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ScienceDirect
Nutrition Research 29 (2009) 82–88
Nutrition Research
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Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men

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Received 24 September 2008; revised 19 November 2008; accepted 21 November 2008

Abstract

Zinc has antioxidative properties and plays an important role in scavenging reactive oxygen species. We hypothesized that in the absence of Zn, the possibility of increased oxidative damage exists that would contribute to poor sperm quality. Therefore, measurement of seminal Zn in the seminal plasma of males with a history of subfertility or idiopathic infertility is necessary and can be helpful in fertility assessment. The primary objective of the present study was to assess the relationship between Zn levels in seminal plasma with sperm quality in fertile and infertile men. Semen samples were provided by fertile (smoker [n = 17], nonsmoker [n = 19]) and infertile men (smoker [n = 15], nonsmoker [n = 21]). After semen analysis, concentrations of Zn, Mg, Ca, Na, and K in the seminal plasma of all groups were determined by atomic absorption spectroscopy. Element concentrations in seminal plasma of all groups were in the order Na > K > Ca > Zn > Mg. Fertile subjects, smoker or not, demonstrated significantly higher seminal Zn levels than any infertile group ($P < .001$). A trend was observed for a lower Zn levels in seminal plasma of smokers compared with nonsmokers. Seminal Zn in fertile and infertile (smokers or nonsmokers) males correlated significantly with sperm count ($P < .01$) and normal morphology of sperm ($P < .001$). There was a significantly positive correlation between seminal Zn with Ca ($P < .01$) and K ($P < .01$) levels in all specimens. In conclusion, poor Zn nutrition may be an important risk factor for low quality of sperm and idiopathic male infertility.
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Keywords: Male infertility; Trace elements; Zinc; Seminal plasma; Sperm quality
Abbreviation: ROS, reactive oxygen species.

1. Introduction

Human seminal plasma contains several trace elements that play an important role in the normal function of sperm. Recent reports summarized the role of trace elements in human reproductive potential. Intracellular calcium (Ca) is essential for sperm motility [1,2], metabolism [3], and acrosomal reaction [4,5]. Magnesium (Mg) is involved in many biologic processes and has an important function in enzymatic reactions and ejaculation [6]. Magnesium was found in high concentrations in the prostate gland and released into seminal fluid. Dramatic reduction in Mg concentrations in semen may therefore lead to disorders in male fertility [7]. Sodium (Na) and potassium (K) are present in seminal plasma at high concentration [8,9]. High levels of

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doi:10.1016/j.nutres.2008.11.007

K^+ improve the rate of acrosome reaction in human sperm in vitro and may be affected by increasing levels of intracellular Ca [10].

The concentration of zinc (Zn) in human seminal plasma is higher than in other tissues [11]. Zinc is a metalloprotein cofactor for DNA-binding proteins with Zn fingers. It is part of copper (Cu)/zinc superoxide dismutase and several proteins involved in the repair of damaged DNA (eg, P_{53}, which is mutated in half of human tumors) and in the transcription and translation processes of DNA [12,13]. Zinc has an important role in testes development, sperm physiologic functions and decreasing of its levels causing hypogonadism, decrease in testes volume, inadequate development of secondary sexual characteristics, and atrophy of seminiferous tubules (and hence spermatogenesis failure) [14-17]. Recent studies hypothesized that insufficient intake of Zn can impair antioxidant defenses and may be an important risk factor in oxidant release, compromising the mechanism of DNA repair, and making the sperm cell highly susceptible to oxidative damage [18,19].

Infertile and smoker men are very susceptible to oxidative damage induced by free radicals [20]. High levels of free radicals may overwhelm the antioxidant strategies (especially the effective concentrations of seminal Zn), which associated with low quality of sperm. In the present study, we hypothesized that major changes in the level of seminal trace elements, especially Zn levels, are related to low quality of sperm and poor fertilizing capacity. Therefore, this study focused primarily on Zn levels in the seminal plasma of fertile and infertile subjects (smokers and nonsmokers). Association of Zn (and to a certain degree Mg and Ca) in the seminal plasma of all groups was evaluated.

2. Methods and materials

2.1. Semen collection and semen populations

Seventy-two semen samples were collected from fertile nonsmokers (n = 19), fertile smokers (n = 17), infertile nonsmokers (n = 21), and infertile smokers (n = 15) referred to the Fatemeh Zahra In Vitro Fertilization center in Babol, Iran. Semen samples were obtained by masturbation into a sterile container after sexual abstinence for 2 to 3 days. Before semen analysis, a questionnaire was distributed to obtain information on smoking habits; alcohol use; use or abuse of other substances and drugs; and a history of orchitis, testicular trauma, sexually transmitted disease, varicocele, surgery for inguinal hernia, and cryptorchism. Consent was obtained from the subjects. Fertile and infertile patients who smoked cigarettes regularly or who were nonsmokers (never previously smoked) were included in the study.

2.2. Semen parameters analysis

After collection, semen specimens were allowed to liquefy at room temperature for 30 minutes and used for analysis. On microscopic examination, sperm count, percentage of motile sperm, and sperm with normal morphology were objectively evaluated. Sperm count and percentage of motile sperm were evaluated according to standards set by the World Health Organization [21]. Sperm morphology was evaluated according to the criteria by Kruger [22].

2.3. Measurement of Ca, Mg, Zn, K, and Na in semen

Semen samples were centrifuged at 600*g* for 10 minutes. After centrifugation, supernatants were diluted 10-fold by deionized water. Levels of Ca, Mg, Zn, Na, and K were measured by atomic absorption spectroscopy (Perkin Elmer model 2380). For this method, concentrations (milligram per 100 mL) of Ca (10-30), Mg (1-10), Zn (1-20), K (50-100), and Na (100-250) were used to plot the standard curve.

2.4. Statistical analysis

All data are reported as means ± SD. An independent *t* test was considered to compare the scores of each of the measures and mean of the parameters data between the 2 groups. The analysis of variance model was used for statistical analyses of element concentration between 4

Table 1
Age and semen parameters of subject in the 2 study groups

Variable	Fertile group		Infertile group	
	Nonsmoker (n = 19)	Smoker (n = 17)	Nonsmoker (n = 21)	Smoker (n = 15)
Age (y)	31.26 ± 4.03	29.52 ± 3.6	29.65 ± 3.42	30.53 ± 4.53
Volume (mL)	4.42 ± 1.16	4.44 ± 1.23	4.12 ± 1.58	3.1 ± 0.94
Sperm count ($\times 10^6$ mL)	87.63 ± 13.16	77.64 ± 16.4 *	38.25 ± 30.21 †	31.53 ± 19.52 ‡
Total sperm ($\times 10^6$)	396.31 ± 153.34	347.64 ± 138.58 *	158.4 ± 137.93 †	97.33 ± 64.96 ‡
Motility (%)	67.36 ± 8.87	64.7 ± 8.74	42.05 ± 24.6 §	42.33 ± 20.77 ‖
Normal morphology (%)	15.78 ± 3.95	12.04 ± 4.81 *	5.7 ± 3.56 †	4.06 ± 2.15 ‡

Values are presented as means ± SD.
* $P < .05$ by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of fertile smokers are compared with fertile nonsmokers.
† $P < .001$ by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile nonsmokers are compared with fertile nonsmokers.
‡ $P < .001$ by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile smokers are compared with fertile smokers.
§ $P < .01$ by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile nonsmokers are compared with fertile smokers.
‖ $P < .01$ by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile smokers are compared with fertile smokers.

groups. The Pearson correlation test and linear regression were used to analyze and examine the relationship between the sperm parameters and elements concentrations scores. A probability of less than 0.05 was considered as significant. Data were analyzed using SPSS, version 11.5 (SPSS Inc, Chicago, Ill) (FAQs).

3. Results

Mean values of sperm parameters in fertile and infertile groups can be seen in Table 1. No significant differences between the groups were observed in age and semen volume. Sperm count, motility, and normal morphology in fertile group (smokers or nonsmokers) were significantly higher than those in infertile group. A trend toward a higher quality of sperm was seen for nonsmokers compared with smokers (Table 1).

Mean concentrations of Zn, Mg, Ca, Na, and K in the seminal plasma of all samples can be seen in Table 2. Element concentrations in seminal plasma were in the order Na > K > Ca > Zn > Mg. No significant differences were seen in mean concentrations of Na, K, Ca, and Mg between groups. However, a trend (P = .09) was observed for a lower mean Mg levels in seminal plasma of infertile smokers compared with infertile nonsmokers. Moreover, a trend (P = .07) toward higher Mg levels was seen in seminal plasma of fertile nonsmokers compared with fertile nonsmokers (Table 3).

Fertile groups (smokers or nonsmokers) demonstrated significantly higher Zn levels in their seminal plasma than any infertile groups (P < .001). A trend was observed for a lower mean Zn levels in seminal plasma of smokers compared with nonsmokers (Table 2). These comparisons can be seen in Fig. 1A and B. fertile nonsmokers had significantly high levels of Zn in their seminal than infertile nonsmokers (P < .001); moreover, fertile smokers had significantly high levels of Zn in their seminal plasma compared with infertile smokers (P < .001). A trend (P = .07) was observed for a lower mean Zn levels in seminal plasma of fertile smokers compared with fertile nonsmokers. Moreover, a trend (P = .06) toward higher mean Zn levels was seen in seminal plasma of infertile nonsmokers compared with infertile smokers (Fig. 1A).

Correlation between Zn and Ca levels can be seen in Fig. 2. There was a significantly positive correlation (P < .01) between Zn and Ca levels in seminal plasma (Fig. 2). Correlation between Zn levels and sperm quality is presented in Fig. 3. Seminal Zn was positively correlated with sperm count (Fig. 3A; P < .01) and normal morphology (Fig. 3B; P < .001).

Table 3
Comparison of elements levels between all subjects by P

P				Elements
Fns vs IFns	Fs vs IFs	IFns vs IFs	Fns vs Fs	
.32	.16	.19	.63	Ca
.07	.13	.09	.13	Mg
<.001	<.001	.06	.07	Zn
.19	.99	.72	.36	Na
.55	.25	.29	.95	K

Fs indicates fertile smoker; Fns, fertile nonsmoker; IFs, infertile smoker; IFns, infertile nonsmoker men. An independent samples t test was considered to compare the difference between the 2 groups. A P < .05 is significant. A trend (P = .06) was observed for a lower mean Zn levels in seminal plasma of infertile smokers compared with infertile nonsmokers. A trend (P = .07) was observed for a lower mean Zn levels in seminal plasma of fertile smokers compared with fertile nonsmokers. A trend (P = .07) was observed for a lower mean Mg levels in seminal plasma of infertile nonsmokers compared with fertile nonsmokers. A trend (P = .09) was observed for a lower mean Mg levels in seminal plasma of infertile smokers compared with infertile nonsmokers.

4. Discussion

Calcium regulates the motility of ejaculated sperm, hyperpolarization [23,24], chemotaxis [25], acrosome reaction, and capacitation [26] by several signal transductions [27]. Many studies have shown that decreasing seminal Ca leads to low motility of sperm [5,28], but the relationships between Ca levels and sperm motility are controversial. Some authors indicated that the concentration of seminal Ca between fertile and infertile men is significantly different

Table 2
Concentrations of Ca, Mg, Na, K, and Zn in seminal plasma of the subjects

Elements (mg/100 mL)	Fertile group		Infertile group	
	Nonsmoker (n = 19)	Smoker (n = 17)	Nonsmoker (n = 21)	Smoker (n = 15)
Ca	24.49 ± 5.5	23.31 ± 7.91	21.96 ± 6.81	19.81 ± 5.46
Mg	6.85 ± 1.89	5.58 ± 2.25	5.83 ± 2.15	5.07 ± 2.13
Zn	14.08 ± 2.01	12.43 ± 2.64 *	10.32 ± 2.98 †	8.07 ± 2.65‡,§
Na	222.78 ± 43.49	210.39 ± 36.36	208.6 ± 33.9	206.55 ± 31.36
K	77.93 ± 25.20	74.48 ± 20.48	73.59 ± 20.24	66.54 ± 16.71

Values are presented as means ± SD.
* A trend (P = .07) was observed for a lower mean Zn levels in seminal plasma of fertile smokers compared with fertile nonsmokers.
† P < .001 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile nonsmokers are compared with fertile nonsmokers.
‡ P <.001 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile smokers are compared with fertile smokers.
§ A trend (P = .06) was observed for a lower mean Zn levels in seminal plasma of infertile smokers compared with infertile nonsmokers.

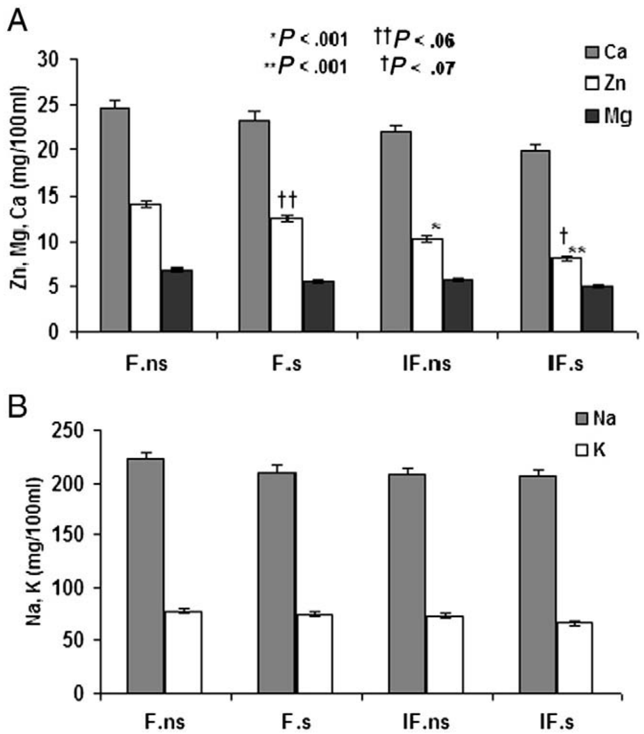

Fig. 1. Zinc concentrations decrease in seminal plasma of infertile and smoker men. A, Fertile men, smoker (Fs), or nonsmoker (Fns) have significantly high levels of Zn in their seminal than infertile groups ($P < .001$). A trend ($P = .07$) was observed for a lower mean Zn levels in seminal plasma of fertile smokers compared with fertile nonsmokers. Moreover, a trend ($P = .06$) toward higher mean Zn levels was seen in seminal plasma of infertile nonsmokers compared with infertile smokers. The concentrations of (A) Ca and Mg and (B) Na and K were not significantly different between groups.

[29-31], whereas other reports did not show a significant difference [32,33]. In our study, there was no significant difference in Ca levels in seminal plasma between all groups, and seminal Ca did not correlate with sperm quality.

Potassium has a key role in hyperpolarization of the sperm membrane. Potassium-dependent hyperpolarization is essential for induction of Ca influx activated by depletion of the Ca store and for the acrosome reaction [27]. Hyperpolarization of the plasma membrane depends on activation of K efflux, possibly through Ca-activated K channels that have a fundamental role in regulating the biologic events leading to the acrosome reaction. It may therefore have a primary role in fertilization [27,34]. Some researchers showed that the level of Na in fertile men is higher than in infertile men and had a positive relationship with sperm motility [35]. Gusani et al [35] measured Na and K in the seminal plasma of healthy and unhealthy men. A decreased level of Na was seen in the latter group, and a positive correlation was found between Na and motile sperm in both groups. Seminal K was negatively correlated with the percentage of motile sperm in the groups studied. In the present study, we did not observe a significant difference between Na and K levels in the seminal plasma of all

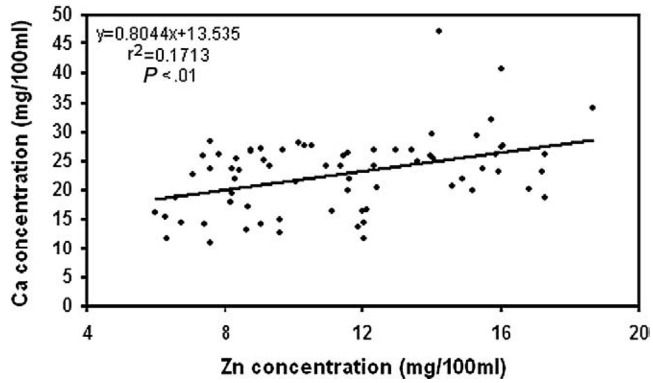

Fig. 2. Correlation between Zn and Ca contents in seminal plasma. Seminal plasma Zn and Ca concentrations were significantly correlated ($r^2 = 0.17$; $P < .01$). The Pearson correlation test was used to analyze and examine the relationship between Zn and Ca levels.

groups, and there was no significant correlation between seminal Na and K and sperm quality.

Magnesium is necessary for many enzymatic reactions [6]. Edorh et al [7] analyzed differences in Mg and Zn levels in the seminal plasma of 213 (48 normozoospermic, 30

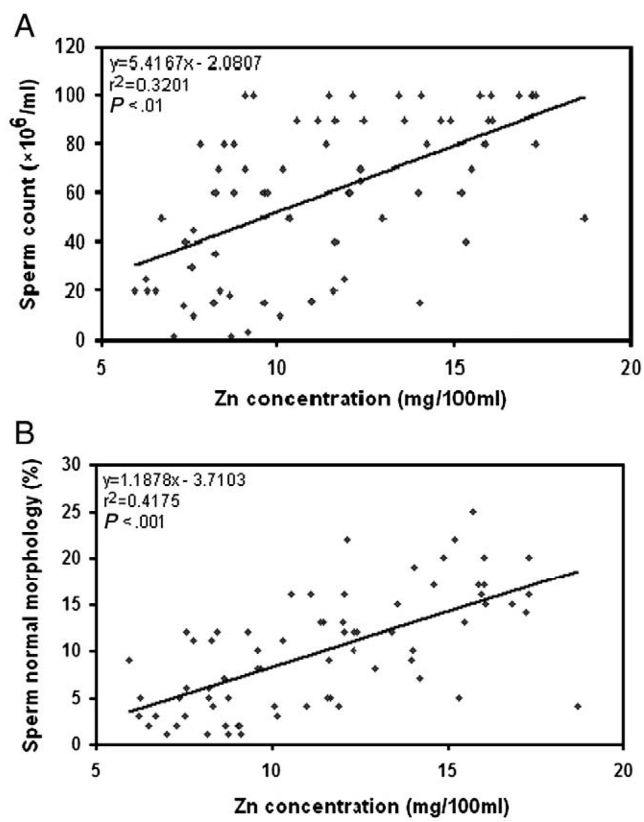

Fig. 3. Correlation between the seminal plasma Zn content with (A) sperm counts and (B) normal morphology. Seminal plasma Zn was positively correlated with sperm counts ($r^2 = 0.32$; $P < .01$) and normal morphology ($r^2 = 0.41$; $P < .001$). The Pearson correlation test was used to analyze and examine the relationship between Zn with sperm counts and normal morphology.

azoospermic, 28 oligoasthenozoospermic, 22 asthenozoospermic, 85 chronic prostatitis) males. The Zn levels in seminal plasma did not correlate with sperm count or the percentage of pathologic forms, but Mg concentration in seminal plasma was significantly reduced in patients with chronic prostatitis. In other reports [33,36], Mg concentration between subfertile and fertile groups was not different, and weak correlation was demonstrated between Mg levels in seminal fluid and sperm count. Colleen et al [37] observed no significant difference between patients and controls regarding the concentration of Mg or Zn, and no correlation was found between Mg or Zn and the number of sperm or the percentage of abnormal sperm; our results were in accord with the data of Colleen et al [37]. In this study, some infertile patients had significantly low levels of Mg in their seminal plasma compared with fertile groups.

Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of sperm [38]. There is extensive evidence that human seminal Zn has an important role in the physiologic functions of sperm and that reduced levels result in low quality of sperm and reduced chances of fertilization [38,39]. Total content of Zn in mammalian semen is high and has been found to be critical to spermatogenesis, but there have been conflicting reports on the effect of seminal Zn on sperm quality. Some studies indicated that there is no significant difference between Zn content in fertile and infertile men [33,36,37,40,41], but others found a significant difference between them [42-44], which was in agreement with our results. In our study, fertile subjects (smokers or not) had significantly higher levels of Zn in their seminal plasma than infertile groups. Some authors have reported high concentration of Zn to be associated with enhanced sperm parameters, including sperm count [7,41-43], motility [38,41,45], and normal morphology [7,43], whereas another study [46] reported a high concentration of Zn to be associated with poor motility of sperm. Zhao et al [44] observed a positive relationship between poor production of sperm and poor sperm motility with a lower content of Zn in the seminal plasma of infertile subjects. Many studies could not find a significant association between total Zn in seminal plasma and sperm quality [33,36,37,47,48]. Deng et al [49] reported that biologic zinc treatment has a positive effect on sperm motility, and supplementation of biologic zinc was an effective method for the treatment of infertile males with chronic prostatitis. In our study, seminal Zn had a significantly positive correlation with sperm count and normal morphology in all groups. We found a significant positive correlation between seminal Zn with Ca and K contents in all samples that were comparable with other research [33]. Weak correlation was found between seminal Zn and Mg that was comparable with other studies [30,36,37]. The Zn levels in the seminal plasma of smokers and nonsmokers, in addition to fertile and infertile men, were compared. Nonsmokers (fertile or infertile) had higher levels of Zn in their seminal plasma than smokers. This difference was not significant, but our results suggested that smokers are susceptible to Zn deficiency in their seminal fluid.

The mechanism by which Zn in the seminal plasma of smokers and infertile patients is depleted has not been fully elucidated. Many studies have shown that Zn has antioxidative properties, and has an important role in scavenging reactive oxygen species (ROS) [18,50,51]. Reactive oxygen species (eg, superoxide [O_2^-]) are unstable compounds with a short half-life that can adversely affect certain cellular processes [12]. High levels of ROS were detected in the semen of 25% to 40% of infertile patients, which affected sperm function by oxidation of lipids, proteins, and DNA [52]. Evidence suggests that Zn has antioxidative effects, and one consequence of Zn deficiency can be an increase in oxidative damage induced by ROS [19,53]. Zinc is an essential component of Cu/Zn superoxide dismutase, which has antioxidative properties for sperm function [12]. Zinc, through its competition with Cu and iron for membrane binding sites, reduces the potential for formation of the hydroxyl radical via redox cycling [18]. A small amount of ROS is necessary for sperm to acquire fertilizing capabilities [53], but it appears that high levels of seminal ROS may decrease the effective concentration of seminal Zn [53]. Increased ROS in the seminal plasma of infertile men may decrease the effective concentration of Zn, increasing the harmful effects of ROS to sperm cells that are associated with abnormal sperm parameters [53]. Several studies support this hypothesis, noting that decrease in Zn concentration can lead to an increase in oxidation of DNA, proteins, and lipids and that Zn has an important role in inhibition of oxidative damage [18,51,53,54].

Men who smoke inhale a host of toxic substances such as ROS that can be absorbed, so a causal relationship is suspected. Recently, studies have shown that cigarette smoking lead to increased seminal ROS by several mechanisms as follows: (i) cigarette smoking itself contains high levels of ROS and (ii) smoking metabolites may induce an inflammatory reaction in the male genital tract with a subsequent release of chemical mediation of inflammation that can recruit and activate leukocytes. Activated leukocytes can generate high levels of ROS in semen, and (iii) toxic metabolites of cigarette smoke may impair spermatogenesis, resulting in the production of abnormal spermatozoa, which is an important source of ROS and oxidative stress. High levels of ROS induced by smoking may overwhelm the antioxidant strategies (especially Zn effective concentration), resulting in oxidative stress that associated with low quality of sperm in smokers [20].

Results of this study may have also been influenced by the conditioning level of the subjects. Furthermore, dietary information may be a precise estimate of seminal plasma Zn levels. There is also the potential for dietary confounders because foods that are high in Zn may also be high in other substances such as antioxidant vitamins or other yet-to-be identified beneficial nutrients. However, correlation between Zn and sperm parameters is not dependent to dietary condition.

In conclusion, association of seminal Zn with other parameters of semen quality indicates that decrease of seminal Zn can be a risk factor for sperm abnormality and idiopathic male infertility. Smokers were susceptible to Zn deficiency in their seminal fluid. These data suggest that poor Zn nutrition may be an important risk factor for low quality of sperm and idiopathic male infertility. Routine determination of Zn levels during infertility investigation is therefore recommended.

Acknowledgment

The authors would like to thank a dedicated group of subjects. This study was supported by a grant from the University of Mazandaran (Babolsar, Iran).

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