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Effects of the Seminal Plasma Iron and Lead Content on Semen Quality of Water Buffalo *(Bubalus bubalis)* Bulls

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Abstract

In order to determine iron and lead content of seminal plasma in water buffalo and to study their associations with the semen characteristics, 54 semen samples were collected from 10 buffalo bulls. The semen characteristics were evaluated; its iron and lead content were estimated by atomic absorption spectrophotometer. The iron and lead content of the seminal plasma (Mean ± SEM) was recorded as 40.68 ± 0.75 mg L^{-1} and 0.026 ± 0.008 mg L^{-1}, respectively. The mean iron value was highly associated with sperm progressive motility, gross motility and viability, negatively with lead content, and had a negative association with semen volume. The mean lead value was highly negatively associated with sperm progressive motility, gross motility, viability and positively associated with sperm abnormal morphology.

For further clarification of these associations, the results 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, Go and Mo group was 92.24 ± 0.51 %, 81.66 ± 0.62 %, and 71.66 ± 1.05 % respectively. The mean iron and lead values and their associations with other parameters in these groups are discussed.

The results show that seminal plasma iron content is associated with the motility and viability of the spermatozoa after ejaculation, but its lead content has an adverse effect on these parameters.

Key words: Buffalo; Seminal Plasma, Iron, Lead

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Introduction

Iron is an essential component of a group of heme proteins that function in oxygen transport or as enzymes in redox systems. A small amount of iron is contained in several nonheme metalloenzymes.[1] The major complexes coordinating Fe with the cell are heme and heme-containing proteins, hemosiderin and ferritin.[2]

Iron is indispensable for life, being part of heme and non-heme iron proteins that play a vital role in a broad range of cellular functions. These proteins are essential for such processes as oxygen transport, electron transfer, DNA synthesis and nitrogen fixation. In vertebrates, iron is transported within the organism between sites of absorption, storage and utilization by transferrin (m.w.80 kDa) which can bind up reversibly to two atoms of iron very tightly. Absorption and metabolism of iron ions in the cell has been investigated. [3-5]

Lead can create oxidative, genetic, metabolic and enzymatic damage, and can produce adverse effects on virtually every organ in the body. It can damage kidneys, the nervous system, reproductive system and causes blood pressure.[6] Lead has been reported to cause oxidative cellular damage in reproductive system tissues of adult male rats, which may be closely associated with ROS production.[7]

No information is available about seminal plasma iron and lead content of buffalo bulls. This study was carried out to: (1) estimate the iron and lead 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 2007. Each bull had 5 to 6 semen collections during this period of time.

Semen evaluation. Immediately after collection, the ejaculate was placed in a 37˚C water bath and the volume was recorded. Semen motility was evaluated immediately after collection. Gross motility was scored from 0 to 5 on a wet mount of neat semen at 100x magnification (0 = cells present without motion; 5 = very rapid dark swirls). The percentage of progressively motile spermatozoa was estimated by microscopic examination at 400x 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).[8] 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 1000x magnification using eosin-nigrosin staining method of Barth (2007).[9] The same method was used to evaluate sperm abnormal morphology.[9] The semen samples were cooled to room temperature and transported to the laboratory within 2 hours.

Preparation of Seminal Plasma. Fresh semen was centrifuged (Clements 2000, England) 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 mineral contents. Seminal plasma was diluted (1:10) by double de-ionized water and its iron and lead content were measured by atomic absorption spectrophotometer (Shimadzu Asc-6100, Japan).

Data analysis. The data was analyzed using SPSS software (Version 16.5 for Windows; SPSS Inc., Chicago, IL, USA) computer program. Results are quoted as arithmetic mean ± standard error of mean

(S.E.M) and significance was set 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 mineral contents of the seminal plasma in groups of samples was carried out by one-way ANOVA, variance homogeneity of samples was examined by Levene's test, Then, the results were categorized in three groups of excellent (Ex), good (Go) and moderated (Mo) according to their motility rates, which is the most important factor in the bull's semen fertility, and Duncan's test was used for the multiple comparison and LSD values were calculated in all the groups.

Results

The results of the semen evaluation as well as iron and lead contents of seminal plasma of 54 samples are summarized in Table 1. The mean value of iron content of the seminal plasma was recorded as 40.68 ± 0.75 mg L^{-1}, while, for the lead values it was 0.026 ± 0.008 mg L^{-1}. The mean iron content of the seminal plasma was highly positively associated with sperm motility (gross [r = 0.587] and progressive [r = 0.826]) and viability (r = 0.767) ($P = 0.000$ for all), highly negatively associated with seminal plasma lead content (r = -0.720, $P = 0.000$) and negatively with semen volume (r = -0.287, $P = 0.036$). However, it showed a negative non-significant association with sperm abnormal morphology.

The mean lead value had a highly negative association with sperm progressive motility (r = -0.806) and viability (r = -0.742) as well as seminal plasma iron content (r = -0.720) ($P = 0.000$ for all), and gross motility (r = -0.453. $P = 0.001$) and was positively associated with sperm abnormal morphology (r = 0.295, $P = 0.030$).

In order to have a better insight into these results, and make the range of variations narrower, the results 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 92.24 ± 0.51 % in Ex, 81.66 ± 0.62 % in Go, and 71.66 ± 1.05 % in Mo groups, which were significantly different ($P < 0.05$ for all). The comparison of the data of the three groups is presented in Table 2. The mean iron value in Ex group (44.19 ± 0.48 mg L^{-1}) was highly positively associated with sperm progressive motility (r = 0.833, $P = 0.000$), sperm viability (r = 0.766, $P = 0.000$), and negatively associated with seminal plasma lead values (r = -0.464, $P = 0.007$). The mean iron (36.40 ± 1.37 mg L^{-1}) and lead (0.0331 ± 0.0018 mg L^{-1}) values in Go group were not associated with any semen parameters, but in Mo group iron (34.12 ± 1.22 mg L^{-1}) had a significant association with gross motility (r = 0.818, $P = 0.047$) only and the mean lead value (0.0333 ± 0.0017 mg L^{-1}) had no association with any parameter.

Table1. Characteristics of the buffalo semen (Mean ± SEM) n = 54

Ejaculate volume (ml)	3.07 ± 0.17
Sperm concentration ($\times 10^6$ cells/ml)	1374.14 ± 61.22
Progressive motility (%)	87.02 ± 1.06
Gross motility (Score)	3.59 ± 0.16
Abnormal morphology (%)	6.53 ± 0.32
Viability (%)	89.68 ± 0.94
Seminal Plasma Iron (mg L^{-1})	40.68 ± 0.75
Seminal Plasma Lead (mg L^{-1})	0.026 ± 0.008

Table 2. Comparison of the results of the different groups of samples (Mean ± SEM)

	Groups		
	Excellent(n = 33)	***Good(n = 15)***	***Moderate(n = 6)***
Gross motility (Score)	4.03 ± 0.14^{a}*	3.23 ± 0.33^{b}	2.4 ± 0.35^{c}
Progressive motility (%)	92.24 ± 0.51^{a}	81.66 ± 0.62^{b}	71.66 ± 1.05^{c}
Viability (%)	94.00 ± 0.48^{a}	85.26 ± 0.95^{b}	77.00 ± 2.94^{c}
Abnormal morphology (%)	6.06 ± 0.36^{a}	6.81 ± 0.62^{a}	8.45 ± 1.17^{b}
Concentration ($\times 10^6$ cells/ml)	1376.84 ± 65.10^{a}	1584.86 ± 125.66^{a}	859.5 ± 150.78^{b}
Volume (ml)	2.76 ± 0.15^{a}	3.86 ± 0.47^{b}	$2.83 \pm 0.30^{a\,b}$
Seminal Plasma Iron (mg L^{-1})	44.19 ± 0.48^{a}	36.40 ± 1.37^{b}	34.12 ± 1.22^{b}
Seminal Plasma Lead (mg L^{-1})	0.022 ± 0.0007^{a}	0.0331 ± 0.0018^{b}	0.0333 ± 0.0017^{b}

*Different superscripted letters (a, b and c) denote a significant difference ($P < 0.05$).

Discussion

Little information is available about seminal plasma iron and lead content in buffalo bulls, and this study was performed to gain more information in this field. The total iron content of the buffalo seminal plasma obtained in this study was recorded as 40.68 ± 0.75 mg L^{-1} which was highly associated with sperm motility and viability and poorly associated with seminal plasma lead values. Pesch *et al.* (2006) reported that the iron content of seminal plasma of the stallion was 1.9 µmol $L^{-1} \approx 0.106$ mg L^{-1}.[10] Massanyi *et al.* (2003) compared semen iron content in the bull, ram, stallion, boar and fox, and reported that seminal iron concentration was significantly higher in the ram (40.32 ± 10.81 mg kg^{-1}), bull (38.04 ± 22.07 mg kg^{-1}), and fox (33.16 ± 24.36 mg kg^{-1}) than that in the boar (16.14 ± 10.35 mg kg^{-1}) and stallion (12.68 mg kg^{-1}).[11]

Associations between iron content of seminal plasma and sperm motility and viability was best depicted in the Ex group while in the Go and Mo groups, iron values, which were lower than that in the Ex group, had no association with semen parameters, and in the Mo group showed an association with lead content only.

Frazer and Anderson (2003) reviewed detailed mechanisms involved in iron absorption in the intestine and its regulation in the body.[12]

Excessive amounts of reactive oxygen species (ROS) play a role in male infertility by impairing spermatozoal motility.[13] Epididymal antioxidant enzymes 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.[13]

Rajesh Kumar *et al.* (2002) reported that oxidative stress in testicular milieu is associated with DNA damage and produces high frequency abnormal sperms with significant impact on male fertility.[14]

Hydrogen peroxide (H_2O_2) is a reactive oxygen species that at low concentration is toxic to sperm. H_2O_2 not only inhibits sperm viability but it also fails the acrosome reaction, sperm-egg binding, and oocyte penetration. Antioxidants activate the decomposition of H_2O_2 into water and oxygen, thus removing an initiator of free radical chain reactions leading to lipid per-oxidation.[15, 16] Turner and Lysiak (2008) reported that hydrogen peroxide can undergo a reaction with heavy metals such as iron to form ferric ions and hydroxyl ions. Hydroxyl ions have nanosecond half-lives but are damaging inside the cell because they can cause the covalent cross-linking with a variety of biological molecules as well as the propagation of other free radicals through more complex reactions.[16] This could be considered as an antioxidant role for iron in the cell.

The total lead content of the seminal plasma of buffalo bulls in this study was 0.026 ± 0.0008 mg L^{-1}, which was highly negatively associated with sperm motility, viability, and iron content, and had a positive association with sperm abnormal morphology. Massanyi *et al.* (2003) reported that the seminal lead concentration was the highest in the ram (0.35 ± 0.68 mg kg^{-1}),[11] which was much higher than in fox (0.08 ± 0.06 mg kg^{-1}), bull (0.06 ± 0.04 mg kg^{-1}), stallion (0.05 ± 0.05 mg kg^{-1}) and boar (0.02 ± 0.03 mg kg^{-1}). Massanyi *et al.* (2004) observed a higher occurrence of pathological spermatozoa in ram semen (17.17 ± 3.76 %) in comparison with the semen of the bull (11.79 ± 4.88 %) which was related to its higher lead content.[17] The total sperm abnormal morphology in this study (6.53 ± 0.32 %) is also lower than that in their report. In this study we observed that the lower lead content (0.022 ± 0.0007 mg L^{-1} in Ex group) the higher motility (92.24 %) and viability (94.00 %), and the lower sperm abnormal morphology rate (6.06 %) (Table 2).

Lead can be concentrated in the nucleus and perturbs cell proliferation and DNA synthesis in vivo, so experimental lead treatment could affect germinal cells during pre-and postnatal development, when pro-spermatogonia undergo mitosis, or when Sertoli cells appear. During spermatogenesis, histones are replaced by protamines which condense and protect sperm DNA, and zinc contributes to sperm chromatin stability. Lead intoxication during spermatogenesis can delay spermiation as well as release of immature spermatogenic cells in the tubules of testis. Low level of exposure may cause testicular atrophy, cellular degeneration, reduction in seminiferous tubule diameter and sperm count. Lead also affects the conduction in the autonomous nervous system in the epididymis because of the duct wall tension and the relaxation of AChE and MAO activities. [18]

Corpas *et al.* (2002) reported that reproductive system targets of lead intoxication are not only testes, it results in inhibition of testicular, epididymal and seminal vesicles function altering biochemical composition of these organs and consequently affecting the normal development of germinal cells.[18] This could be the reason of lower motility and viability and higher abnormal morphology observed in group Go and Mo in this study.

Al-Saleh *et al.* (2008) reported that blood lead level was negatively associated with IVF fertilization outcome.[19]

Reglero *et al.* (2009) reported the lead value in the testes of red deer in a lead mine area in Spain, with a liver and blood lead values below the toxic level, as 0.037 mg kg^{-1} which was associated with lower sperm acrosome integrity, membrane viability but not with sperm motility.[20]

Neonatal exposure of male mice to lead reduces fertility in the sexually mature animals without reducing sperm count, but

with an effect on the number of testicular macrophages and somatic cells.[21]

Vahter *et al.* (2007) working with human subjects found that boys are more susceptible to neurotoxic effects of lead following exposure early in life and females are more susceptible to immunotoxic effects of lead,[22] and White *et al.* (2007) have reviewed the neurotoxicology of lead.[23] Saaranen *et al.* (1987)[24] reported that the seminal plasma lead level in infertile men was significantly lower than that in the blood but Xu *et al.* (1993)[25] found no such relationship. Finally, Singh Rana (2008)[26] believes that lead induces apoptosis through immunosuppressive mechanisms.

It can be concluded that the iron content of seminal plasma in buffalo bulls is important for the preservation of sperm motility, and viability after ejaculation, and its presence in the seminal plasma will help spermatozoa to maintain their functions as long as it is necessary, while seminal plasma lead content has an adverse effect on these parameters which are considered to play an important role in the fertility of the semen.

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References

1. Kaplan A, Jack R, Opheim KE, et al. Mineral and trace elements. In: Clinical chemistry, 4th ed. Baltimore: Williams and Wilkins, 1995; 358.
2. Sarafanov AG, Todorov TI, Kajdacsy-Balla A, et al. Analysis of iron, selenium and cadmium in paraffin-embedded prostate tissue specimens using inductively coupled plasma mass-spectrometry. J Trace Elem in Med Biol, 2008; 22: 305-314.
3. Nevo Y, Nelson N. The NRAMP family of metal-ion transporters. Biochem Biophys Acta, 2006; 1763: 609-620.
4. Wallander ML, Leibold EA, Eisenstein RS. Molecular control of vertebrate iron regulatory proteins. Biochem Biophys Acta , 2006; 1763: 668-689.
5. Ponka P, Lok CN. The transferrin receptor: role in health and disease. Inter j Biochem Cell Biol, 1991; 31: 1111-1137.
6. Bagchi D, Preuss HG. Effects of acute and chronic oval exposure of lead on blood pressure and bone mineral density in rats. J Inorg Biochem, 2006; 99: 1155-1164.
7. Marchlewicz M, Michlska T, Wiszniewska B. Detection of lead-induced oxidative stress in the rat epididymis by chemiluminescence. Chemosphere, 2004; 57: 1553-1562.
8. Ax RL, Dally M, Didion BA, et al. Semen evaluation, In: Reproduction in Farm Animals, 7th edition, Hafez B and Hafez ESE (eds), Philadelphia, Lippincott Williams & Wilkins, 2000; 365-375
9. Barth AD. Evaluation of Potential Breeding Soundness of the Bull. In: Current Therapy in Large Animal Theriogenology, 2nd edition, Youngquist RS and Threlfall W R (eds), W.B. Saunders, St. Louis, 2007; 235.
10. Pesch S, Bergmann M, Bostedt H. Determination of some enzymes and macro-and microelements in stallion seminal plasma and their correlations to semen quality. Theriogenol, 2006; 66(2): 307-313.
11. Massanyi P, Trandzik J, Nad P, et al. Seminal concentrations of trace elements in various animals and their correlations. Asian J Androl, 2003; 5 (2): 101-104.
12. Frazer DM, Anderson GJ. The orchestration of body iron intake: how

do erythrocytes receive their cues? Blood cell Mol Dis, 2003; 30: 288-287.
13. Zubkova EV, Robaire B. Effects of glutathione depletion on antioxidant enzymes in the epididymis, seminal vesicles, and liver, and on spermatozoa motility in the aging Brown Norway rat. Biol Reprod, 2004; 71: 1002-1008.
14. Rajesh Kumar T, Doreswamy K, Shrilatha B, et al. Oxidative stress associated DNA damage in testis of mice: induction of abnormal sperms and effects on fertility. Mut Res, 2002; 513: 103-111.
15. Lapointe S, Sullivan R, Sirard MA. Binding of bovine oviductal fluid catalase to mammalian spermatozoa. Biol Reprod, 1998; 58: 747-753.
16. Turner TT, Lysiak JJ. Oxidative stress: A common factor in testicular dysfunction. J Androl, 2008; 29(5): 488
17. Massanyi P, Trandzik J, Nad P, et al. Concentration of copper, iron, zinc, cadmium, lead, and nickel in bull and ram semen and relation to the occurrence of pathological spermatozoa. J. Environ. Sci. Health A Tox. Hazard Subs. Environ. Eng, 2004; 39(11&12): 3005-3014.
18. Corpas I, Castillo M, Marquina D, et al. Lead intoxication in gestational and lactation periods alters the development of male reproductive organs. Ecotoxic Envir safety, 2002; 53: 259-266.
19. Al-Saleh I, Coskun S, Mashhour A, et al. Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. Int j Hyg Envion Health, 2008; 211: 560-579.
20. Reglero MM, Taggart MA, Castellanos P, et al. Reduced sperm quality in relation to oxidative stress in red deer from a lead mining area. Environ Pol, 2009; 8-9: 2209-2215.
21. Pace BM, Lawrence DA, Behr MJ, et al. Neonatal lead exposure changes quality of sperm and number of macrophages in testis of BALB/c mice. Toxicol, 2005; 210: 247-256.
22. Vahter M, Aksson A, Liden C, et al. Gender differences in the disposition and toxicity of metals. Environ Res, 2007; 104: 85-95.
23. White LD, Cory-Slechta DA, Gilbert ME, et al. New and evolving concepts in the neurotoxicology of lead. Toxicol Applied Pharmacol, 2007; 225: 1-27.
24. Saaranen M, Suistomaa U, Kantola M, et al. Lead, magnesium, selenium and zinc in human seminal plasma fluid: comparison with semen parameters and fertility. Hum Reprod, 1987; 2(6): 475-479.
25. Xu B, Chia SE, Tsakok M, et al. Trace elements in blood and seminal plasma and their relationship to sperm quality. Reprod Toxicol, 1993; 7 (6): 613-618.
26. Singh Rana SV. Metal and apoptosis: recent developments. J Trace Elem Med Biol, 2008; 22 (4): 262-284.