (Sidhu and Guraya, 1985), which make them more susceptible to lipid peroxidation. They added that poor quality semen samples of buffalo was more susceptible to lipid peroxidation than high quality semen. Formation of lipid peroxidase in spermatozoa is accompanied by loss of lipid from the membrane and rapid decline in motility (Jones and Mann, 1977). It is suggested that selenium plays a specific role in maintaining the structural and functional integrity of the sperm cell (Wue *et al.*, 1973) which interpret the significant decrease of seminal total lipids and free fatty acid values after Se-injection. Moreover,

it has been known that selenium is necessary for synthesis of the selenium dependent enzyme glutathione peroxidase (GSH-Px) in bovine seminal plasma (Smith *et al.*, 1979). This enzyme protects cellular and subcellular membranes from peroxidative damage by destruction of peroxides (Chow, 1979). Slaweta *et al.* (1988) mentioned that, fresh semen samples containing significantly lower selenium concentration and total GSH-PX activity had higher levels of lipid peroxidase.

On the other hand, rise in total cholesterol levels and their percentages, out of total lipids after Se-injection, (Table 2 & Figure 1) indicates the role of this element on accessory sex glands activity and on lipid metabolism. The percentage of cholesterol out of total lipids (34.78%) was similar to the result of Chaudhary and Gangwar (1979) in buffalo. Cholesterol is important in forming the reasonably impermeable and cohesive membrane structure which overcomes the environmental stress. Therefore, the lower seminal concentration of cholesterol might constitute one of the factors of poor preservability of spermatozoa in buffalo semen (Chaudhary and Gangwar, 1979).

From the afore-mentioned results, we concluded that selenium injection can correct the abnormal level of seminal enzymes and lipids of the sub-fertile bulls to be nearly similar to those of fertile bulls. The positive correlation of seminal enzymes and cholesterol, and the negative correlation of total lipids and free fatty acid, with some parameters of the physical character of semen (concentration, motility, alive sperm and total abnormalities) confirmed the role of these components in evaluation of semen in buffalo-bulls.

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تأثير حقن السيلينيوم على بعض انزيمات ودهون السائل المنوي لطلائق جاموس منخفضة الخصوبة

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أجريت هذه الدراسة على خمسة طلائق جاموس ناضجة جنسيا وبها ضعف فى الخوص الطبيعية للسائل المنوي بالمقارنة مع خمسة طلائق أخرى بحالة جيدة (كمجموعة ضابطة) فى مركز تجميد السائل المنوي بالعباسية. تم حقن الحيوانات المنخفضة الخصوبة بمحلول سيلينيت الصوديوم (تسعين مليجرام لكل طلوقة مقسمة على ثلاث جرعات متساوية بفواصل اسبوع بين كل جرعة وأخرى).

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