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Comparison of Zinc Concentrations in Blood and Seminal Plasma and the Various Sperm Parameters Between Fertile and Infertile Men

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ABSTRACT: The aim of the study was to examine the relationships between concentrations of zinc in blood and seminal plasma and sperm quality among infertile and fertile men. One hundred seven male (infertile group) partners of couples who were undergoing investigation for infertility with no known cause for the infertility and 103 men (fertile group) whose wives were pregnant at the time of the study were recruited. The subjects' blood and seminal plasma concentration of zinc were determined by atomic absorption spectroscopy. Except for semen volume, all the other semen parameters for the infertile men were significantly lower than those for the fertile group. The geometric means of the seminal plasma zinc concentration were significantly lower in the infertile group compared with those in the fertile group; 183.6 mg/L (range, 63–499) versus 274.6 mg/L (range, 55–420). There were no significant differences in the geometric means of the blood zinc concentration between the 2 groups. Seminal plasma zinc concentration was significantly correlated with sperm density ($r = 0.341$, $P < .0001$), motility ($r = 0.253$, $P < .0001$), and viability ($r = 0.286$, $P < .0001$). On the basis of the findings of this study and those of other reports, zinc may contribute to fertility through its positive effect on spermatogenesis.

Key words: Sperm density, sperm motility, sperm viability, correlation.

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Several trace elements have been shown to be essential for testicular development and spermatogenesis. Zinc in human semen seems to play an important role in the physiology of spermatozoa. Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules (Bedwal and Bahuguna, 1994). The gonads are the most rapidly growing tissues in the body, and vital enzymes involved in nucleic acid and protein synthesis are zinc metalloenzymes (Bedwal and Bahuguna, 1994). Zinc has also been reported to be the primary factor responsible for the antibacterial activity of the seminal plasma. Other studies have suggested that zinc may have a role in sperm production and/or viability, in the prevention of spermatozoa degradation, and in sperm membrane stabilization (Lewis-Jones et al, 1996).

Most studies that examine the effects of zinc on spermatogenesis have recruited men who attended infertility/fertility clinics. There are limitations inherent in the selection of this group of people. The selection criteria for fertility are based on the men's semen parameters. The quality limit of semen parameters designed to discriminate infertile from fertile men is constantly being reexamined and redefined. Indeed, reports have appeared in the literature of pregnancies occurring with men whose semen analyses were well below the ''subfertile'' threshold (both not listed Van et al, 1975; Smith et al, 1977). In a 5-year follow-up study of 1089 infertile couples, there was no significant difference in any of the semen variables between couples who remained infertile and those who later conceived (Polansky and Lamb, 1988).

Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation (Plante et al, 1994; Irvine, 1996). Thus, it seems that seminal plasma, because of its high content of zinc, will exert protective, antioxidant-like activity sufficient to cope with the excessive amount of superoxide anions (Gavella and Lipovac, 1998). Reports have shown that infertile men have a higher percentage of abnormal spermatozoa (Menkveld et al, 1990; Liu and Baker, 1992). The abnormal spermatozoa would be a source of superoxide anions that bind with zinc present in the seminal plasma and thus reduce the zinc levels. Therefore, it may not be useful to use infertile subjects only to study the association between semen parameters and zinc concentrations in seminal plasma and blood.

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The present study was based on a group of men (103) of proven fertility (wives who are pregnant at the time of providing the semen). The findings are more likely to be a more accurate reflection of the association of zinc on sperm parameters and fertility. The aim of this study, therefore, was to examine the relationships between concentrations of zinc in blood and seminal plasma and sperm quality among infertile and fertile men.

Materials and Methods

Male partners of couples who were undergoing investigation for infertility in an obstetric and gynecology department of a general hospital in Singapore were randomly selected for inclusion in the study. Each of the subjects was interviewed by a trained interviewer. A questionnaire was used to elicit the following information: 1) occupational exposure to agents that are known to affect spermatogenesis, 2) alcoholic consumption, 3) smoking history, and 4) past medical history. The smoking history was classified into the following: 1) nonsmoker: an individual who has never smoked a cigarette before, 2) exsmoker: an individual who has quit smoking for more than a year, 3) smoker: an individual who is currently smoking. Smokers were also asked for the duration of their smoking behavior (in years) and for the average number of cigarettes smoked per day. None gave a history of taking any hallucinatory drugs. This was followed by a clinical examination by a gynecologist.

A total of 107 male partners fulfilled our criteria and were thus included in the study. These criteria were as follows: 1) an absence of factors in the individual's history that have a possible influence on male infertility, as suggested by the World Health Organization (WHO) Task Force on the Diagnosis and Treatment of Infertility (eg, history of diabetes mellitus, long-term medication, urinary tract infection, sexually transmitted disease, or testicular injury), 2) no abnormality detected clinically (none of the men had small testes—volume <10 mL—or varicoceles), 3) more than 1 year of failed attempts at conception, and 4) exclusion of female factors (eg, tubal occlusion, endometriosis, pelvic inflammatory diseases, endocrine and ovulation defects).

The fertile men in this study were recruited earlier in a study to determine what constitutes a normal seminal analysis (Chia et al, 1998). Females attending the antenatal clinic at the same department were approached by a female obstetric nurse. The purpose of the study was explained to the pregnant woman by the nurse. If the woman agreed to the study, she would then seek her husband's consent to participate. Only couples who had never attended an assisted reproductive program were recruited for the study. This information was verified separately with both the husband and wife. All who agreed to participate in the study had to sign a consent form. Approval for the study has earlier been obtained through the Hospital Ethics Committee. These fertile men were similarly interviewed by a trained interviewer with the same questionnaire that was used for the infertile men.

Semen Collection and Analysis

The men were asked to collect their semen at home in the morning by masturbation into a sterile, wide-mouth plastic container after 3 days of abstinence. The samples were brought into the hospital within 1 hour of collection. Time of ejaculation, abstinence period, spillage (if any), and fever during the last 3 months were recorded by the subject. All semen samples were processed and analyzed blind by experienced laboratory assistants at the Fertility Clinic of the Singapore General Hospital within 1 hour of receipt. Volume, total sperm count, sperm viability, proportion of progressively motile sperm, and proportion of normal and abnormal sperm forms were examined according to the WHO guidelines for the examination of human semen (WHO, 1992). Intra- and interassays for all the above parameters consistently gave values within a 10% variance.

Analysis of Zinc in Blood and Seminal Plasma

A blood sample was obtained by venipuncture with metal-free disposable syringes and stored in metal-free bottles. The concentrations of zinc in blood and seminal plasma were analyzed for all the infertile men. However, in the fertile group, only 103 of the men's samples were analyzed. The 103 men were randomly selected from the total 243 subjects recruited. These samples would be representative of the group of fertile men.

All reagents used in the present study were of high-purity analytical grade for trace elements analysis. Glassware and containers were soaked in 20% nitric acid overnight and rinsed thoroughly with double-distilled water.

Graphite furnace atomic absorption spectroscopy technique was used to determine zinc in blood and seminal plasma. The spermatozoa were separated from seminal plasma by centrifugation at 7000*g* for 5 minutes. The supernatant was then used for analysis of zinc content. Standards addition method was used throughout the whole analysis of all the samples. The coefficient of variation (CV) for zinc in blood (ZnB) is 1.5% for within-day runs and 4.3% for day-to-day runs. The CV for zinc in seminal plasma (ZnS) is 2.2% for within-day runs and 2.7% for day-to-day runs.

Statistical Analysis

As the data for zinc levels and sperm density were skewed, natural logarithmic transformation was used for the statistical analysis of these parameters. Comparison between groups was carried out using Student's *t* test, the chi-square test (χ^2), or Fisher's exact test. The relationships between parameters were examined by Pearson's correlation coefficients. All statistical analysis was performed by SPSS 7.5 for Windows (SPSS, 1997). The level of statistical significance was $P < .05$.

Results

Table 1 shows the demographic characteristics of the fertile and infertile men. The men were similar in the mean age groups. Most of the fertile men's wives were in their first or second trimester of pregnancy (range, 2–39 weeks gestation). There are significant differences in the ethnic distribution among the fertile and infertile men. In our earlier study, we showed that there were no significant differences in the sperm parameters in the different ethnic

Table 1. *Characteristics of the study population*

Characteristic	Fertile Men	Infertile Men
Number of men	103	107
Mean age (SD), y	34.2 (4.3)	34.8 (5.3)
Alcohol intake		
Social drinkers†	36 (35.0%)	46 (43.0%)
Nondrinker	67 (65.0%)	61 (57.0%)
Smoking history**		
Yes	28 (27.2%)	73 (68.2%)
No	75 (72.8%)	34 (31.8%)
Ethnic groups*		
Chinese	58 (56.3%)	85 (79.4%)
Malays	21 (20.4%)	11 (10.3%)
Indians	19 (18.4%)	8 (7.5%)
Others	5 (4.9%)	3 (2.8%)
Mean week of pregnancy (SD) for men's wives	17.7 (10.2)	—

† Social drinkers refer to those who drink less than once a month, each time consuming no more than 2 large bottles of beer.
* $P < .01$. ** $P < .001$.

Table 3. *Correlation coefficient between the blood and seminal plasma zinc concentrations and sperm parameters*

	LogZnB	LogZnS
LogZnB	—	0.103
LogZnS	0.103	—
Log volume	−0.072	−0.056
Log density	0.127	0.341**
Motility	0.009	0.253**
Viability	0.149*	0.286**
Log (normal morphology)	0.089	0.096

* $P < .01$. ** $P < .0001$.

groups (Chia et al, 1998). Therefore, ethnicity in the 2 groups is unlikely to be a potential confounder. The percentage of smokers was significantly higher among the infertile men compared with the fertile men ($P < .001$). Although the percentage of drinkers among the infertile men was greater than among the fertile men, this difference was not statistically significant. No subjects were on medication of any kind at the time of the study.

Table 2 shows the mean semen parameters and geometric mean blood and seminal plasma concentrations for zinc among the fertile and infertile men. Except for semen volume, all the other semen parameters for the infertile men were significantly lower than the fertile group. ZnS was significantly lower in the infertile group (Table 2). These observations were still valid after adjusting for smoking habits of the men. Thus, the observed effects are unlikely to be confounded by the difference in the smoking habits of the fertile and infertile men.

ZnS were significantly correlated with sperm density ($r = 0.341$, $P < .001$), motility ($r = 0.253$, $P < .0001$), and viability ($r = 0.286$, $P < .0001$; Table 3). Figures 1 and 2 show the relationships between ZnS and sperm density and motility.

Discussion

This study, to our knowledge, is the largest study correlating seminal plasma and blood zinc concentrations with semen parameters from both fertile and infertile men. The findings are more likely to be an accurate reflection of semen parameters and fertility. Ideally, it would have been better to obtain the men's semen as soon as their wives were found to be pregnant, but this arrangement would be very difficult logistically. Thus, it would be true to say that the sperm parameter measured in the present study may not be an absolute reflection of the subjects' sperm quality at the time of conception. But the time lag is not too long, considering that the median and mode were 16 and 8 gestation weeks, respectively.

Sperm counts are subject to wide variation; hence,

Table 2. *Mean semen parameters and mean blood and seminal plasma zinc concentrations for fertile and infertile men*

	Fertile Men (n = 103)		Infertile Men (n = 107)	
Semen Parameters	Mean (SD)	Range	Mean (SD)	Range
Volume (mL)	3.2 (1.4)	0.8–8.7	3.1 (1.4)	0.9–9.9
Density (million/mL)†	42.9 (2.8)	2–433	12.9 (5.8)**	0.1–227
Motility (%)	53.7 (16.4)	0–90	42.5 (19.1)**	0–78
Viability (%)	70.8 (14.8)	0–96	55.0 (22.0)**	0–99
Normal morphological (%)	20.9 (10.4)	0–43	16.1 (10.4)*	
Zinc in blood (mg/L)†	7.57 (1.0)	5.3–9.8	7.13 (1.0)	0–45
	7.59‡		7.11‡	3.3–9.9
Zinc in seminal plasma (mg/L)†	274.6 (1.4)	54.7–420.2	183.6 (1.6)**	63.0–499.0
	264.2‡		184.9‡,**	

† Geometric means and standard deviation (SD).
‡ Values adjusted for smoking history by analysis of variance.
* $P < .001$. ** $P < .0001$.

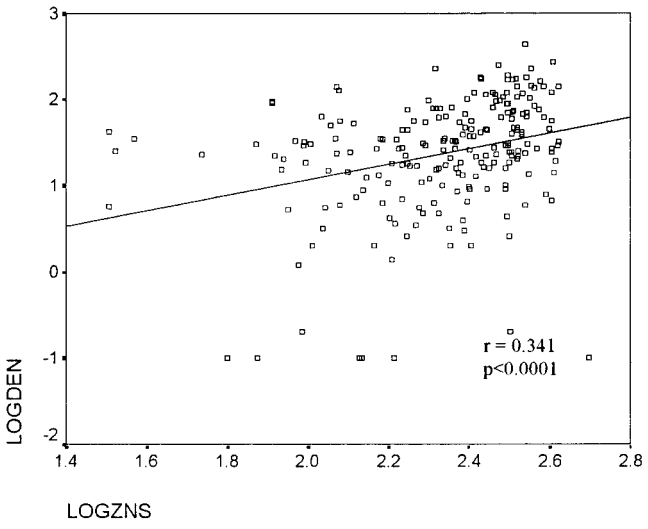

Figure 1. Relationship between seminal plasma zinc concentration and sperm density.

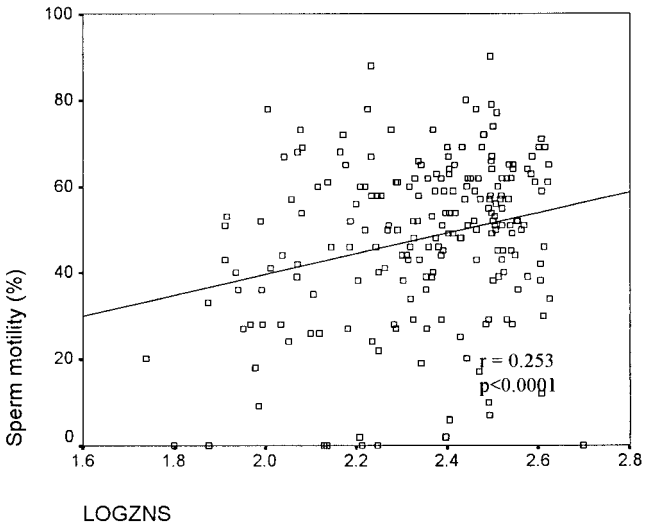

Figure 2. Relationship between seminal plasma zinc concentration and sperm motility.

some researchers have questioned the use of a single sample for semen analysis. However, Whorton and Meyer (1984) reported on sperm analysis in a large cohort of American chemical/agricultural workers and suggested that a single sample will suffice for an occupational study. Frequency of ejaculation is also a factor that affects the semen analysis result and that consequently risks evaluation. This would not apply in our study. All the subjects had a 3-day abstinence period, which was confirmed by the staff when the semen samples were collected.

It is clear that the infertile group had poorer semen parameters compared with the fertile group. Using the WHO criteria (1992), all the men in the infertile group had mean semen parameter values, except for semen volume, that were below the WHO-designated normal values (Table 2). This finding could explain why the couples had been unable to conceive for at least a year.

Several trace elements have been shown to be essential for testicular development and spermatogenesis. Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules (Bedwal and Bahuguna, 1994). The gonads are the most rapidly growing tissues in the body, and some of the vital enzymes involved in nucleic acid and protein synthesis are zinc metalloenzymes (Bedwal and Bahuguna, 1994).

Seminal plasma zinc concentrations varies depending on the reports. Lewis-Jones et al (1996) reported a mean seminal plasma zinc concentration of 117.6 mg/L (interquartile range of 75.2–176.5) in 1178 patients referred for fertility treatment. However, it must be noted that azoospermic patients (16.5% of the study population) were excluded from the analysis. Umeyama et al (1986) did a comparative study of seminal trace elements in fertile (n = 22) and infertile (n = 69) men and reported mean seminal plasma zinc concentrations of 124 mg/L and 129 mg/L, respectively. In their study, there were no significant differences between the 2 groups. It must be noted that there were only 22 fertile men, defined as men who had fathered a child in the last 2 years. There was no indication as to the distribution of these fertile men across that 2-year range. Thus, a fertile occurence might have been 2 years ago. As such, this was not a good recent indicator of proven fertility.

The mean seminal plasma zinc concentrations of infertile and fertile men were 184 mg/L and 275 mg/L, respectively. These values were higher compared with those in the 2 earlier studies. These differences may be due to the slightly different techniques used in the measurement of zinc concentration. These differences could also be due to the difference in the cigarette-smoking habits between the groups. Studies have been reported in which smokers had significantly lower seminal plasma zinc concentrations compared with nonsmokers (Oldereid et al, 1994; Pakrashi and Chatterjee, 1995). Neither the Umeyama et al (1986) nor the Lewis-Jones et al (1996) study provided information on the smoking habits of their subjects.

There was a significant difference between the mean seminal plasma zinc concentrations of the fertile and infertile groups (Table 2). Studies have demonstrated that zinc therapy results in significant improvement in sperm quality with increases in sperm density, progressive motility, and improved conception and pregnancy outcome (Tikkiwal et al, 1987; Kynaston et al, 1988; Omu et al, 1998). Zinc has been shown to have membrane-stabilizing and antioxidant activity and to maintain sperm viability by inhibiting DNAases (Aitken and Clarkson, 1987). Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa

and/or leukocytes in human semen after ejaculation. Thus, it seems that seminal plasma, because of its high content of zinc, exerts protective, antioxidant-like activity sufficient to cope with the excessive amount of superoxide anions (Gavella and Lipovac, 1998).

Our study demonstrated that seminal plasma zinc concentrations were significantly correlated with sperm density, motility, and sperm viability. These observations are supported by other studies. Saaranen et al (1987) found zinc concentrations to increase with increasing sperm density. This observation was supported by Stankovic and Mikac-Devic (1976), who also reported increased sperm motility with increased seminal plasma zinc concentrations. In a recent study, Lewis-Jones et al (1996) studied 1178 patients referred for fertility treatment. They reported that seminal plasma zinc concentration were poorly correlated ($r = 0.039$) with motile sperm concentration. The authors concluded that ''results from this much larger study have demonstrated a wide range of seminal zinc concentrations within the study population with no significant correlation with either motile sperm concentration or patient's age'' (p. 2466). The data for the seminal zinc concentration and motile sperm number were all skewed (Lewis-Jones et al, 1996, figure 1b). If the data were expressed in logarithmic scale, one might better appreciate the correlation and could have improved the correlation coefficient (r) between seminal zinc concentration and motile sperm concentration. There was also no mention of the smoking habits of the study's subject. Cigarette smoking has been associated with poorer sperm motility (Makler et al, 1993; Sofikitis et al, 1995; Gandini et al, 1997). As such, the study's findings could have been confounded by cigarette smoking among the study subjects.

In summary, on the basis of the findings of this study and that of other reports, zinc may contribute to fertility through its positive effect on spermatogenesis.

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