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Zinc and Iron Concentration and SOD Activity in Human Semen and Seminal Plasma

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Abstract The aim of the present study was to measure zinc (Zn) and iron (Fe) concentration in human semen and superoxide dismutase (SOD) activity in seminal plasma and correlate the results with sperm quality. Semen samples were obtained from men (N=168) undergoing routine infertility evaluation. The study design included two groups based on the ejaculate parameters. Group I (n=39) consisted of males with normal ejaculate

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(normozoospermia), and group II (n=129) consisted of males with pathological spermiogram. Seminal Zn and Fe were measured in 162 samples (group I, n=38; group II, n=124) and SOD activity in 149 samples (group I, n=37; group II, n=112). Correlations were found between SOD activity and Fe and Zn concentration, and between Fe and Zn concentration. SOD activity was negatively associated with volume of semen and positively associated with rapid progressive motility, nonprogressive motility, and concentration. Negative correlation was stated between Fe concentration and normal morphology. Mean SOD activity in seminal plasma of semen from men of group I was higher than in seminal plasma of semen from men of group II. Fe concentration was higher in teratozoospermic males than in males with normal morphology of spermatozoa in group II. Our results suggest that Fe may influence spermatozoa morphology.

Keywords Iron · Zinc · Superoxide dismutase · Male infertility · Semen · Seminal plasma · Ejaculate parameters · Spermatozoa

Introduction

About 30–40% of infertility cases are caused by male factor [1]; however, most of them are due to a small quantity or to inferior quality of spermatozoa. The role of trace elements in sperm and whole semen quality may have significant influence upon men infertility [2]. Simultaneously, Aydemir et al. [3] reported that oxidative stress in the reproductive system is thought to have significant effect on the fertilizing ability of sperm. Zinc (Zn) is present both in spermatozoa and seminal plasma, wherein its concentration is considerably higher than in other body fluids. It takes part in formation of sperm motility [4–6] and influence directly on sperm morphology [7]. Zn may contribute to fertility through its positive effect on spermatogenesis [8], plays an important part in capacitation, and may be a regulatory factor in this process [9, 10]. Zn with iron (Fe) takes part in processes of oxidation and reduction, whereas Zn with copper (Cu) prevents deleterious effects of reactive oxygen species on spermatozoa as a cofactor of Cu–Zn superoxide dismutase (SOD) [11–14]. Moreover, deficiency of Zn may lead to degeneration changes in cells creating spermatozoa

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after meiotic division [15]. There are, however, reports in which no significant correlation between Zn concentration in seminal fluid and sperm density or motility was found [2, 16, 17].

It should be emphasized that Fe and Fe compounds are not essentially toxic for human and animals. However, disturbances can appear due to pathological conditions or prolonged intake of high doses of Fe in the regulative mechanism of absorption. In these cases, Fe binding in hemosiderin (ferric form phosphate) or with proteins occurs, and also the postponement of Fe in the liver [12–14]. Fe can bear negatively on morphology of spermatozoa [7]. Similarly, Huang et al. [18] suggested that Fe^{2+} may induce lipid peroxidation to inhibit sperm motility. On the other hand, Fe participates in oxygenation and reduction processes, entering into the composition of many enzymes and metalloprotein compounds. Generally, the elementary function of Fe in cells that ties in with protective effect ahead toxic products of oxygenation reaction [3] suggests that Fe might be the mediator of oxidative damage effects and plays an essential role in spermatogenesis and male infertility. Simultaneously, both absorption and metabolic function of Fe are linked with the influence of many chemical elements. Particularly important is the antagonistic activity of Cd, Mn, Pb, Zn, and Cu. In relation to Cu, this dependence has additionally complex arrangement and frequently synergetic character in connection with their cooperation in redox processes. So, although Fe and its compounds are not toxic for human and animals, its overload can cause an increase in sperm DNA damage [19]. However, significant differences in Fe concentration between sperm of several teratospermia and normozoospermia subjects were not found [20].

Simultaneously, prolonged exposure of sperm cells to reactive oxygen species may cause peroxidation of cell lipids membrane; alter the structure of protein receptors, enzymes, and transporter proteins; and affect the sperm DNA fragmentation [21–26]. Oxidative stress in human spermatozoa has been associated with a reduction in sperm motility and viability and in sperm–oocyte fusion [22, 23, 27]. Then, SOD catalyzes the reduction of superoxide anions to hydrogen peroxide and plays a critical role in the defense of cells against toxic effects of oxygen radicals [24]. SOD plays also a major role in maintaining sperm viability. The SOD activity in spermatozoa is positively correlated with duration of sperm motility [28].

The above-mentioned research concerning the influence of chemical elements upon spermatozoa demonstrated frequently conflicting results. The knowledge in this area is thus still incomplete and often antagonistic and so demands complementation. Thus, the aim of the present study was to measure Zn and Fe concentration in human semen and SOD activity in seminal plasma, and then correlate the results with sperm quality.

Materials and Methods

Human Semen

Semen samples were obtained from men (N=168) undergoing routine infertility evaluation. Each of the subjects was interviewed, and a questionnaire was used to elicit the following information: (1) occupational exposure to agents that are known to affect spermatogenesis, (2) alcoholic consumption, and (3) smoking habit. We have taken account of the principles and criteria of the World Health Organization (WHO) procedures for sperm collection, analysis, and definitions [29] in our studies. Thus, after 3 to 7 days of abstinence, semen samples were collected into sterile containers. After liquefaction, semen analysis was performed according to the WHO guidelines to obtain volume, sperm concentration,

motility, and morphology [1, 29] using the Makler® Chamber. The study design included two groups based on their ejaculate parameters. So, group I consisted of males with normal ejaculate (normozoospermia; $n=39$) and served as the control group. Group II consisted of males with abnormal volume of semen, abnormal concentration, morphology, or motility of spermatozoa; males with more than one abnormal semen variables; and males with no spermatozoa in the ejaculate ($n=129$).

Zinc and Iron Analysis in Semen

Seminal Zn and Fe were measured in 162 of 168 samples (group I, $n=38$; group II, $n=124$). Zn and Fe concentrations in semen were determined by inductively coupled plasma–mass spectrometry (ICP-MS), i.e., 7500CE plasma ICP-MS spectrophotometer from Agilent Technologies Inc. (Palo Alto, CA, USA). We used the stove of Czylok firm and aluminum mineralizer of Tusnovics firm. Test tubes, from boron and silicon glass each of 25-ml volume, used in the procedure were an advantage. An aliquot of whole available volume, i.e., 1–1.5 ml, was mineralized before measuring. Semen sample was evaporated in a mineralizer at the temperature of 105°C. Evaporated semen sample was burned at the temperature of 450°C within 14 h of order (4 h of access to the temperature of burning). After self-cooling, the sample was poured with 3 ml of 69.0–70.0% nitric acid (Baker Instra analyzed). Then, sample was mixed and located in aluminum mineralizer (heated electrically block up to 400°C supplied with the adjuster and the measure of the temperature). First, mixture was heated at the temperature of 100°C (1 h, 15 min of access to the temperature of heating); then, temperature was raised to 150°C (1 h). After self-cooling to room temperature and addition of 1 ml of H_2O_2 (35%), sample was again heated at the temperature of 100°C (1 h). After self-cooling, solution was made up of 6 ml of bi-distilled water (0.5 μS/cm), mixed up, and then poured to polyethylene tightly closed containers and determined by ICP-MS method. The concentration of elements was given in terms of milligrams per kilogram dry weight (ppm dw).

Superoxide Dismutase Assay in Seminal Plasma

SOD activity was measured in 149 of 168 samples (group I, $n=37$; group II, $n=112$). The fresh semen was centrifuged at 2000 rpm for 15 min. The supernatant (seminal plasma) was collected and stored at −80°C until assayed. SOD activity in the seminal plasma was measured by enzyme analysis reactions using an SOD Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). Before determination of SOD activity, seminal plasma was diluted (1:10) by adding to SOD Assay Kit Sample Buffer.

Statistical Analysis

The obtained data was analyzed by using Statistica StatSoft data analysis software system, version 8.0., StatSoft, Inc. (2008) computer program. The results are summarized as arithmetic mean values and standard deviation (SD). The differences between the mean values of Zn and Fe concentration in semen and SOD activity in seminal plasma were analyzed for statistical significance by Mann–Whitney U test (Z) and by Kruskal–Wallis test. Probability level values at $P<0.05$ were regarded as significant. The relationships between SOD activity and Fe and Zn concentration and semen parameters were examined by Spearman's rank correlation coefficients.

Results

Statistical analyses were performed in three groups of males: group of all individuals, group of normozoospermic males (group I), and group of males with pathological spermiogram (group II).

Zn concentration in semen of men from group I was 1,091.594 (±455.059)mg/kg dry weight, and it was higher than in semen of men from group II (1,064.294±827.829 mg/kg dry weight), but the difference was not significant. No significant difference was also detected between Fe concentration in semen of men from group I (19.583±7.014 mg/kg dry weight) and of men from group II (20.379±9.929 mg/kg dry weight). The mean SOD activity in seminal plasma of semen from men from group I was 4.173 (±1.587)U/ml, and it was significantly higher than SOD activity in seminal plasma of semen from men from group II (3.552±2.402 U/ml; Tables 1, 2, and 3).

In the group of males with pathological spermiogram (group II), the concentration of Fe was significantly higher in teratozoospermic males (n=56; 22.742±10.909 mg/kg dry weight) than in males with normal morphology of spermatozoa (n=67; 18.265±8.599 mg/kg dry weight). The concentration of Fe in semen of men from group II was also significantly higher in smokers (n=32; 23.863±12.439 mg/kg dry weight) than in nonsmokers (n=88; 19.078±8.818 mg/kg dry weight), and in males with consumption of alcohol in their life history (n=90; 20.740±10.367 mg/kg dry weight) than males without consumption of alcohol (n=24; 16.125±6.922 mg/kg dry weight; see also Tables 1, 2, and 3).

Statistical analyses of relationships between examined variables performed in group of all individuals showed significant positive correlations (P<0.05) between SOD activity in seminal plasma and Fe concentration in semen, between SOD activity in seminal plasma and Zn concentration in semen, and between Fe and Zn concentration. SOD activity was negatively associated with volume (milliliters) of semen, and it was positively associated with rapid progressive motility (category A), nonprogressive motility (category C), and concentration ($\times 10^6$/ml). There was significant negative correlation between Fe concentration in semen and normal morphology (percentage of normal spermatozoa; Table 4).

In the group of normozoospermic males (group I), significant correlations (P<0.05) were demonstrated between SOD activity and Fe concentration in semen, whereas SOD activity and Zn concentration and Fe and Zn concentrations were not correlated. SOD

Table 1 Characteristic of men semen and seminal plasma—group of normozoospermic males (group I; mean±SD)

Semen characteristic	Number	Mean±SD	Minimum–maximum
Ejaculate volume (ml)	39	3.860±1.203	2.00–6.00
Sperm concentration ($\times 10^6$/ml)	39	133.414±68.982	27.00–250.00
Rapid progressive motility, category A (%)	39	34.307±19.146	0.00–62.96
Slow progressive motility, category B (%)	39	33.390±20.389	5.91–75.00
Nonprogressive motility, category C (%)	39	5.435±3.790	0.00–15.80
Immotility, category D (%)	39	27.112±8.064	12.50–41.03
Progressive motility, categories A+B (%)	39	67.797±9.199	51.36–84.11
Normal morphology (%)	39	88.773±7.735	84.11–99.76
Zn concentration (mg/kg dry weight)	38	1,091.594±455.059	351.86–2,864.90
Fe concentration (mg/kg dry weight)	38	19.583±7.014	9.72–38.52
SOD activity (U/ml)	37	4.173±1.587	1.22–9.90

Table 2 Characteristic of men semen and seminal plasma—group of males without normal values of semen (group II; mean±SD)

Semen characteristic	Number	Mean±SD	Minimum–maximum
Ejaculate volume (ml)	129	3.980±1.847	0.40–10.25
Sperm concentration ($\times 10^6$/ml)	129	48.714±60.088	0.00–343.00
Rapid progressive motility, category A (%)	129	12.199±14.551	0.00–64.280
Slow progressive motility, category B (%)	129	15.985±14.189	0.00–60.00
Nonprogressive motility, category C (%)	129	7.748±8.345	0.00–55.00
Immotility, category D (%)	129	52.940±28.020	0.00–100.00
Progressive motility, categories A+B (%)	129	28.455±22.206	0.00–83.19
Normal morphology (%)	129	65.499±29.415	0.00–100.00
Zn concentration (mg/kg dry weight)	124	1,064.294±827.829	97.24–6,694.13
Fe concentration (mg/kg dry weight)	124	20.379±9.929	2.56–69.76
SOD activity (U/ml)	112	3.552±2.402	0.57–16.80

activity was positively associated with concentration of spermatozoa ($\times 10^6$/ml), and there was a significant negative correlation between Fe concentration in semen and normal morphology (percentage of normal spermatozoa; Table 4).

In the group of males with pathological spermiogram (group II), significant correlations ($P<0.05$) were demonstrated between SOD activity and Fe concentration, between SOD activity and Zn concentration, and between Fe and Zn. SOD activity was negatively associated with volume of semen (milliliters), and it was positively associated with nonprogressive motility (category C) and concentration ($\times 10^6$/ml). We also stated significant negative correlation between Fe concentration in semen and normal morphology (percentage of normal spermatozoa) of males from this group (Table 4).

Discussion

In the present study, we examined the concentration of Zn and Fe in semen and SOD activity in seminal plasma in samples obtained from normozoospermic males and males

Table 3 Comparison of the results of groups of semen samples ($P<0.05$)

Semen characteristic	Z	P
Ejaculate volume (ml)	−0.14839	0.882
Sperm concentration ($\times 10^6$/ml)	**−6.58194**	**0.000**
Rapid progressive motility, category A (%)	**−6.07853**	**0.000**
Slow progressive motility, category B (%)	**−4.99281**	**0.000**
Nonprogressive motility, category C (%)	0.86595	0.386
Immotility, category D (%)	**5.98273**	**0.000**
Progressive motility, categories A+B (%)	**−8.31384**	**0.000**
Normal morphology (%)	**−4.96088**	**0.000**
Zn concentration (mg/kg dry weight)	−1.12850	0.259
Fe concentration (mg/kg dry weight)	0.18380	0.854
SOD activity (U/ml)	**−2.83177**	**0.005**

Table 4 Correlations between Fe, Zn concentration in semen, SOD activity in the seminal plasma, and sperm parameters in semen from males in group of all individuals, group of normozoospermic males (group I), and group of males without normal values of semen (group II; $P<0.05$)

	All individuals			Group I			Group II		
	SOD activity (U/ml)	Fe concentration (mg/kg dry weight)	Zn concentration (mg/kg dry weight)	SOD activity (U/ml)	Fe concentration (mg/kg dry weight)	Zn concentration (mg/kg dry weight)	SOD activity (U/ml)	Fe concentration (mg/kg dry weight)	Zn concentration (mg/kg dry weight)
Ejaculate volume (ml)	−0.233*	−0.110	−0.072	−0.132	−0.300	−0.079	−0.248*	−0.069	−0.085
Rapid progressive motility, category A (%)	0.210*	−0.085	0.128	0.270	−0.368*	0.262	0.054	−0.046	0.057
Slow progressive motility, category B (%)	0.147	0.109	−0.055	−0.154	0.436*	−0.283	0.113	0.022	−0.069
Nonprogressive motility, category C (%)	0.163*	−0.084	−0.006	−0.028	−0.088	0.026	0.226*	−0.096	−0.011
Immotility, category D (%)	0.088	−0.056	−0.067	0.021	−0.020	0.112	0.236*	−0.066	−0.016
Progressive motility, categories A+B (%)	0.157	−0.008	0.072	−0.022	−0.016	−0.091	0.019	−0.004	0.007
Normal morphology (%)	0.101	−0.299*	0.079	−0.016	−0.531*	0.020	0.045	−0.269*	0.030
Sperm concentration ($\times 10^6$/ml)	0.344*	−0.083	−0.018	0.653*	0.101	−0.080	0.239*	−0.121	−0.088
SOD activity (U/ml)	1.000	0.342*	0.362*	1.000	0.498*	0.194	1.000	0.316*	0.394*
Fe concentration (mg/kg dry weight)	0.342*	1.000	0.486*	0.498*	1.000	0.229	0.316*	1.000	0.542*
Zn concentration (mg/kg dry weight)	0.362*	0.486*	1.000	0.194	0.229	1.000	0.394*	0.542*	1.000

Statistical analysis was conducted using the Spearman's rank correlation coefficients (R_s values are shown in the table)

*$P<0.05$ was considered statistically significant

with pathological spermiogram. We cannot establish the differences in the concentrations of Zn in semen from man in this study between normozoospermic males and males with pathological spermiogram. However, this finding confirms previous reports [4, 30, 31] which investigated the relation of these elements to male factor sub-fertility semen parameters. On the other hand, Chia et al., Huang et al., and Zhao and Xiong [8, 32, 33] found the lower concentration of Zn in seminal plasma of the infertile group. We have also stated no significant differences between normozoospermic males and males with pathological spermiogram in the concentrations of Fe in semen from man in this study. However, Fe concentration in the studies of Aydemir et al. and Huang et al. [3, 32] was higher in seminal plasma of sub-fertile group than in fertile one. They suggest that Fe plays an essential role in spermatogenesis and male infertility. Fe might be the mediator of the effects of oxidative damage and induce lipid peroxidation to inhibit sperm motility.

We have stated significant higher level of SOD activity in normozoospermic group than in group of males with pathological spermiogram. These findings are in accordance with the study of Murawski et al. [34]. It must be emphasized that SOD seminal plasma provides protection against lipid peroxidation of phospholipid and phospholipid-bound fatty acids in normozoospermic samples [35, 36]. The results of other studies, however, indicate that SOD activity in seminal plasma is lower in men with normozoospermia than in men with pathological spermiogram [37, 38].

We observed significantly higher concentration of Fe in teratozoospermic males than in males with normal morphology of spermatozoa in the group of males with pathological spermiogram, which might be connected with Fe-induced lipid peroxidation [18]. Moreover, in agreement with the results of Slivkova et al. [39], we found significant negative correlations between Fe concentration in semen and normal morphology of spermatozoa. Our study also shows significant correlation between Fe and Zn, which is in accordance with the results obtained by [32].

The correlations were also found between SOD activity and rapid progressive motility, nonprogressive motility, and concentration of spermatozoa in the present study. These detailed findings are in accordance with studies of Murawski et al. [34] and seem to confirm that decreased seminal plasma scavenger antioxidant capacity, particularly in the form of low SOD activity, can be responsible for male infertility. Additionally, Kobayashi et al. [40] explained a significant role of SOD in sperm motility. It seems that lipid peroxidation of human spermatozoa may cause the loss of motility and that SOD may inhibit this lipid peroxidation. The findings from studies of Ben Abdallah et al. and Kumar et al. [41, 42] are in contrast with our data. We also find a significant negative correlation between SOD activity and volume of human semen, but we did not find any information for and against these findings.

Additionally, a significant correlation between SOD activity and Zn concentration (this paper) was also stated, and this finding confirms the report of Yeung et al. [43]. This correlation might be explained by the prostatic origin of SOD, especially of tissue-specific Cu–Zn from SOD [44]. Moreover, we observed a significant correlation between SOD activity and Fe, but we could not find any literature data for comparison with this result. However, the possible oxidative stress-induced effect of Fe might be connected with its negative effect on spermatozoa morphology and explains positive correlation between Fe concentration and SOD activity—defensive agent of cells against toxic effects of oxygen radicals.

Solely, in the case of Fe concentration in the group of males with pathological spermiogram, we observed a significantly higher concentration in smokers than in nonsmokers and in drinkers than in nondrinkers; however, we did not find any information

concerning human semen and spermatozoa for and against these findings. On the other hand, the investigations by Zhang et al. [45] confirm that medium, heavy, and long-term smoking adversely affected the semen quality in a population of men.

Summarizing our results, we could not unequivocally talk about Fe concentration and SOD activity influence and relationships with sperm quality as a whole. We suggest that Fe might bear negatively on morphology of spermatozoa, and SOD activity is positively related to motility and concentration of spermatozoa and negatively related to volume of human semen. SOD activity survey could thus improve the diagnosis of male infertility. Simultaneously, the relationships between spermatozoa Zn content and the function of sperm production and sperm motility are still not clear. Therefore, further investigations in this field, particularly concerning also other elements and enzymes, are recommended.

Conclusions

1. Fe might influence negatively on the morphology of spermatozoa, and determinations of Fe levels in semen during infertility are recommended.
2. SOD activity is related to motility, and concentration of spermatozoa and volume of human semen and SOD activity survey could improve the diagnosis of male infertility.
3. Further investigations of Zn and Fe impact on spermatozoa, and relationships with SOD activity and the parameters of human semen are needed.

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References

1. Vayena E, Rowe PJ, Griffin PD (2002) Current practices and controversies in assisted reproduction: report of a WHO meeting. Medical, ethical and social aspects of assisted reproduction (2001: Geneva, Switzerland). World Health Organization, Geneva
2. Abou-Shakra FR, Ward NI, Everard DM (1989) The role of trace elements in male infertility. Fertil Steril 52(2):307–310
3. Aydemir B, Kiziler AR, Onaran I, Alici B, Ozkara H, Akyolcu MC (2006) Impact of Cu and Fe concentrations on oxidative damage in male infertility. Biol Trace Elem Res 112(3):193–203
4. Carpino A, Siciliano L, Petrone MF, DeStefano C, Aquila S, Ando S (1998) Low seminal zinc bound to high molecular weight proteins in asthenozoospermic patients: evidence of increased sperm zinc content in oligoasthenozoospermic patients. Hum Reprod 13(1):111–114
5. Henkel R, Bittner J, Weber R, Huther F, Miska W (1999) Relevance of zinc in human sperm flagella and its relation to motility. Fertil Steril 71(6):1138–1143
6. Kumar H, Verm RP, Singh LP, Varshney VP, Dass RS (2006) Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) bulls. Reprod Nutr Dev 46:663–675
7. Massányi P, Trandzik J, Nad P, Korenková B, Skalisk M, Toman R, Lukac N, Halo M, Strażak P (2004) 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 39(11–12):3005–3014
8. Chia SE, Ong CN, Chua LH, Ho LM, Tay SK (2000) Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. J Androl 21 (1):53–57

9. Andrews JC, Nolan JP, Hammerstedt RH, Bavister BD (1994) Role of zinc during hamster sperm capactation. Biol Reprod 51:1238–1247
10. Bilaspuri GS, Babbar BK (2007) Effect of albumin and zinc on capacitation and acrosome reaction of buffalo spermatozoa. Indian J Anim Sci 77(8):688–692
11. Gavella M, Lipovac V (1998) In vitro effect of zinc on oxidative changes in human semen. Andrologia 30:317–323
12. Kabata-Pendias A, Pendias H (1999) Biogeochemia pierwiastków śladowych. Wyd. Nauk. PWN, Warszawa, p 399
13. Kabata-Pendias A, Mukherjee AB (2007) Trace elements from soil to human. Springer, Heidelberg, p 600
14. Semczuk M, Kurpisz M (2006) Andrologia. Wyd. Lek. PZWL, Warszawa
15. Cigankova V, Mesaros P, Bires J, Ledecky V, Ciganek J, Tomajkova E (1998) The morphological structure of the testis in stallions with zinc deficiency (in Slovak). Slov Vet J 23:97–100
16. Madding CI, Jacob M, Ramsay VP, Sokol RZ (1986) Serum and semen zinc leveles in normozoospermic and oligozoospermic men. Ann Natur Metab 30:213–218
17. Stanwell-Smith R, Thompson SG, Haines AP, Ward RJ, Cashmore G, Stedronska J (1983) A comparative study of zinc, copper, cadium and lead levels in fertile and infertile men. Fertil Steril 40:670–677
18. Huang YL, Tseng WC, Lin TH (2001) In vitro effects of metal ions (Fe^{2+}, Mn^{2+}, Pb^{2+}) on sperm motility and lipid peroxidation in human semen. J Toxicol Environ Health A 23(62, 4):259–267
19. Perera D, Pizzey A, Campbell A, Katz M, Poter J, Petrou M, Irvine DS, Chatterjee R (2002) Sperm DNA damage in potentially fertile homozygous β-thalassaemiapatients with iron overload. Hum Reprod 17 (7):1820–1825
20. Kwenang A, Kroos MJ, Koster JF, van Eijk HG (1987) Iron, ferritin and copper in seminal plasma. Hum Reprod 2(5):387–388
21. Davies KJ (1987) Protein damage and degradation by oxygen radicals I: general aspects. J Biol Chem 262:9895–9901
22. de Lamirande E, Gagnon C (1992) Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. J Androl 13:368–378
23. de Lamirande E, Gagnon C (1992) Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J Androl 13:379–386
24. Frączek M, Kurpisz M (2005) System redoks w nasieniu męskim i peroksydacyjne uszkodzenia plemników. Postepy Hig Med Dosw 59:523–534
25. Griveau JF, Dumont E, Renard P, Callegari JP, Le Lannou D (1995) Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. J Reprod Fertil 103:17–26
26. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T (1997) Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril 68:519–524
27. Aitken RJ, Buckingham D, Harkiss D (1993) Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa. J Reprod Fertil 97:441–450
28. Alvarez JG, Touchstone JC, Blasco L, Storey BT (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa: superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 8(5):338–348
29. World Health Organization (1999) WHO laboratory manual for the examination of human semen and sperm—cervical mucus interaction. Cambridge University Press, Cambridge
30. Krupej J, Tomala J, Zych F, Bakon I, Orgacka H, Machalski T (1994) Zinc levels in semen of men from childless marriages. Ginekol Pol 65(5):239–243
31. Wong WY, Flik G, Groenen PM, Thomas CM, Copius-Peereboom HJ, Merkus HM, Steegers-Theunissen RP (2001) The impact of calcium, magnesium, zinc, copper in blood and semina plasma on semen parameters in men. Reprod Toxicol 15:131–136
32. Huang YL, Tseng WC, Cheng SY, Lin TH (2000) Trace elements and lipid peroxidation in human seminal plasma. Biol Trace Elem Res 76(3):207–215
33. Zhao RP, Xiong CL (2005) Zinc content analysis in serum, seminal plasma and spermatozoa of asthenozoospermic and oligoasthenozoospermic patients. Zhonghua Nan Ke Xue 11(9):680–682
34. Murawski M, Saczko J, Marcinkowska A, Chwiłkowska A, Gryboś M, Banaś T (2007) Evaluation of superoxide dismutase activity and its impact on semen quality parameters of infertile men. Folia Histochem Cytobiol 45(1):123–126
35. Tavilani H, Goodarzi MT, Vaisi-raygani A, Salimi S, Hassanzadeh T (2008) Activity of antioxidant enzymes in seminal plasma and their relationship with lipid peroxidation of spermatozoa. Int Braz J Urol 34(4):485–491

36. Tavilani H, Goodarzi MT, Doosti M, Vaisi-Raygani A, Hassanzadeh T, Salimi S, Joshaghani HR (2008) Relationship between seminal antioxidant enzymes and the phospholipid and fatty acid composition of spermatozoa. Reprod Biomed Online 16(5):649–656
37. Bykova M, Titova N, Sharma R, Agarwal A (2007) Association of classical semen parameters with superoxide dismutase and catalase activities in human semen. Fertil Steril 88(suppl 1):302–303
38. Tkaczuk-Włach J, Kankofer M, Jakiel G (2002) Activity of superoxide dismutase and glutatione peroxidase in human semen in normozoospermia and spermatopathy. Ann Univ Mariae Curie Sklodowska Med 57(2):369–375
39. Slivkova J, Popelkova M, Massanyi P, Toporcerova S, Stwarz R, Formicki G, Lukac N, Putała A, Guzik M (2009) Concentration of trace elements in human semen and relation to spermatozoa quality. J Environ Sci Health A Tox Hazard Subst Environ Eng 44(4):370–375
40. Kobayashi T, Miyazaki T, Natori M, Nozawa S (1991) Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod 6(7):987–991
41. Ben Abdallah F, Dammark I, Attia H, Hentati B, Ammar-Keskes L (2009) Lipid peroxidation and antioxidant enzyme activities in infertile men: correlation with semen parameter. J Clin Lab Anal 23 (2):99–104
42. Kumar R, Venkatesh S, Kumar M, Tanwar M, Shasmsi MB, Kumar R, Gupta NP, Sharma RK, Talwar P, Dada R (2009) Oxidative stress and sperm mitochondrial DNA mutation in idiopathic oligoasthenozoospermic men. Indian J Biochem Biophys 46(2):172–177
43. Yeung CH, Cooper TG, De Geyter C, Rolf C, Kamischke A, Nieschlag E (1998) Studies on the origin of redox enzymes in seminal plasma and their relationship with results of in vitro fertilization. Mol Hum Reprod 4(9):835–839
44. Nonogagi T, Noda Y, Narimoto K, Shiotani M, Mori T, Matsuda T, Yoshida O (1992) Localization of CuZn-superoxide dismutase in the human male genital organs. Hum Reprod 7(1):81–85
45. Zhang JP, Meng QY, Zhang LJ, Mao YL, Sun ZX (2002) Study of correlation and effect of smoking on semen quality of men. Zhonghua Nan Ke Xue 8(1):35–37