Turkish Journal of Medical Sciences

Volume 43 | Number 6 Article 21

1-1-2013

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TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2013) 43: 1000-1007
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doi:10.3906/sag-1211-54

Influence of trace elements and their correlation with semen quality in fertile and infertile subjects

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Received: 16.11.2012 • **Accepted:** 16.01.2013 • **Published Online:** 02.10.2013 • **Printed:** 01.11.2013

Aim: There has been increasing curiosity about the appraisal of essential trace elements present in human body fluids and their correlation to human health. Human seminal plasma contains several trace elements that have an imperative role in the normal functioning of the semen. As a result, it is very important to evaluate Zn and other trace elements present in the seminal plasma for the assessment of male infertility. The main objective of this research is to evaluate Zn concentrations and their intercorrelations with semen parameters such as sperm concentration, sperm motility, and normal morphology of the sperm, towards their contribution to human fertility.

Materials and methods: The concentrations of trace elements such as Zn, Mg, Ca, and Na in human seminal plasma were estimated by atomic absorption spectroscopy.

Results: Zinc concentration was highly significant in the control samples from fertile subjects when compared with all the categories of semen samples. The concentration of Zn in the seminal plasma positively correlated with sperm concentration, sperm motility, and normal sperm morphology.

Conclusion: Alliance of the concentration of Zn in the seminal plasma with the semen quality parameters indicates that Zn could be an indicator for sperm anomalies and male infertility.

Key words: Zinc, seminal plasma, correlation, atomic absorption spectroscopy, trace elements, fertility

1. Introduction

There has been increasing interest in the evaluation of essential trace elements present in different concentrations in the human body fluids and their correlation to the human health. Trace elements play important roles in a number of body functions (1). All the essential trace elements have their own range of adequacy. Smaller levels result in various abnormalities because of their specific biochemical changes (2). The analysis of essential trace elements will serve 2 purposes: first, to determine the concentrations and profiles of the various trace elements, and, second, to determine and detect the presence of potential toxic metals (3). Several research studies over the past 2 decades have suggested that the quality of semen that includes the sperm quality as well as the sperm quantity is declining in the industrialized countries throughout the world. This raises serious concern about male fertility (4). The decline in the semen quality in terms of sperm concentration and volume in men over a short period of time suggests that causes for human male infertility are more of environmental concern than genetic concern.

Human seminal plasma contains several trace elements that play an important role in the semen functions, including in sperm capacitating, metabolism, and the acrosome reaction. Zinc is an important micronutrient for human health. It is associated with many physiological functions, including its role in metalloenzymes that relate to intermediary metabolism (5). Zinc plays a major role in semen ejaculation as well as being a cofactor for the DNA-binding proteins with Zn fingers. Zinc is thought to be the part in superoxide dismutase for the repair of damaged DNA. It plays a major role in the transcription and translational process. Zinc in the seminal plasma stabilizes the cell membrane and nuclear chromatin of the sperm. The total content of zinc in human semen is very high and is found to have a critical role in spermatogenesis. However, there is controversy about zinc content and sperm quality. Zinc is thought to be one

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of the major factors that affect spermatozoa motility and morphology. It controls the effects by modulating the activity of the Ca^{2+} ATPase enzyme. Zn plays an important role in the development of testes and secondary sexual characteristics, and in a few sperm physiologic functions. Zinc acts as a growth factor, an immune-regulator, and a cryoprotectant with antiinflammatory effects (6). Decrease in the concentration of Zn in the seminal plasma causes hypogonadism, decrease in the size of the testes, inadequate development of secondary sexual characteristics, and atrophy in somniferous tubules. Consequently, these result in the spermatogenesis failure (7).

Calcium is an important element for determining the sperm metabolism, sperm motility, sperm vitality, and acrosome reaction (8,9). Calcium plays a vital role in the regulation of the motility, chemotaxis, capacitation, and hyperpolarization (10,11). Magnesium is thought to be in high concentrations in the prostate gland and is released into the semen during ejaculation. Drastic reduction in the magnesium concentration in the seminal plasma will therefore result in male reproductive disorders (12). Sodium is present in the seminal plasma of humans at higher concentrations (13). The development of apposite and steadfast analytical techniques such as atomic absorption spectroscopy enabled determination of multivariate elements in a much faster way (14). The objective of the current study is to analyze the effect of different trace elements in the human seminal plasma (Zn, Mg, Na, and Ca) and their correlation with different semen parameters, and to compare their correlations with different categories of infertile (oligoasthenozoospermia, asthenozoospermia, oligozoospermia, azoospermia, and normozoospermia) and fertile semen samples.

2. Materials and methods

2.1. Semen population

Semen samples were collected from infertile subjects, including cases of oligoasthenozoospermia (n = 10), asthenozoospermia (n = 10), azoospermia (n = 6), normozoospermia (n = 10), and oligozoospermia (n = 8), and fertile subjects (n = 8) at the Bangalore Assisted Conception Centre Pvt. Ltd., Bangalore, India.

2.2. Inclusion criteria

Males leading normal lives with regular unprotected sex without conception for 1 year or more were included in the study.

2.3. Exclusion criteria

A brief medical history of the patients was performed before semen analysis. The patients who were already using supplementary antioxidants or any other medication for male infertility were not included in this study. The patients who came for the first time for this reason were accepted for this study. In addition, subjects with testicular varicocele, genital infection, leukocytospermia, sexually transmitted diseases, chronic illness and serious systemic diseases, alcoholism, or smoking history were excluded from the study because of the well-known high seminal reactive oxygen species levels that decrease antioxidant activity, which results in decreased motility and abnormal morphology.

2.4. Semen collection and research ethics

This research study is part of a major research project, for which the human ethical approval and clearance was obtained from the VIT University Institutional Human Ethical Committee, Ref. No. VIT/UHEC-3/NO.11. We collected the samples from patients who were receiving semen analysis at the BACC in Bangalore, Karnataka, India. These patients gave their verbal consent to participate in the study. We are not able to get written statement from the patients, and this was also approved by the VIT University Institutional Human Ethical Committee, Ref. No. VIT/UHEC-3/NO.11. The patients were not willing to give written information because male infertility in India is creating issues personally as well as socially. They were not willing to reveal that they are infertile to anyone outside the center. Upon documenting the verbal consent, we noted the sample donor's name, address, and background. The semen samples were obtained by masturbation and collected in a clean, sterile, and wide-mouthed container made up of plastic that was confirmed as nontoxic for spermatozoa. The sample container was kept at ambient temperature (37 °C), thus avoiding the large changes in temperature that may affect the spermatozoa after ejaculation. After collection, the specimen was labeled with name of the patient, identification number, date, and time of collection. The semen container was placed in an incubator at 37 °C while the semen liquefied. If the sperm-rich part was missing from the sample, the sample was thought to be incomplete and a new sample was collected from the same person after an abstinence period of 3 days.

2.5. Semen analysis tests

The liquefied sample was taken for further analysis of semen parameters. Semen parameter analysis includes physical appearance, volume, viscosity, pH, and microscopic analysis. Microscopic analysis of the semen included sperm concentration, count of motile sperms, and count of morphologically normal sperm (15).

2.6. Evaluation of trace elements in seminal fluid

Initially, semen samples were centrifuged at 1500 rpm for 10 min at 4 °C. The supernatant was transferred to another Eppendorf tube and centrifuged at 1500 rpm for 10 min to completely remove any debris or spermatozoa. Subsequently, the samples were taken for the estimation of trace elements like Zn, Mg, Ca, and Na using atomic absorption spectroscopy. Standards used for the analysis

were purchased from Hi-Media, Mumbai, India. $ZnCl_2(H_2O)_4$ at 1–25 mg/100 mL, $MgCl(H_2O)_4$ at 1–15 mg/100 mL, $Ca_2Cl(H_2O)_2$ at 5–25 mg/100 mL, $NaCl(H_2O)_4$ at 90–180 mg/100 mL, and the standard curve were plotted. The seminal plasma was diluted 10 times using Millipore distilled water. Trace element concentrations were estimated using the standard curve.

2.7. Statistical analysis

Semen analysis was completed according to the standards set by the World Health Organization (15). Data reported here are the mean ± standard error of mean (duplicated). Statistical t-test was done to compare the scores of each of the measures and the mean data of each semen parameter between the control and infertile groups. One-way ANOVA modeling was used for the comparison of the trace element concentrations in the seminal fluids of the infertile groups.

3. Results

The values of the semen parameters including volume, pH, sperm concentration, total motility, and normal morphology of fertile and various infertile groups are represented in Table 1. The results reveal that the volume of samples varied from person to person irrespective of fertility or infertility. The pH of the semen seemed to be slightly alkaline (pH 7.1–8). Generally, minor variation in the pH of different samples was observed, but the pH was never acidic in any of the semen samples. Sperm concentration data reveal that this is the ultimate parameter for the determination of infertility. As denoted by azoospermia, there is no sperm count; in normozoospermia, there is a close relation in sperm concentration to that of the fertile samples. In oligozoospermia, asthenospermia, and combinational defects like oligoasthenozoospermia, the sperm concentration was significantly low. In our research, Zn concentration positively correlated with sperm concentration, sperm motility, and sperm normal morphology (Figure 1). The concentration of Zn was compared between the infertile and fertile categories and was found to be significantly different between these 2 categories. The concentration of Zn was found at the same level in the case of oligoasthenozoospermia and asthenospermia (Figure 2). Sperm concentration was found to be significantly less in all classes of the infertile category when compared to the control (fertile samples), except for the normozoospermia samples (Figure 3). Motility testing revealed that in the fertile and normozoospermia groups, the sperms were highly motile; they were moderately motile in the asthenospermia and oligozoospermia groups; and they were relatively immotile in the oligoasthenozoospermia group. Morphologically the sperms were found to be identical in all cases, except oligoasthenozoospermia. In general, progressive forward movement of the sperm by motility test should reveal that about 25% of the sperms are expected to be fast and progressive. In our observation, the oligozoospermia category's sperms were progressive like fertile sperms. In all other categories, the progressiveness of the sperms was negligible (Table 1). The hypoosmotic swelling test was used to determine vitality, rather than using dye exclusion, and it was found to be very low in cases of oligoasthenozoospermia and oligozoospermia. The mean concentrations of trace elements including Zn, Na, Mg, and Ca in the human seminal plasma of infertile and fertile subjects are given in Table 2. A significantly low

Table 1. Comparison of semen parameter scores between infertile groups and fertile group*.

Semen parameters	Infertile groups					Fertile (control group)
	Oligoasthenozoo-spermia	Asthenozoo-spermia	Azoo-spermia	Normozoo-spermia	Oligozoo-spermia	
Volume	2.49 ± 0.43	1.89 ± 0.16	2.16 ± 0.42	2.44 ± 0.25	2.06 ± 0.15	3.075 ± 0.24
pH	7.7 ± 0.08	7.5 ± 0.07	7.41 ± 0.11	7.58 ± 0.04	7.78 ± 0.11	7.65 ± 0.09
Sperm concentration (millions/mL)	9.6 ± 1.7	44.40 ± 3.75	0.00	92.5 ± 15.5	9.98 ± 1.74	107.1 ± 13.2
Total motility (%)	16.56 ± 3.5	36.07 ± 4.99	0.00	70.02 ± 3	37.3 ± 2.70	65.61 ± 6.34
Rapid progressive (%)	1.88 ± 0.91	5.80 ± 1.02	0.00	17.04 ± 2	28 ± 5.14	29.01 ± 2.68
Normal morphology (%)	9.70 ± 2.24	18.9 ± 0.72	0.00	25.3 ± 1.10	34.7 ± 2.90	17.04 ± 1.50
Hypoosmotic swelling (%)	16.3 ± 4.13	39.9 ± 3.07	0.00	77.1 ± 2.84	25 ± 3.16	79.25 ± 3.33

Scores are represented as mean ± standard error of mean.

*Footnote: pH was found to be alkaline and no samples were acidic. The percentage of rapid progression of control was almost 15 times greater for oligoasthenozoospermia. The sperm concentration was found to be almost the same in the cases of oligozoospermia and oligoasthenozoospermia. Total motility percentage was found to be higher in the normozoospermia group than in control fertile subjects.

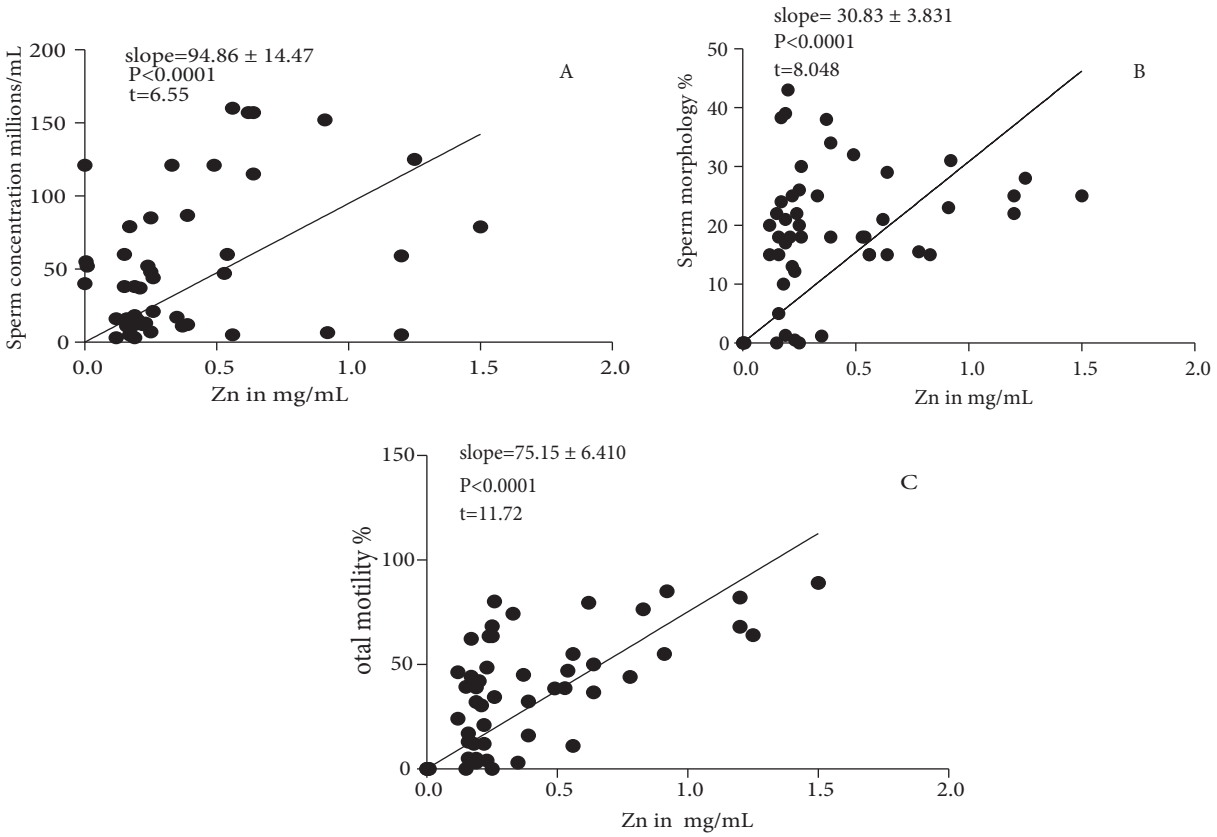

Figure 1. Correlation and linear regression of human seminal plasma Zn concentration with A) sperm concentration in millions/mL, B) normal morphology, and C) total motility. These semen parameters were correlated with the concentration of trace elements in the seminal plasma of each category. The parameters were positively correlated with Zn concentration.

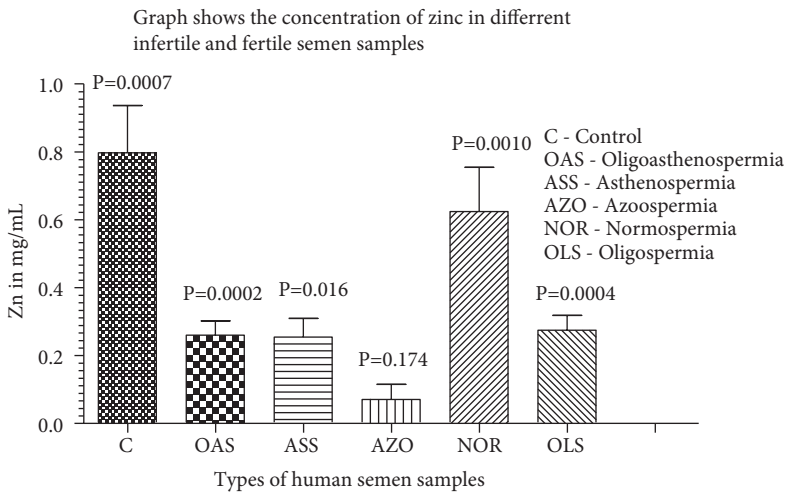

Figure 2. Assessment of Zn concentration between controls and various categories of infertile semen samples. The concentration of Zn was compared between the infertile and fertile categories and was found to be significantly different between these categories. The concentration of Zn was found at the same level in the cases of the oligoasthenozoospermia and asthenozoospermia groups.

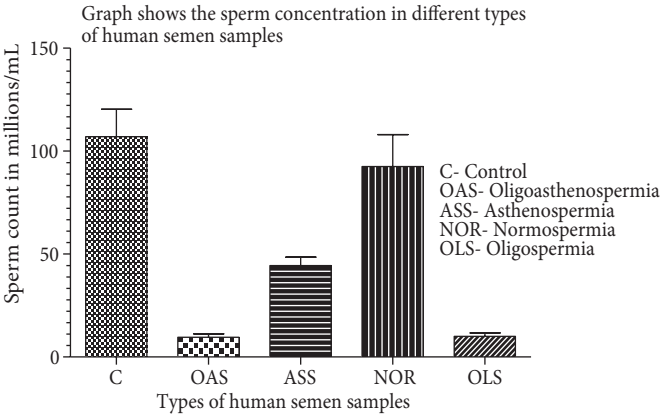

Figure 3. Comparison of sperm concentration between control and infertile samples. Sperm concentration was found to be significantly different for all classes of infertile categories when compared to fertile samples, except for normozoospermia samples. Sperm concentration was found to have significant difference between all the other infertile categories. Normozoospermia is thus considered to be normal within every semen parameters and is therefore considered to be subfertility.

concentration of zinc was present in all infertile groups, except normozoospermia. In azoospermia, the zinc content was almost nil. This was found to be an important observation for the therapy of zinc supplements. The overall observation reveals that all the mineral contents are required for the normal functioning of the sperms, which are not found in the other body fluids. Sodium content seemed to be little higher in the fertile group, and in all other cases identical concentrations were found. Our observation shows that magnesium and calcium contents of the seminal fluid did not have significant differences between the fertile and infertile categories. No significant differences were seen between concentrations of Zn, Na, Mg, and Ca in the control and normozoospermia groups. The significant values were justified as Gaussian approximation for all categories except normozoospermia. The significant level of normozoospermia was an exact calculation. In the case of all trace elements, 76.06% of total variation was observed when comparing all the subjects by using 2-way ANOVA. The concentrations of trace elements were found in the order of Na > Ca > Zn > Mg in the seminal plasma. Wilcoxon rank testing was done for all the trace elements and results were compared for all the subjects. A significant difference was found between all the subjects for zinc. In the case of Mg concentration, there was no significant difference in the oligozoospermia and azoospermia groups when compared with the control.

4. Discussion

Available literature reveals that Zn is essential for living organisms, because it is thought that more than 350 enzymes require Zn for their activity, including the

Table 2. Comparison of trace element concentrations between infertile and fertile group*.

Trace elements (mg/ml)	Infertile groups					Fertile group
	Oligoasthenozoospermia	Asthenozoospermia	Azoospermia	Normozoospermia	Oligozoospermia	
Zn	0.26 ± 0.04	0.25 ± 0.05	0.07 ± 0.04	0.62 ± 0.13	0.27 ± 0.04	0.79 ± 0.13
Na	3.218 ± 0.30	3.62 ± 0.39	0.75 ± 0.30	3.61 ± 0.75	3.00 ± 0.28	5.36 ± 1.02
Mg	0.154 ± 0.04	0.18 ± 0.05	0.53 ± 0.32	0.41 ± 0.11	0.15 ± 0.06	0.29 ± 0.11
Ca	0.411 ± 0.05	0.41 ± 0.10	0.62 ± 0.13	0.76 ± 0.08	0.39 ± 0.04	0.63 ± 0.12

Scores are represented as mean ± standard error of mean.
*Footnote: The concentration of Zn was found to have significant differences between the fertile and infertile categories. All trace elements evaluated here were found with almost the same concentration in the oligoasthenozoospermia and asthenozoospermia groups. The concentration of trace elements were found in the order of Na > Ca > Zn > Mg in the seminal plasma.

oxidant defense system (4). There exists strong evidence that Zn has an important physiologic function in semen, which includes the sperm motility and maintenance of the normal morphology of the sperm cell, and reduction in Zn level will yield low quality of the sperm as well as semen, which automatically reduces the chances of the fertilization (16). Moreover, Zn plays an important role in the regulation of the Ca^{2+} pumps through its activity in controlling the Ca^{2+} ATPase activity that regulates the intracellular Ca^{2+} in animal cells (7). Zinc is required to maintain the molecular information of many enzymes, RNA, DNA, and protein molecules (17). The total content of Zn in the human seminal plasma is high and there exists conflicting information on the effect of Zn on the semen parameters. Some research shows that there is no significant difference in the concentration of Zn between fertile and infertile men (18,19). However, Zhao et al. (20) reported a significant difference in the content of Zn. In our study, the concentration of Zn differed significantly between fertile and infertile (oligoasthenozoospermia, asthenozoospermia, oligozoospermia, azoospermia) semen samples at a significance level of $P = 0.0007$.

The high concentrations of Zn in the seminal plasma will be helpful in the enrichment of sperm quality, including the sperm count or concentration, sperm motility, and sperm morphology (21). However, decrease in the motility of sperm was observed (poor motility) when the Zn concentration was present at higher concentrations than usual (22). Nadeem et al. concluded that sperm motility is decreased by regular smoking and finally concluded that more studies need to be done regarding sperm motility (23).

Volume and pH were found to be same in all categories of infertile and fertile samples. The same kind of data were observed in the work done by Zaho et al. (20). Sperm concentration was found to be significant between all classes of infertile categories when compared to the control (fertile samples), except for the normozoospermia samples. Sperm concentration was significantly different between all the other infertile categories. Thus, cases of normozoospermia are considered to be normal in all semen parameters, and so normozoospermia is considered to be subfertility instead of infertility. Deficiency of Zn was thought to have an important role in growth retardation and sexual development in geophagy and thalassemia (24). There exists linear growth and sexual maturation following zinc therapy in geophagy and partially in thalassemia (25). Infertile men are more inclined to oxidative damage induced by the free radicals, and high levels of the free radicals might devastate the antioxidant strategies, which is associated with poor quality of semen, including sperm quantity and sperm quality (26). In the study conducted by Colager et al. (21), it was hypothesized that major changes in the level of the seminal trace elements are related to poor sperm count and poor fertilizing capacity. Biologic zinc treatment has a positive effect on the sperm motility, and the supplementation of zinc is thought to be the best treatment for infertile patients with prostatitis (27). In our research, Zn concentration was positively correlated with sperm concentration, sperm motility, and sperm normal morphology (Figure 1). None of the semen parameters were negatively correlated with Zn concentration. Zinc has an antioxidative protective nature and was found to have the property of scavenging reactive oxygen species. There exists strong evidence that paucity of Zn in the human seminal plasma increased oxidative damage that was initially thought to be done by reactive oxygen species (28). Turhan et al. concluded that progressive sperm motility is an important and powerful factor in determining the chance of pregnancy in oligoasthenozoospermic patients. Our results also lead to the same conclusion as in that study (29).

The literature reveals that a decrease in the seminal Ca leads to limited motility of the sperm (30). 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 biological events leading to the acrosome reaction. It may therefore have a primary role in fertilization. The estimation of Ca concentration in the human semen is of great interest as a consequence of its relation to sperm morphology, metabolism, acrosome reaction, and fertilization itself (31). Only a small portion, 2%–4%, of the calcium in semen is present in ionized form (32). Calcium also binds to the sperm surface, which can lead to differences between measurements on whole semen versus seminal plasma (33). The relationship between the seminal plasma and Ca is controversial. Some authors suggest that there is no significant difference between Ca concentration in the fertile and infertile categories. In our, research there exists a low level of significance between those 2 main groups, but there exists a strong significance difference between the control and asthenospermia groups. Magnesium is thought to play an imperative role in enzymatic reactions and in ejaculatory functions. Magnesium concentration was found to be reduced in the infertile patients. A low concentration was also found in patients with chronic prostatitis (34). Plasma membrane K^+ channels and plasma membrane hyperpolarization may be the important determinants in the cascade of events leading to Ca^{2+} influx and perhaps to the activation of capacitative Ca^{2+} entry in human spermatozoa. 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. Calcium regulates the motility of the sperm, fertilization, capacitation, and acrosome reaction. The acrosome reaction is the key component of the fertilization process, and its proper timing is very important for the proper fertilization. During capacitation, various trace elements present in the seminal plasma and spermatozoa were removed and rearranged, and the whole process will end in the proper fertilization.

In conclusion, the concentration of Zn in the seminal plasma is positively correlated with sperm concentration, sperm motility, and normal sperm morphology. In normozoospermia a normal range of Zn level was observed in the seminal plasma and there was no significance difference with the fertile group. Correlation and linear regression analysis indicated that Zn concentration and sperm concentration are positively correlated. Alliance of the concentration of Zn in the seminal plasma with the semen quality parameters revealed that Zn deficiency is a menace for sperm anomalies and male infertility. Lack of Zn in the diet is therefore also a major cause for male infertility; the concentration Zn present in the seminal plasma should thus be evaluated.

Acknowledgment
The authors are thankful to the Management of the Vellore Institute of Technology, Vellore, as well as to the Management of the Bangalore Assisted Conception Centre, Bangalore.

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