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THE IMPORTANCE OF ZINC AND VITAMIN E IN BUFFALO SEMINAL PLASMA

(With 4 Tables)

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أهمية وجود الزنك وفيتامين ه فى بلازما السائل المنوى للجاموس

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تم تجميع عدد ١٢١ عينة سائل منوي من خمس طلائق جاموسي ثم قيمت بالنسبة لحجم القذفة، تركيز الحيامن /مللي، الحركة الجماعية والفردية، النسبة المئوية للحيامن الحية، مسافة اختراق الحيامن، النسبة المئوية للعيوب المورفولوجية، درجة تركيز أيون الهيدروجين. وكذلك تم قياس الخصائص الكيميائية للسائل المنوي التي تتمثل في قياس الوقت اللازم لاختزال الميثيلين الأزرق، تركيز سكر الفركتوز، إنزيم الفوسفاتيز القاعدي، أنزيم اللاكتيت ديهيدروجينيز، تركيز الزنك وفيتامين ه في السائل المنوي وسيرم الدم وكذلك الهرمون الخصوى في مصل الدم. وقد أسفرت النتائج عن وجود علاقة معنوية طردية بين تركيز الزنك في بلازما السائل المنوي وكل من تركيز الحيامن /مللي، تركيز سكر الفركتوز، تركيز فيتامين ه في السائل المنوي. بينما وجدت علاقة معنوية عكسية بين تركيز الزنك في بلازما السائل المنوي وكل مندرجة تركيز أيون الهيدروجين وكذلك النسبة المئوية للنقط البروتوبلازمية العليا. أما بالنسبة للعلاقة بين تركيز فيتامين ه في السائل المنوي وخصائص السائل المنوي أسفرت عن وجود علاقة معنوية طردية بين فيتامين ه والحركة الجماعية للحيامن، تركيز سكر الفركتوز في السائل المنوي، تركيز الزنك في السائل المنوي وكذلك إنزيم الفوسفاتيز القاعدى. بينما وجدت علاقة معنوية عكسية بين تركيز فيتامين ه في بلازما السائل المنوي والنسبة المئوية لتشوهات القلنسوة. كما أسفرت النتائج أن تركيز الزنك وفيتامين ه في بلازما السائل المنوي يرتفع معنويا عنه في مصل الدم ويوجد بين تركيزهما في بلازما السائل المنوي ومصل الدم علاقة طردية غير معنوية. وتخلص هذه الدراسة إلى أهمية وجود الزنك وفيتامين ه في علائق طلائق الجاموس المستخدمة في التلقيح الاصطناعي وذلك لانتاج سائل منوي ذو خصائص جيدة.

SUMMARY

A total number of 121 semen samples from Five buffalo bulls were collected and evaluated for volume, mass motility, percentage of individual motility, percentage of live spermatozoa, sperm concentration, percentage of sperm abnormalities, acrosomal defects, pH

and Modified sperm penetration test. Also, biochemical properties in semen were evaluated as mehtylene blue reduction time, initial fructose concentration, seminal palsma zinc concentration, seminal palsma alkaline phosphatase activity, Seminal palsma lactate dehydrogenase, Seminal palsma vitamin E concentration. Scrotal measurements as scrotal circumference and scrotal thickness as well as reaction time were recorded. Zinc and vit. E concentration and testosterone were determined in the blood serum. The present study revealed a significant positive correlation between zinc concentration in seminal plasma and sperm concentration ($r = 0.28$, $P < 0.05$), initial fructose concentration in seminal plasma ($r = 0.31$, $P < 0.01$) and vitamin E concentration in seminal plasma ($r = 0.28$, $P < 0.05$). While, there was a significant negative correlation between zinc concentration in seminal plasma and semen pH ($r = -0.37$, $P < 0.01$) and percentage of proximal protoplasmic droplets ($r = -0.30$, $P < 0.05$). While, vitamin E concentration in seminal plasma correlated significantly in a positive manner with initial fructose concentration in seminal plasma ($r = 0.33$, $P < 0.01$), zinc concentration in seminal plasma ($r = 0.27$, $P < 0.05$), mass movement ($r = 0.31$, $P < 0.05$) and alkaline phosphatase activity ($r = 0.33$, $P < 0.01$). On the other hand, vitamin E concentration in seminal plasma correlated significantly with the percentage of acrosomal abnormalities in a negative manner ($r = -0.38$, $P < 0.01$).Also, The obtained results revealed that zinc and vit. E in seminal plasma were significantly ($P < 0.01$) higher than that of blood serum. A non significant positive correlation coefficient between zinc or vitamin E concentration in seminal plasma and that of blood serum was observed. It was concluded that the level of zinc and vitamin E in seminal plasma master most of the physicochemical buffalo semen characters. So that A.I. bulls should be provided with sufficient amount of zinc and vitamin E in their rations for better semen characteristics.

Key wards: *Zinc, vit. E, physicochemical semen characteristics, buffalo semen.*

INTRODUCTION

Zinc and vitamin E have been recognized as indispensable for normal fertility, particularly in male animal (Chaney, 1992). They control the activity of 3-ß hydroxy steroid dehydrogenase which was the key enzyme of steroidogenesis and androgen metabolism at cellular level (Lees *et al.*, 1982 and Hafiez *et al.*, 1989). Also, they were involved in

biosynthesis and metabolism of nucleic acid and in gene transcription and expression at cellular level (Clegg *et al.*, 1989 and Brown, 1992).

For that reason both zinc and vitamin E are essential for normal testicular growth and size (Putnam and Comben, 1987 and Cigankova *et al.*, 1994) as well as some physical semen characteristics like volume (Petryankin *et al.*, 1987; Misra *et al.*, 1989b and Ibrahim *et al.*, 1996), sperm concentration (Misra *et al.*, 1989a; Zinat Rabie, 1992; El-Anwar and Badr, 1996 and Ibrahim *et al.*, 1996), sperm motility (Si *et al.*, 1990 and Badr *et al.*, 2003), live sperm percentage (El-Masry *et al.*, 1994; Ibrahim *et al.*, 1996) and total sperm abnormalities (Zinat Rabie, 1992and Abd El- Moneim and Tharwat, 1996).

Moreover, they are responsible for improving the biochemical characteristics of bull and buffalo spermatozoa (El-Menoufy, 1974; Misra *et al.*, 1989b; Abdel- Malak *et al.*, 1995 and Ibrahim *et al.*, 1996).

It was proved that zinc and vit. E were present in semen and blood of bulls and buffalo bulls (Keen and Graham, 1989; Eissa *et al.*, 1992; Surai, 1992 and Daghash *et al.*, 1993).

However, the relationship between the concentrations of zinc and vitamen E in blood and semen as well as their effect on the physiochemical characteristics of semen are not clear in buffalo bull. Therefore the present study aimed at clarifying this phenomena.

MATERIALS and METHODS

1- Experimental animals:

Five healthy buffalo bulls aged 3-4 years were used in the present study. They were raised on the Animal Reproduction Research Institute farm (ARRI).

2- Semen collection:

A total number of 121 semen samples were collected by means of artificial vagina set up to optimal conditions. Immediately after collection, semen samples were evaluated for volume (ml), mass activity, individual motility (%), percentage of live spermatozoa, sperm concentration (x 10^6 sperm / ml) and pH according to El Menoufy (1974), as well as morphological sperm abnormalities in smears stained by eosin-nigrosin (Harasymowyez *et al.*, 1976) and acrosomal defects in smears stained by Fast Green (Wells and Awa, 1970).

Modified sperm penetration test (mm) was recorded according to Suttiyotin *et al.* (1995).

3-Evaluation of biochemical properties in semen:

- Mehtylene blue reduction time (min) was determined in ejaculates according to Beck and Salisbury (1943).
- Initial fructose concentration (mg/ 100 ml) in ejaculates according to Mann (1948).
- Seminal palsma zinc concentration (ppm) according to Smith *et al.*, (1979).
- Seminal palsma alkaline phosphatase activity (iu/L) according to Tietz (1976).
- Seminal palsma lactate dehydrogenase (u/L) according to Kachmar and Moss (1976).
- Seminal palsma vitamin E concentration (ppm) according to Quaife and Dju (1949).

4-Evaluation of reproductive performance:

Scrotal measurements as scrotal circumference and scrotal thickness (cm) were recorded for each animal as well as reaction time (sec.) (Rollinson *et al.*, 1970).

5- Blood samples:

Blood samples were collected at 2 weeks interval through jugular venipuncture. After collection of blood samples from each bull, the sample was left to coagulate at room temperature for 30 minutes then refrigerated for separation of serum. The sample was centrifuged at 3000 rpm for 10 minutes and the split serum into 3 portions. The first and second portions were stored at – 20 °C for determination of zinc concentration (Smith *et al.*, 1979) and testosterone (ng/ dl) concentration by double antibody radioimmunoassay (Yen and Jaffe, 1978) using kits obtained from DPC (Diagnostic Production Corporation, Los Angeles, CA, USA). While the third portion was stored at -70 °C to determine the concentration of vitamin E (Quaife and Dju, 1949).

6- Statistical analysis:

The obtained data were analyzed statistically using Costat Computer Program, version 3.03 copyright (1986) Cottort software.

RESULTS

The physical characteristics of buffalo bulls semen are shown in Table (1). Analysis of variance revealed a significant difference among five bulls with mass movement ($P < 0.05$), percentage of individual motility ($P < 0.01$), percentage of live spermatozoa ($P < 0.05$) as well as sperm concentration ($P < 0.05$). While non significant difference

between bulls was observed with semen volume, sperm penetration distance and semen pH.

Data gathered in Table (2) illustrates the percentages of sperm abnormalities and testicular measurements. Analysis of variance indicated that there was a significant difference with percentages of proximal and distal protoplasmic droplets and abnormal tail ($P < 0.05$).

On the other hand, no significant variation between bulls was recorded with the percentages of abnormal head, abnormal midpiece and abnormal acrosome.

Table "3" reported semen and blood biochemical properties of buffalo bulls. Methylene blue reduction time and initial fructose concentration in seminal plasma varied significantly between bulls ($P < 0.01$ and $P < 0.05$, respectively). While no significant variation between bulls was observed in the other semen biochemical properties.

Regarding the blood biochemical properties only serum testosterone showed highly significant difference ($P < 0.01$) between bulls while no significant different between them was observed with serum zinc or vitamin E.

Regarding to the reproductive performance (Table 4) analysis of variance showed a highly significant difference among bulls ($P < 0.01$) with testicular measurements as scrotal circumference and scrotal thickness as well as the reaction time ($P < 0.01$).

Regarding to the correlation coefficient between zinc concentration in seminal plasma and buffalo semen characteristics, there was a significant positive correlation between zinc concentration in seminal plasma and sperm concentration ($r = 0.28$, $P < 0.05$), initial fructose concentration in seminal plasma ($r = 0.31$, $P < 0.01$) and vitamin E concentration in seminal plasma ($r = 0.28$, $P < 0.05$).

While, there was a significant negative correlation between zinc concentration in seminal plasma and semen pH ($r = -0.37$, $P < 0.01$) and percentage of proximal protoplasmic droplets ($r = -0.30$, $P < 0.05$).

In this connection, there was a significant positive correlation between vitamin E concentration in seminal plasma and initial fructose concentration in seminal plasma ($r = 0.33$, $P < 0.01$), zinc concentration in seminal plasma ($r = 0.27$, $P < 0.05$) , mass movement ($r = 0.31$, $P < 0.05$) and alkaline phosphatase activity ($r = 0.33$, $P < 0.01$) .

On the other hand, vitamin E concentration in seminal plasma correlates significantly negative with the percentage of acrosomal abnormalities ($r = -0.38$, $P < 0.01$).

It is worthy to note that, the allover mean concentration (Table 3) of zinc in seminal plasma (3.87 ± 0.13 ppm) was significantly ($P < 0.01$) higher than that of blood serum (1.06 ± 0.04 ppm). The same trend was observed between vitamin E concentration in seminal plasma and blood serum (40.59 ± 1.18 and 15.52 ± 0.54 ppm, respectively).

The data obtained revealed a non significant positive correlation coefficient between zinc or vitamin E concentration in seminal plasma and that of blood serum.

DISCUSSION

Zinc is one of the essential trace elements that has been detected to be present at a high concentration in mammalian semen (El-Anwar and Badr, 1996 and Gur *et al.*, 1998). It is involved initially in many aspects of sperm morphology, physiology and biochemistry (Stoltenberg *et al.*, 1997). The biological effect of zinc on sperm kinematic activity depends upon its concentration in spermatozoa (Mann and Iutwak-Mann, 1981).

Vitamin E is a chain-breaking antioxidant that has been reported to be present in mammalian semen including seminal plasma and spermatozoa (Therond *et al.*, 1996 and Khalifa, 1997). It can inhibit the propagation of lipid peroxidation within the biological membranes without influencing the initiation of this process (Aitken *et al.*, 1989). So, it provide sperm protection against reactive oxygen species (ROS) which induce DNA damage (El-Sheltawi *et al.*, 1999; Cerolini *et al.*, 2000 and Badr *et al.*, 2003).

The current study revealed a significant positive correlation coefficient between zinc concentration in seminal plasma and sperm concentration ($r = 0.28$, $P < 0.05$). In agreement with our data, Nour (1985), Misra *et al.* (1989b) and Khalifa (1997) found a significant positive correlation between zinc content and sperm concentration of buffalo bull semen. While the same results were obtained by El-Anwar and Badr (1996) on goat semen. In contrast Dyrendahl (1959) did not find any relationship between them in bull semen. On the other hand, Swarup and Sakhon (1976) recorded an inverse relationship between them. Zinc was thought to be essential for the germinal epithelium and sperm production. In addition, the histochemical of some zinc metalloenzymes including alkaline phosphatase in buffalo testis revealed the importance of these enzymes in relation to onset of spermatogenesis (Antoniou *et al.*, 1977). Also, zinc exerted pronounced stimulatory

influence on biosynthesis and metabolism of prostaglandin $F_{2\alpha}$ (Mc Dowell, 1989) which was found to increase sperm cell concentration by acceleration of sperm passage from testes to ejaculates of bull semen (El Azab *et al.*, 1996).

The present study indicated that there was a negative correlation between the percentage of proximal protoplasmic droplets and zinc concentration in buffalo seminal plasma($r = -0.30$, $P < 0.05$). A fact that come in agreement with Misra *et al.*, 1989b) on bull semen. In contrast, Misra *et al.* (1989a) on buffalo semen detected a significant positive correlation between zinc content and sperm abnormalities. It was thought to be kinoplasmic droplet shared a number of characteristic enzymatic properties with the lysosomes of other tissues, so zinc might reduce the incidence of protoplasmic droplet and subsequently this could enhance sperm maturation which is a prerequisite for sperm motility and fertilizing ability (Mann and Lutwak-mann, 1981).

The present study indicated that there was an inverse relationship between zinc concentration in seminal plasma and semen pH ($r = -0.37$, $P < 0.01$). This result agrees with Khalifa (1997). Zinc was found to increase blood testosterone concentration (Zinat Rabie, 1992 and El-Masry *et al.*, 1994) which might increase the secretion of ascorbic and citric acid from seminal vesicles and subsequently might lead to reduction in pH of buffalo semen (Eltohamy and Younis, 1991 and Nagorna-Stasiak *et al.*, 1993).

In the matter of seminal fructose, our findings revealed a highly significant positive correlation between each of zinc and vitamin E concentrations in seminal plasma and initial fructose concentration ($r = 0.33$, $P < 0.01$). This finding come in agreement with Khalifa (1997). In contrast, in human semen Lindholmer and Eliasson (1972) did not find any relationship between them. Zinc has been shown to be an important element in glucose metabolism (Sandstead *et al.*, 1967) because it is considered as a metalloenzyme (Vallee, 1983 and Tiez, 1986) which help in conversion of glucose to fructose in seminal vesicles of bulls (Mann and Iutwak-Mann, 1981). In agreement with Khalifa (1997) the present results indicated that zinc and vitamin E concentrations in seminal plasma were correlated significantly in a positive manner.

With respect to sperm motility, the present work demonstrated a significant positive correlation coefficient between mass motility and vitamin E concentrations in seminal plasma ($r = 0.31$, $P < 0.05$). A fact that explained the significant positive correlation that exert between vitamin E concentration and mass activity of buffalo spermatozoa as

well as in Khalifa (1997).Vitamin E was reported to be involved in the normal phosphyrelation reaction, especially of high energy phosphate compounds such as ATP (Putnam and Comben, 1987) which was found to be involved in metabolism activity in the motility of bovine spermatozoa (Mann and Iutwak-Mann, 1981 and Mc Dowell, 1989).

It was proved from the present study that there was a highly significant negative correlation between vitamin E concentrations in seminal plasma and the acrosomal defects ($r = -0.38$, $P < 0.01$), which come in agreement with Misra *et al.* (1989b) and El-Sheltawi *et al.* (1999). The reason behind this fact that vitamin E protect the polyunsaturated fatty acids content of phospholipids within cell membrane from oxidative degeneration of hydrogen peroxides. So it is exerting a protective effect on sperm membrane (Combs *et al.*, 1975; Beconi *et al.*, 1993; Badr *et al.*, 2003 and Khalifa *et al.*, 2004).

Looking to the present study, it was proved that there was a significant positive correlation between vitamin E concentrations in seminal plasma and alkaline phosphatase activity ($r = 0.33$, $P < 0.01$). Similar observation was recorded by Khalifa (1997). Vitamin E might increase the synthesis or release of tetsosterone, which might enhance the secretory activity of the accessory sex glands and subsequently might increase the alkaline phosphatase activity of semen (Hidiroglou *et al.*, 1979; Mann and Iutwak-Mann, 1981 and Pond *et al.*, 1995).

Vitamin E and zinc concentration showed a significant increase in seminal plasma than the concentration in blood serum. Similar observations were recorded by El-Anwar and Badr (1996) which they found zinc concentrations in seminal plasma of goat semen is high than in blood serum. As well as Surai (1992) and Surai and Ionov (1992) found the vitamin E concentration in seminal plasma is higher than that of turkey and geese blood serum.

In contrast, Kaludin *et al.* (1989) and Blesbois (1993) found the vitamin E concentrations in seminal plasma is lower than that of serum of ram.

It was concluded that the level of zinc and vitamin E in seminal plasma master most of the physicochemical buffalo semen characters. So that A.I. bulls should provided with sufficient amount of zinc and vitamin E in their rations for better semen characteristics.

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Table 1: Semen physical characters of buffalo bulls (Mean ± S.E.).

Bull number	Volume (ml)	Mass activity (%)	Individual motility (%)	Live spermatozoa (%)	Sperm concentration ($x10^6$ sperm/ml)	Semen pH	Sperm penetration distance (mm)
1	2.14±0.21^{a}	2.92±0.14^{b}	70.38±0.88^{b}	73.84±1.76^{b}	581.53±57.38^{b}	6.88±0.05^{a}	32.38±3.60^{a}
2	2.31±0.31^{a}	3.15±0.19^{ab}	71.54±1.31^{b}	78.85±1.86^{ab}	809.23±97.16^{a}	6.85±0.06^{a}	33.69±2.74^{a}
3	2.48±0.25^{a}	3.61±0.14^{a}	75.77±0.95^{a}	80.08±1.49^{a}	772.30±101.40^{ab}	6.84±0.05^{a}	35.15±7.82^{a}
4	1.85±0.18^{a}	3.00±0.19^{b}	69.61±1.05^{b}	75.00±1.22^{ab}	638.46±60.04^{ab}	6.93±0.04^{a}	36.33±3.77^{a}
5	2.00±0.21^{a}	3.08±0.17^{b}	71.54±1.31^{b}	76.08±2.08^{ab}	561.53±40.78^{b}	6.88±0.06^{a}	32.92±2.54^{a}
Overall mean	2.16±0.11	3.15±0.08	71.77±0.55	76.77±0.79	672.61±34.92	6.88±0.02	34.07±1.37

* Figures with different alphabetical superscripts are significantly different at least (P<0.05).

Table 2: Percentages of sperm abnormalities and of buffalo bulls (Mean ± S.E).

Bull number	Abnormal head (%)	Abnormal midpiece (%)	Proximal protoplasmic droplet (%)	Distal protoplasmic droplet (%)	Abnormal tail (%)	Abnormal acrosome (%)
1	2.15±0.19^{a}	2.46±0.49^{a}	0.31±0.17^{b}	0.54±0.31^{bc}	19.00±1.63^{a}	2.85±0.27^{a}
2	1.31±0.39^{a}	2.23±0.59^{a}	0.62±0.27^{ab}	1.69±0.49^{a}	15.23±0.93^{b}	2.77±0.38^{a}
3	1.85±0.55^{a}	2.62±0.44^{a}	1.15±0.36^{a}	1.31±0.44^{ab}	14.38±0.75^{b}	2.38±0.37^{a}
4	1.31±0.43^{a}	2.23±0.51^{a}	0.38±0.21^{b}	0.23±0.12^{c}	16.54±1.43^{ab}	2.23±0.26^{a}
5	2.62±0.60^{a}	2.85±0.44^{a}	0.23±0.20^{b}	0.08±0.07^{c}	14.92±1.10^{b}	2.08±0.26^{a}
Overall mean	1.85±0.21	2.48±0.22	0.54±0.12	0.77±0.16	16.02±0.56	2.46±0.14

* Figures with different alphabetical superscripts are significantly different at least (P < 0.05).

Table 3: Semen and blood biochemical properties of buffalo bulls (Mean ± S.E.).

Bull number	MBRT (sec)	Initial fructose conc. (mg/100ml)	Seminal zinc conc. (ppm)	Seminal vit E conc. (ppm)	Alkaline phosphatase (iu/ml)	Lactate dehydrogenase (u/L)	Serum vit E conc. (ppm)	Serum zinc conc. (ppm)	Serum testosterone (ng/dL)
1	267.54 ±17.90^{a}	504.84 ±59.94^{ab}	3.38 ±0.30^{a}	40.29 ±3.31^{a}	383.54 ±39.10^{a}	258.92 ±24.05^{a}	17.84 ±1.43^{a}	1.05 ±0.07^{a}	81.44 ±2.29^{a}
2	236.15 ±21.22^{ab}	591.92 ±53.04^{a}	4.15 ±0.25^{a}	43.74 ±2.77^{a}	354.15 ±39.00^{a}	292.69 ±22.98^{a}	15.05 ±1.12^{a}	0.96 ±0.14^{a}	73.28 ±3.66^{a}
3	188.77 ±13.78^{b}	466.35 ±36.99^{ab}	4.10 ±0.22^{a}	39.99 ±2.18^{a}	394.69 ±42.05^{a}	290.08 ±28.29^{a}	14.18 ±1.10^{a}	1.06 ±0.09^{a}	91.24 ±14.53^{a}
4	279.31 ±17.68^{a}	554.99 ±51.24^{a}	3.82 ±0.37^{a}	41.76 ±2.95^{a}	377.54 ±43.06^{a}	229.08 ±16.31^{a}	15.32 ±1.21^{a}	1.18 ±0.15^{a}	70.53 ±6.66^{a}
5	228.23 ±15.31	395.86 ±37.93^{b}	3.89 ±0.37^{a}	37.19 ±1.78^{a}	347.77 ±38.86^{a}	250.23 ±22.32^{a}	15.23 ±1.15^{a}	1.04 ±0.06^{a}	48.48 ±3.42^{b}
Overall mean	240.0 ±8.51	502.79 ±22.74	3.87^{a} ±0.13	40.59^{A} ±1.18	371.54 ±17.66	264.2 ±10.45	15.52^{B} ±0.54	1.06^{b} ±0.04	72.99 ±3.72

* Figures with different alphabetical superscripts are significantly different at least ($P < 0.05$).

Table 4: Reproductive performance of buffalo bulls (Mean ± S.E.).

Bull number	Reaction time (sec)	Scrotal circumference (cm)	Scrotal thickness (cm)
1	42.84±6.44^{b}	31.22±0.04^{a}	0.91±0.05^{a}
2	47.07±8.02^{b}	30.04±0.08^{b}	0.69±0.01^{b}
3	32.00±5.91^{b}	31.04±0.35^{a}	0.70±0.05^{b}
4	36.76±4.01^{b}	29.18±0.03^{c}	0.51±0.01^{c}
5	70.62±11.92^{a}	27.03±0.05^{d}	0.73±0.01^{b}
Overall mean	45.86±3.74	29.79±0.21	0.71±0.02