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## Trace elements levels in the serum, urine, and semen of patients with infertility

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**Background/aim:** Studies suggest that trace elements may have an adverse impact on male reproduction, even at low levels. We tried to investigate the relationships between these metals and semen quality in various body fluids among men with infertility.

**Materials and methods:** A total of 255 samples of blood, semen, and urine were collected from 85 men suffering from infertility. Inductively coupled plasma-optical emission spectrometry was used for the determination of 22 trace elements. We compared the results of the semen parameters with the results of the element determinations.

**Results:** Because of the high proportion of samples with values lower than the limit of detection for a number of the elements, only 8 of a total 22 trace elements were determined in the samples. When the concentrations of sperm were classified according to the World Health Organization's guidelines for normospermia, oligospermia, and azoospermia, statistically significant differences were found among Zn, Ca, Al, Cu, Mg, Se, and Sr concentrations in various serum, sperm, and urine samples ( $P < 0.05$ ).

**Conclusion:** In the present study, we found significant correlations between concentrations of Zn, Ca, Al, Cu, Mg, Se, and Sr and semen parameters in various body fluids.

**Key words:** Male infertility, seminal plasma, serum, trace elements, urine

### 1. Introduction

Elements can be divided into two groups: the essential elements of the first group are necessary for metabolic and life processes, such as calcium (Ca), chromium (Cr), copper (Cu), manganese (Mn), magnesium (Mg), and zinc (Zn), and the nonessential second group includes elements that are toxic to humans, such as aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb), and thallium (Tl). Trace elements are essential for testicular growth and development. Among the essential elements, Cu and Zn play a particular role in the reproductive system. Zn and Cu were first studied and evaluated in semen (1). Exposure to inorganic lead is detrimental to human semen quality (2). Leads do not only affect the spermatozoa count but also damage the spermatozoa structure and function (3).

Humans are exposed to elements at low concentrations either voluntarily via supplementation or involuntarily via intake or contact with contaminated materials. Some elements, such as Cd, Pb, As, and mercury (Hg), are nonessential and can be measured in most of the general population (1,4). Pb may adversely affect sperm morphology and motility (5, 6). Hg is associated with sperm abnormalities in subfertile males (7). Other

elements such as Cu, Mn, molybdenum (Mo), selenium (Se), and Zn are essential for good health but may be harmful above certain levels (3,8). For example Cr, Mn, and Cu, which act as cofactors for a variety of important enzymes, have been associated with reduced semen quality (9–11). On the other hand, lower levels of Cu, Se, and Zn may have protective effects on male reproductive outcomes and may assist in counteracting the effects of Cd and Pb (1,4,12–14).

Studies suggest that trace elements may have an adverse impact on the male reproductive system, even at relatively low levels. Although seminal plasma is a well-preserved environment, trace elements can be detected in the serum and even in the urine before reaching the semen. We tried to explore the relationship between these metals and semen quality in various body fluids among men with infertility.

### 2. Materials and methods

All volunteering males of infertile couples who participated in the study attended the infertility clinic of our department. Men with diabetes, thyroid or adrenal disorders, known genetic disorders related to fertility,

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testicular cancer, and unilateral orchiectomy or those taking hormone therapy were excluded. Written consent from each subject was obtained. The study was approved by the local ethics committee of our university (number: SAU200504).

### 2.1. Collection of blood and urine samples

Blood and urine samples were collected from participants during a morning visit: 10 mL of venous blood was collected into plastic tubes containing EDTA using stainless needles and 10 mL of urine was stored in sterile polyethylene tubes. After extracting serum samples from whole blood, 5 mL of serum and urine samples were stored at  $-20\text{ }^{\circ}\text{C}$  until the analysis.

### 2.2. Collection of semen and semen analysis

Semen samples were collected after masturbation into sterile cups following 3 days of sexual abstinence. After 30 min of liquefaction, the standard semen parameters were immediately evaluated according to the World Health Organization (WHO) guidelines (15). Specimens were analyzed for spermatozoa morphology using a modified Papanicolaou stain. Sperm samples were stored at  $-20\text{ }^{\circ}\text{C}$  and subsequently mineralized in the laboratory.

### 2.3. Determination of elements

All the material from each sample was placed in separate mineralization tubes. The glassware and plastic containers were soaked in 10%  $\text{HNO}_3$  overnight and rinsed thoroughly with distilled deionized water (Milli-Q Millipore, 18.2  $\text{M}\Omega\text{cm}$ ). All reagents used in the present study were of high purity and analytical grade for trace elements analysis. Standard solutions ( $1000\text{ }\mu\text{g mL}^{-1}$ ) of each element were used with inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Analytical Instruments), purchased from Merck. Deionized water (Millipore) was used throughout the study for serial dilution of standards. ICP-OES was used for the determination of elements (Al, As, beryllium (Be), bismuth (Bi), Ca, Cd, cobalt (Co), Cr, Cu, iron (Fe), Hg, Mg, Mn, Mo, nickel (Ni), Pb, antimony (Sb), Se, Tl, titanium (Ti), vanadium (V), Zn). Semen samples were prepared and dissolved by using the Milestone Ethos PLUS microwave dissolution system Milestone Ethos D (maximum pressure 1450 psi, maximum temperature  $300\text{ }^{\circ}\text{C}$ ).

### 2.4. Calibration graphs

Calibration standards at 5, 10, 100, 300, 600, and 1200  $\text{ng mL}^{-1}$  concentrations were prepared. At the same time, in order to prevent interference to calibration solutions, 500  $\text{ng mL}^{-1}$  indium solution was added as a matrix modifier. Prior to the analysis, the instrument was calibrated according to the manufacturer's recommendation. Blank digestion was also carried out by completion of a full analytical procedure without the sample. ICP-OES calibration curve parameters are shown in Table 1.

**Table 1.** Experimental conditions of ICP-OES instrument.

Instrumental conditions	
RF power	1400 W
External gas flow rate	$1.0\text{ L min}^{-1}$
Cooling gas flow rate	$13\text{ L min}^{-1}$
Nebulizer gas flow rate	$0.8\text{ L min}^{-1}$
Nebulizer type	Modified Lichte
Spray chamber	Cyclonic
Sample aspiration rate	$2.0\text{ mL min}^{-1}$
Sample pump speed	25 rpm

### 2.5. Sample digestion procedure

The urine samples were analyzed directly; the blood serum and semen samples were digested by using the Milestone Ethos D instrument at 600 W for 60 min. One milliliter of semen and blood serum samples was taken and 1 mL of concentrated  $\text{HNO}_3$  acid was added to the samples, which were then were digested in a microwave instrument for 60 min at 600 W. After this procedure, samples were put into the polypropylene-made autosampler tubes soaked in 10%  $\text{HNO}_3$  solutions overnight. The samples were made up to 6 mL using distilled-deionized water. The indium standard was used as the internal standard. Ten examples of blank samples were analyzed.

### 2.6. Validation of method

The Seronorm Trace Elements in Urine, Level 2 (SERO210705) certified reference material was used for validation of method.

### 2.7. Statistical analysis

The statistical analyses were carried out using linear regression analysis, Pearson's correlation coefficient with log transformation, analysis of variance, and Student's t-test as appropriate. Statistics were computed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was used to reject the null hypothesis.

## 3. Results

We recruited a total of 85 men and a total of 255 samples into the study. The mean age of the subjects was  $30.77 \pm 5.06$  years. The relationship between the sperm parameters and the subjects' characteristics can be seen in Table 2. Because a high proportion of the samples were lower than the detection limits of ICP-OES for a number of the elements, only 8 of the total 22 trace elements were determined in the samples. The mean concentration of the detectable 8 elements (Fe, Zn, Ca, Al, Cu, Mg, Se, and Sr) in the serum, urine, and semen are shown in Table 3.

**Table 2.** General characteristics of the subjects.

Sperm parameters (n)	Age	Occupational exposure		Dental implant		Comorbidity	
		+	-	+	-	+	-
Normospermia (n = 53)	30.62 ± 5.06	18 (34%)	35 (66%)	26 (49.1%)	27 (50.9%)	6 (11.3%)	47 (88.7%)
Oligospermia (n = 17)	31.89 ± 5.6	6 (35.3%)	11 (64.7%)	9 (52.9%)	8 (47.1%)	2 (11.8%)	15 (88.2%)
Azoospermia (n = 15)	30.56 ± 5	4 (26.7%)	11 (73.3%)	5 (33.3%)	10 (66.7%)	1 (6.7%)	14 (93.3%)
Total (n = 85)	30.77 ± 5.06	28	57	40	45	9	76

**Table 3.** The mean concentration of 8 detectable elements (Fe, Zn, Ca, Al, Cu, Mg, Se, Sr) in the serum, urine. and semen.

	Serum			Urine			Semen			P
	NS	OS/AS	AzS	NS	OS/AS	AzS	NS	OS/AS	AzS	
Fe	272.995	272.210	269.800	LOD	LOD	LOD	250.820	246.400	258.10	>0.05
Zn	309.893	219.885	256.865	210.735	140.794	572.540	138.000	106.350	87.930	0.045
Ca	678.398	745.591	634.743	LOD	LOD	LOD	694.763	694.230	694.56	0.046
Al	LOD	LOD	LOD	LOD	LOD	LOD	21.741	22.120	28.009	0.021
Cu	259.005	243.099	271.174	LOD	LOD	LOD	LOD	LOD	LOD	0.033
Mg	5754	5898	5810	LOD	LOD	LOD	4720	3204	3083	0.036
Se	41,461	44,044	43,440	LOD	LOD	LOD	LOD	LOD	LOD	0.046
Sr	17,274	17,760	17,540	25,842	11,165	19,886	25,277	25,500	25,760	0.033

LOD: Lower than the level of detection, NS: normospermia, OS/AS: oligoasthenozoospermia, AzS: azoospermia.

We found a similar concentration of Fe in the serum, semen, and urine samples of both the infertile men and the normal group ( $P > 0.005$ ). The concentration of Zn in the serum was lower in the subjects with azoospermia than in the patients with normospermia ( $P = 0.045$ ). Similarly, the Zn values were significantly low in urine and semen samples ( $P = 0.019$  and  $P = 0.012$ ). Calcium concentrations were found to be under the normal range in the serum of the subjects with azoospermia as compared to those with normospermia ( $P = 0.046$ ). However, there were no differences in the Ca values in the urine and semen between the normospermia group and the others ( $P > 0.05$ ). The concentration of Al was found to be high in subjects with azoospermia ( $P = 0.021$ ). The values of Al were similar in the subjects with normospermia and oligospermia ( $P > 0.05$ ). The concentration of Cu was higher in the serum

samples of the individuals with asthenozoospermia than in the patients with normospermia ( $P = 0.033$ ). Copper was found to be in the seminal plasma at the same levels in all study groups ( $P > 0.05$ ). The concentration of copper in the urine was lower in the azoospermia patients than in the individuals with normospermia ( $P = 0.042$ ). The concentration of magnesium was lower in the semen of the patients with azoospermia than those with normospermia ( $P = 0.036$ ). We found the same values of Mg in the serum samples of the subjects with normospermia and azoospermia ( $P > 0.05$ ). The analysis of selenium showed that the concentration in the serum was higher in the patients with azoospermia than in those with normospermia ( $P = 0.046$ ). The urine strontium was lower in the asthenozoospermia patients than in the subjects with normospermia ( $P = 0.033$ ).

#### 4. Discussion

Some elements are possible pollutants that may be harmful to sperm production. Occupational exposure to several trace elements is known to impair sperm quality (16,17). Lead and cadmium concentrations have been measured in human semen, seminal plasma, spermatozoa, blood, and urine, and high concentrations have been found to be related to impaired sperm quality (18–25). Many trace elements have also been shown to be harmful in regard to testicular function and sperm production in experimental studies. It appears to be important to measure metal concentrations in the spermatozoa, not just those in seminal plasma, serum, or other tissues (7,16,26).

In the male reproductive system, iron overload can have a deleterious effect. Iron accumulation is associated with either acute or chronic iron overload, and it led to a subtle iron increase in the testes that was associated with oxidative damage to lipids, proteins, and DNA (27). Similarly, iron accumulation secondary to zinc deficiency or to intoxication with dioxin or cadmium may be involved in oxidative damage to the testes (28). We could not find any difference between the concentrations of Fe in any of the body fluids of the subjects.

Zinc levels in human seminal plasma have been noted to be remarkably high in comparison with other body fluids (29). However, the exact mechanism in the function of zinc in the reproductive system is still unclear. Lower zinc concentrations in semen have been reported in infertile men (25). We found that zinc levels were low in all biological fluids in infertile men.

Calcium and magnesium ions, acting as intracellular second messengers, play an important role in sperm physiology. Thus, the Ca content is suggested as being closely related to sperm morphology and reproductive potential, and it is an important indicator of sperm quality. It has been shown that sperm motility is dependent on an influx of free Ca ions. Thus, Ca channel blockers can lead to a reversible loss in sperm motility (30). In the present study, we found that the calcium and magnesium contents were low in the serum in subjects with azoospermia, but we could not find any differences in either the sperm or urine of the patients.

Aluminum may be one of the potential pollutants, because it reduces the weight of the testes and causes decreased epididymal sperm counts in mice (31). The high concentrations of aluminum in the spermatozoa and seminal plasma of some men, and their correlation with decreased sperm motility, was a new finding. Aluminum may therefore be one of the environmental factors affecting sperm quality. It is important to determine the

concentrations of aluminum in the spermatozoa in areas with poorer sperm quality (31).

Although Cu supplementation has been shown to be effective in protecting semen quality in animal studies, caution should be taken when extrapolating this effect to humans (13) because we found evidence of an inverse association between high Cu levels and semen quality, which is consistent with a number of animal and human studies (9,32). We also conversely found low urine Cu levels in the azoospermia patients.

Selenium is well known as a human essential trace element, and its role in human reproduction has been studied extensively. However, findings regarding the relationship between sperm quality and selenium in biological fluids are inconsistent. (25,33,34). We found a high concentration of Se levels in the serum of patients with azoospermia only, but there was no meaningful difference between the infertile or normal subjects.

Strontium treatment, which is proven to induce calcium oscillations in mice, was found to be an effective method for low or absent fertilization after ICSI (35). However, whether strontium is effective in improving the fertilization of abnormal sperm has not yet been reported. We found that urine Sr levels in patients with asthenozoospermia were lower than those with normospermia only. We could not detect any difference in the Sr in other body fluids in our study.

One limitation of this study was the limited number of participants included in the study and the lack of power analysis. Another limitation was the high percentage of values lower than the level of detection of various elements in samples from the body. This calls for the conducting of further evaluations and investigations of the elements. Another potential limitation was the collection of only a single sample of blood, semen, and urine from each participant for the measurement of the metals and semen quality.

In conclusion, when the concentration of sperm was classified according to the WHO guidelines for normospermia, oligospermia, and azoospermia, a statistically significant difference was found between Zn, Ca, Al, Cu, Mg, Se, and Sr concentrations in various serum, sperm, and urine samples. Results of our study show that trace elements have possible effects on the semen of patients with infertility.

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