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Effects of the Seminal Plasma Zinc Content and Catalase Activity on the Semen Quality of Water Buffalo (*Bubalus bubalis*) Bulls

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Abstract: In order to determine zinc and catalase content of seminal plasma in the buffalo and to study their associations with the semen characteristics, 54 semen samples were collected from 10 buffalo bulls; semen volume and sperm concentration, gross and progressive motility and viability were evaluated, seminal plasma was then harvested by centrifugation and its zinc content was estimated by atomic absorption spectrophotometer and its catalase activity determined by using a commercial kit. The zinc content of the seminal plasma (Mean±SEM) was recorded as 154.40±1.74 mg L^{-1}, while, the mean catalase value was 32.00±0.42 U mL^{-1}. The mean zinc values was highly correlated with sperm progressive motility and viability and with catalase values ($p = 0.000$ for all) and also was associated with gross motility ($p = 0.020$) and negatively with abnormal morphology ($p = 0.049$). The catalase values were highly associated with sperm progressive motility, viability and zinc content ($p = 0.000$ for all) and was associated with sperm gross motility ($p = 0.024$). For further clarification of these correlations, the samples were categorized in three groups of excellent (Ex, >90% motile, n = 33), good (Go, 80-89% motile, n = 15) and moderate (Mo, <79% motile, n = 6) according to their percentage of sperm motility. The mean progressive motility in Ex group was 92.54±0.51%, in Go group was 81.66±0.62% and in Mo group was 71.66±1.05%. The mean zinc and catalase values were recorded as 161.07±1.63 mg L^{-1} and 33.41±0.34 U mL^{-1} in Ex, 146.70±1.91 mg L^{-1} and 31.01±0.67 in Go and 136.42±4.97 mg L^{-1} and 26.51±0.87 U mL^{-1} in Mo groups. The mean zinc value in Ex group was highly associated with sperm motility, viability and catalase values, in Go group was associated with catalase values and highly associated with sperm abnormal morphology and in Mo group it was highly associations with catalase values only. The mean catalase value in Ex group, was highly associated with sperm motility and viability, in Go group was associated with zinc content and in Mo groups was highly associated with the zinc content. These results show that seminal plasma zinc and catalase content are correlated with semen characteristics and synergistically act to preserve motility and viability of the spermatozoa after ejaculation.

Key words: Buffalo, semen, microelement, antioxidant

INTRODUCTION

Zinc is involved in a number of functions of importance to sperm physiology (Vallee and Falchuk, 1993). The zinc content of testicular tissue varies in different animals from 20 to 200 μg g^{-1} dry weight, values that are in the range of those for most other organs. In contrast, the content of the prostate gland, the seminal fluid and ejaculated sperm are higher, ranging from ~800 to 3000 μg g^{-1} dry weight. Moreover, the zinc content of sperm increases after exposure to seminal fluid, suggesting that sperms accumulate the metal as they traverse from the testicle to the urethra (Vallee and Falchuk, 1993).

Testicular zinc is critical for spermatogenesis. Zinc deficiency induces atrophy of seminiferous tubules in the rat and failure of spermatogenesis, particularly the last stages when the zinc content of maturing sperm increases (Vallee and Falchuk, 1993).

Reactive Oxygen Species (ROS) play a role in male infertility, where excessive amounts impair spermatozoal motility. Epididymal antioxidant enzymes, including

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catalase, protect spermatozoa from oxidative damage in the epididymal lumen. Antioxidant secretions (superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) from the seminal vesicle into the seminal fluid protect spermatozoa after ejaculation (Zubkova and Robaire, 2004).

Hydrogen peroxide (H_2O_2) is a reactive oxygen species that at low concentration is toxic to sperm. H_2O_2 inhibits not only sperm viability but also the acrosome reaction, sperm-egg binding and oocyte penetration. Catalase activates the decomposition of H_2O_2 into water and oxygen, thus removing an initiator of free radical chain reactions leading to lipid peroxidation (Lapointe *et al*., 1998; Turner and Lysiak, 2008).

Catalase exists in only 1 form and is a highly efficient, intracellular enzyme converting hydrogen peroxide into hydrogen and water (Turner and Lysiak, 2008).

Detailed investigations of zinc and catalase content of seminal plasma in humans have been reported, but there is little information available about zinc and catalase contents in the buffalo seminal plasma. This study was carried out to: (1) estimate the zinc and catalase contents of the seminal plasma in buffalo bulls, (2) test whether any association exists between these parameters and semen characteristics.

MATERIALS AND METHODS

Animals: Fifty four semen samples were collected by a bovine artificial vagina from 10 sexually mature buffalo bulls (4-5 years old) kept in The Buffalo Breeding Center northwest of Iran, Urmia (37° 33′ N, 45° 4′ E) during the summer and autumn of 2007. Samples collected at weekly intervals and between 9 to 11 am. First mount ejaculates were collected, but in cases that it was of very poor quality, the second mount ejaculate was taken.

Semen evaluation: Immediately after collection, the volume was recorded and the ejaculate was placed in a 37°C water bath. Semen motility was evaluated immediately after collection. Gross motility was scored from 0 to 5 on a wet mount of neat semen at ×100 magnification (0 = cells present without motion; 5 = very rapid dark swirls). The percentage of progressively motile spermatozoa was estimated by microscopic examination at x400 magnification on a pre-warmed slide (37°C) and a subjective assessment of the progressive statement was recorded (0 = no motility to 5 = steady rapid forward progression) according to procedure of Ax *et al*. (2000). Sperm concentration was measured using standard hemocytometer methods (Hausser Scientific, Horsham, PA. USA), the percentage of viable spermatozoa was estimated by viewing 200 spermatozoa under x1000 magnification using eosin-nigrosin staining method of Barth (2007). The semen samples were cooled to room temperature and transported to the laboratory within 2 h.

Preparation of seminal plasma: Fresh semen was centrifuged at 5,000 rpm for 10 min, the supernatants were transferred into 1.5 mL tubes, re-centrifuged to eliminate the remaining cells.

Determination of zinc and catalase contents: Seminal plasma was diluted (1:10) by double de-ionized water and the zinc content was measured by atomic absorption spectrophotometry (Shimadzu Asc-6100, Japan). The catalase content of the seminal plasma was determined by using a kit (Catalase Assay Kit, Cayman Chemical Co. Ann Arbor, MI, USA).

Data analysis: The obtained data was analyzed by using SPSS software (version 11.5 for Windows; SPSS Inc., Chicago, IL, USA) computer program. Results are quoted as arithmetic Mean±SEM and significance was attributed at $p<0.05$.

Pearson's correlation coefficient (two tailed) test was used to examine the correlation between all the parameters of the semen. The comparison of the semen parameters and zinc and catalase contents of the seminal plasma in groups of samples was carried out by ANOVA, variance homogeneity of samples was examined by Levene's test, Duncan's test was used for multiple comparison and LSD values were calculated in all the groups.

RESULTS

The mean value of zinc content of the seminal plasma was recorded as 154.40±1.74 mg L^{-1}, while, for the catalase values it was 32.00±0.42 U mL^{-1}. The mean zinc content of the seminal plasma was highly positively associated with sperm progressive motility ($r = 0.743$, $p = 0.000$), viability ($r = 0.689$, $p = 0.000$) and with seminal plasma catalase value ($r = 0.881$, $p = 0.000$) while it was positively associated with gross motility ($r = 0.316$, $p = 0.020$) and negatively with sperm abnormal morphology ($r = -0.269$, $p = 0.049$) (Table 1). The catalase

Table 1: Characteristics of the buffalo semen (Mean±SEM) n = 54

Characteristics	Values
Ejaculate volume (mL)	3.07±0.17
Sperm concentration (×10^6 cells mL^{-1})	1377.14±61.22
Progressive motility (%)	87.20±1.06
Gross motility (score)	3.59±0.16
Abnormal morphology (%)	6.53±0.32
Viability (%)	89.68±0.94
Seminal plasma zinc (mg L^{-1})	154.40±1.74
Seminal plasma catalase (U mL^{-1})	32.00±0.42

Table 2: Comparison of the results of the different groups of samples

Parameters	Groups	No.	Mean±SEM
Gross motility (score)	Excellent	33	4.03±0.14^{a}**
	Good	15	3.23±0.33^{b}***
	Moderate	6	2.08±0.56^{c}**
	Total	54	3.59±0.16
Progressive motility (%)	Excellent	33	92.54±0.51^{a}***
	Good	15	81.66±0.62^{b}***
	Moderate	6	71.66±1.05^{c}***
	Total	54	87.20±1.06
Viability (%)	Excellent	33	94.00±0.48^{a}***
	Good	15	85.26±0.95^{b}***
	Moderate	6	77.00±2.94^{c}***
	Total	54	89.68±0.94
Abnormal morphology (%)	Excellent	33	6.06±0.36^{a}*
	Good	15	6.81±0.62^{a}
	Moderate	6	8.45±1.17^{b}
	Total	54	6.53±0.32
Concentration ($\times10^{6}$ cells mL^{-1})	Excellent	33	1376.80±65.10^{a}***
	Good	15	1584.90±125.66^{a}***
	Moderate	6	859.50±150.78^{b}
	Total	54	1377.10±61.22
Volume (mL)	Excellent	33	2.76±0.15^{a}***
	Good	15	3.86±0.47^{b}
	Moderate	6	2.83±0.30^{b}
	Total	54	3.07±0.17
Seminal plasma zinc (mg L^{-1})	Excellent	33	161.17±1.63^{a}***
	Good	15	146.70±1.91^{b}**
	Moderate	6	136.42±4.97^{c}
	Total	54	154.40±1.74
Seminal plasma catalase (U mL^{-1})	Excellent	33	33.41±0.34^{a}***
	Good	15	31.01±0.67^{b}***
	Moderate	6	26.51±0.87^{c}***
	Total	54	32.00±0.42

Different superscripted letter(s) denote a significant difference: *p<0.05, **p<0.01 and ***p<0.005 level

values were highly associated with sperm motility (r = 0.716, p = 0.000), viability (r = 0.626, p = 0.000) and zinc content (r = 0.881, p = 0.000) and it was associated with sperm gross motility (r = 0.306, p = 0.024). Sperm motility had a highly positive association with gross motility (r = 0.550) and viability (r = 0.888) in addition to the zinc content and catalase values and was negatively associated with sperm abnormal morphology (r = -0.316, p = 0.02); gross motility was highly correlated with sperm motility, viability (r = 0.500, p = 0.000), concentration (r = 0.400, p = 0.003) and was associated with the zinc content (r = 0.316, p = 0.020) and catalase values (r = 0.306, p = 0.024). Sperm abnormal morphology had a highly negative association with sperm viability (r = -0.399, p = 0.003) in addition to its associations with sperm motility and seminal plasma zinc content. Sperm concentration was also highly associated with semen volume (r = 0.421, p = 0.002).

In order to have a better insight of these results and make the range of variations narrower, the samples were categorized in three groups of Excellent (Ex, > 90% motile, n = 33), good (Go, 80-89% motile, n = 15) and moderate (Mo, <79% motile, n = 6) quality according to their progressive motility rates. The mean values for progressive motility were recorded as 92.54±0.51% in Ex, 81.66±0.62% in Go and 71.66±1.05% in Mo groups, which were significantly different (p<0.000 for all). The comparison of the data of the three groups is presented in Table 2. The mean zinc value in Ex group (161.17±1.63 mg L^{-1}) was highly positively associated with sperm progressive motility (r = 0.612, p = 0.000), catalase (r = 0.897, p = 0.000) and sperm viability (r = 0.456, p = 0.008); in Go group (146.70±1.91) was highly negatively associated with sperm abnormal morphology (r = -0.676, p = 0.006) and was associated with catalase (r = 0.631, p = 0.012) and in Mo group (136.42±4.97 mg L^{-1}) was positively associated with catalase values (r = 0.957, p = 0.003). The catalase values in Ex group (33.41±0.34 U mL^{-1}) was highly correlated with sperm motility (r = 0.646, p = 0.000), viability (r = 0.522, p = 0.002) and the zinc content (r = 0.897, p = 0.000); in Go group (31.01±0.67 U mL^{-1}) it was associated with the zinc content (r = 0.631, p = 0.012) only and in Mo group, catalase (26.51±0.87 U mL^{-1}) was highly associated with the zinc content (r = 0.957, p = 0.003) (Table 2).

DISCUSSION

The total zinc content of the buffalo seminal plasma we obtained in this study was recorded as 154.4±1.74 mg L^{-1} (Mean±SEM) which was highly correlated with sperm progressive motility and viability and also with seminal plasma catalase values. Our mean total zinc value is much higher than the value (86.88 μmol L^{-1} ≈ 5.65 mg L^{-1}) reported by Sansone *et al.* (2000) for seminal plasma in buffalo bulls. Massányi *et al.* (2003) compared semen zinc content in the bull, ram, stallion, boar and fox and reported that seminal zinc concentration in the boar was 171.74±65.72 mg L^{-1}, it was 86.20±45.88 mg L^{-1} in the stallion, 83.15±61.61 mg L^{-1} in the bull, 60.47±35.37 mg L^{-1} in the ram and 13.09±5.22 mg L^{-1} in the fox. In this study, the total zinc content of the seminal plasma was highly positively associated with sperm progressive motility and viability and catalase values.

Association between zinc content of seminal plasma and sperm motility was best depicted in the Ex group while in the Go group zinc values, which were lower than that in the Ex group, had a high association with sperm abnormal morphology and with catalase values, but in the Mo group it showed an association with catalase only.

Stoltenberg *et al.* (1997) demonstrated the presence of chelatable zinc in electroejaculated sperm cells and spermatozoa from the epididymis by *in vitro* autometallographic technique (AMG) and described the localization of zinc ions in rat spermatozoa. They

postulated that an exchange of zinc ions takes place between the epididymal epithelium and the sperm cells as they pass along the epididymal duct. Eickhoff *et al.* (2004) reported that macrophage Migration Inhibitory Factor (MIF) plays an important role in the maturation process of rat sperm during epididymal transit by inducing the elimination of zinc and affecting the amount of free sulphydryl groups in the sperm flagella.

Kendall *et al.* (2000) administered supplemental zinc to rams grazed on pastures that were not considered to be deficient in this element and assessed their seminal quality by ejaculate volume, spermatocrit, sperm concentration, abnormal morphology, motility and percentage of live (nigrosin-eosin stain) and seminal fluid zinc concentration evaluation. The supplemented lambs had significant increases in motility and proportion of live sperm. Kumar *et al.* (2006) supplemented crossbred (*Bos indicus*×*Bos taurus*) bulls with different amount of zinc propionate for 6 months and evaluated semen quantitative (ejaculate volume, sperm concentration and sperm number per ejaculate) and qualitative characteristics (semen pH, mass motility, individual motility, sperm livability percent and abnormal sperm percent) and found statistically differences among the bulls of different groups after six months of zinc supplementation. Mean ejaculate volume ($p<0.05$) and sperm concentration, live sperm (%) and motility (%) in zinc supplemented bulls were significantly ($p<0.01$) higher as compared with the control bulls. They concluded that zinc supplementation either in the inorganic or organic form in the diet of crossbred bulls improves qualitative and quantitative attributes of the semen. They also reported that number of sperm per ejaculate, mass motility and semen fertility tests, like bovine cervical mucus penetration, was significantly higher in bull given an organic form of zinc (Zn propionate) as compared to an inorganic form (Zn sulfate).

Massanyi *et al.* (2004) by comparing the zinc content of the bull and ram semen investigated its relation to sperm morphology and reported that the zinc concentration in bull semen was significantly higher than the ram semen and the occurrence of pathological spermatozoa in ram semen was higher than the bull semen (17.17±3.7% versus 11.79±4.88%). (We recorded a total abnormal morphology of 6.53±0.32% and a figure of 6.06±0.36% for the Ex group in this study).

Strzezek and Hopfer (1987) isolated a zinc ion-dependent protein with a special affinity for egg yolk from the boar seminal plasma which was secreted by epithelial cells of seminal vesicle glands and enveloped the spermatozoa after ejaculation, especially in middle-piece area. Strzezek *et al.* (1987) observed that this zinc ion-dependent protein is a factor inactivating the plasmatic inhibitor of sperm motility. Its regulating activity was exhibited at pH range of 7.3-8.2. This protein also inhibited the growth of bacteria, especially Gram-positive species, at the concentration of 4 mg mL^{-1} of the media.

Holtz and Foote (1978) measured the concentration of zinc in seminal plasma of Dutch-belted rabbits collected twice a day for 3 days and reported that it was lower in second ejaculates than the first ejaculates.

Massanyi *et al.* (2005) measured the concentration of zinc in the semen of foxes (*Vulpes vulpes*) as 13.09±5.22 mg mL^{-1} and found a significant negative correlation between the zinc concentrations and the number of spermatozoa with broken flagellum.

These reports support the association of zinc content of seminal plasma and motility observed in this study. The catalase content of seminal plasma in this study was highly positively associated with seminal plasma zinc values in addition to its association with sperm motility and viability. The mean catalase values in Ex group was highly associated with sperm motility and viability and with zinc values, but in Go groups, it was associated and in Mo group it was highly associated with the zinc content only. This means that the catalase content of seminal plasma in buffalo bulls is also an important factor for the sperm motility. Lindmann *et al.* (1988) in an investigation of the effectiveness of certain antioxidants, including catalase, in preserving the motility of bull sperms concluded that oxidation could be a factor in motility loss in living sperm. Lapointe *et al.* (1998) reported on the presence of catalase in the region of the acrosomal cap of the spermatozoa.

The findings on the seminal plasma catalase are controversial. Sanocka *et al.* (1996) showed a decreasing in catalase activity in infertile samples. Ahotupa and Huhtaniemi (1992) reported on a decrease of catalytic activity of catalase in the testis of experimentally cryptorchid rats. Lapointe *et al.* (2000) reported that catalase had a significant positive affect on maintenance of sperm motility in the bovine sperm. Bilodeau *et al.* (2002) observed that *in vitro* addition of catalase to the bovine semen samples overcame the loss of motility caused by 100 μM H_2O_2 and increased intracellular ATP level. Baumber *et al.* (2003) observed that addition of catalase to the equine semen prevented the increase in live acrosome reacted sperms. Verberckmoes *et al.* (2005) reported that addition of catalase to the semen diluents had no effect on sperm quality in the bovine. Cordoba *et al.* (2006) observed that catalase failed to modify oxygen uptake and block capacitation in heparin-treated samples in the bovine. Marti *et al.* (2007) reported that catalase activity was higher in the first ejaculate of

rams in all months of the year and higher in non breeding season and finally, De Graaf *et al.* (2007) found that *in vitro* addition of catalase had no effect on the post thaw sperm motility in the ram.

It can be concluded that the zinc content of seminal plasma in buffalo bulls is important for the preservation of sperm motility and viability, while seminal plasma catalase by protecting spermatozoa from damaging oxidative reactions helps in preserving the semen motility and viability after ejaculation. These two parameters seems to be interrelated and working together.

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