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Reproductive Toxicology 15 (2001) 131–136

Reproductive Toxicology

www.elsevier.com/locate/reprotox

The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men

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Received 9 June 2000; received in revised form 25 September 2000; accepted 16 October 2000

Abstract

To investigate the impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters, 107 fertile and 103 subfertile males provided a standardized blood and semen specimen. Total calcium and magnesium concentrations were determined with colorimetric end point assay procedures. Zinc and copper were determined by flame atomic absorption spectrophotometer (AAS). Semen analysis was performed according to World Health Organization guidelines (1992). The concentrations of calcium, magnesium, zinc, and copper in blood and seminal plasma were not different between the subfertile and fertile group. Weak correlations were demonstrated between blood plasma zinc concentrations and sperm count ($r_s = 0.18$), sperm motility ($r_s = 0.15$), and abnormal sperm morphology ($r_s = 0.13$). Zinc and magnesium concentrations in seminal plasma correlated weakly with sperm count ($r_s = 0.17$ and $r_s = 0.16$, respectively), and copper concentrations in blood plasma with motility ($r_s = 0.25$). Strong correlations were found between calcium, magnesium, and zinc in seminal plasma. Although calcium, magnesium, zinc, and copper play an essential role in spermatogenesis and fertility, the determination of these elements in blood and seminal plasma does not discriminate on the basis of fertility in this group of men.

Keywords: Calcium; Copper; Male fertility; Magnesium; Semen parameters; Zinc

1. Introduction

Human semen contains high concentrations of calcium (Ca), magnesium (Mg), zinc (Zn), and copper (Cu) in bound and ionic forms. The testicular plasma, that is, the fluid composed of the secretions originating in the seminiferous tubules, tubuli recti, rete testis, and ductuli efferentes, and the epididymal plasma serve as a nutrient medium in which maturation of the developing spermatozoa takes place [1]. Contact with toxic substances in the seminal plasma can affect developing spermatozoa. The presence of abnormal levels of Ca and Mg and of trace elements, in particular Zn and Cu, may affect spermatogenesis with regard to production, maturation, motility, and fertilizing capacity of the spermatozoa [2,3].

Calcium is an essential element, which is a crucial regulator of many physiologic processes in every living cell, including spermatozoa. Calcium ion (Ca^{2+}) is the trigger of the acrosome reaction in mammalian spermatozoa, and there is substantial evidence that the calcium ion is differentially involved in sperm motility depending on the stage of sperm maturation [2,4–6]. It has been demonstrated that the prostate, seminal vesicles, and epididymis are also very rich in calcium, which is why several studies have investigated the association between calcium and male subfertility.

Another crucial element in cell physiology is Mg, which is present in a high concentration in semen [7]. Magnesium is an important cation found in nearly all enzymatic sys-

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0890-6238/01/$ – see front matter
PII: S0890-6238(01)00113-7

tems. Magnesium modifies specific enzyme substrates and plays a fundamental role as a cofactor in more than 300 enzymatic reactions involving energy metabolism (ATP) and nucleic acid synthesis. Magnesium may play a role in spermatogenesis, in particular in sperm motility. Furthermore, Mg is regarded as a marker of the secretions of the seminal vesicles and acts as an intracellular calcium antagonist [8].

Total Zn concentration in human seminal plasma is extremely high (i.e. approximately 2.0 mM). It is well-established that the prostate secretes high levels of Zn [1]. The main sources of Zn are meat and seafood. Zinc is a cofactor for more than 200 metalloenzymes in a variety of animal species. Zinc finger proteins among others are involved in the genetic expression of steroid receptors [9,10]. There is evidence that Zn in seminal plasma influences sperm oxygen consumption [11,12], nuclear chromatin decondensation [13], and acrosin activity [14,15]. Zinc deficiency causes hypogonadism [16] and Zn is thought to be important in the stabilization of sperm chromatin [17]. Consensus exists that the Zn concentration in seminal plasma is of importance for spermatogenesis, although the results of several studies are still contradictory [17–19].

The trace element Cu has been identified as a highly toxic element for sperm [3]. In vitro studies of Roblero et al. [20] have demonstrated the effect of Cu in intrauterine devices (IUD) in preventing conception. The only way Cu normally enters mammals and other terrestrial vertebrates is via the alimentary tract. The average diet of adult humans in Western countries contains from 0.6 to 1.6 mg Cu per day with shellfish, organ meats, and seeds as the richest sources of copper. Copper in the cytosol is mainly bound to proteins, i.e. metallothioneins, which also binds the element Zn to render these elements nontoxic in case of excess. Copper is an important element for numerous metalloenzymes and metalloproteins that are involved in energy or antioxidant metabolism. In its ionic form (Cu^{2+}), the trace element rapidly becomes toxic to a variety of cells, including human spermatozoa.

Sperm count, motility, and morphology are parameters used to evaluate potential male fertility. Since Ca, Mg, Zn, and Cu are believed to be important for spermatogenesis, we conducted a case-control study to investigate the relation of these elements to male factor (sub)fertility and semen parameters by quantifying the concentrations in blood and seminal plasma of fertile and subfertile males.

2. Population and methods

2.1. Subjects

The study samples were derived from a randomized controlled trial described in detail by Wong et al. (submitted, 2000). One hundred and seven fertile (mean (SD) age 34.1 ± 4.0 years) and 103 subfertile (34.4 ± 4.1 years) males were included in this study. Fertile males were recruited from nine midwifery practices in Nijmegen and surroundings, and included healthy males without a history of fertility problems whose partners conceived spontaneously within one year and were pregnant at initiation of the study. Subfertile males were recruited from those couples who failed to conceive after one year of regular unprotected intercourse with the same partner and who had a sperm count of less than 20 million/mL, as determined by the first semen analysis after referral to the fertility clinic of the University Medical Centre Nijmegen and the Canisius Wilhelmina Hospital Nijmegen. The choice for the latter criterion was based on its high predictive power for the probability of conception [21,22]. Exclusion criteria for both groups included chromosomal disorders related to a fertility disorder, cryptorchidism, vasectomy, and the use of folic acid and/or Zn supplements or medications within three months before recruitment.

The ethics committee of the University Medical Centre Nijmegen gave its approval for the study protocol and all participants gave their informed consent.

2.2. Specimen collection, storage and analytical procedures

Participants provided semen samples, produced via masturbation, in polypropylene containers at home after an abstinence period of 3 to 5 days. These samples were delivered to the fertility laboratory within 1 h after production. After liquefaction, an aliquot of semen was centrifuged at 1,400 *g* (Hettich 16A, 1323 rotor) for 10 min. The supernatant (seminal plasma) was frozen without preservatives and stored at -20°C until further assay for Ca, Mg, Zn, and Cu. Subsequently, a semen analysis was performed according to the World Health Organization guidelines to obtain volume, pH, sperm concentration, motility, and morphology [22]. Sperm concentration was determined with a Makler® counting chamber, equipped with a 10 × 10 μm compartment frame format. Motility was expressed as the percentage of motile spermatozoa and their mean velocity (scale 1 to 6; 1 = immotile; 6 = progressively motile, i.e. > 100 μm/s). Morphology was determined according to the WHO criteria after incubation of the sample with trypsin (10 min at room temperature), using the methylene blue/eosin staining procedure, feathering, and fixation by flame [22]. At least 100 cells were examined (in duplicate by two independent technicians) at a final magnification of 1000 ×.

The coefficient of variation (CV) for sperm concentration analysis decreased from approximately 30% to 10% for sperm concentrations ranging from 10 to 70 × 10^6/mL. In the case of sperm morphology, the CV was approximately 35%, with an interval of 65% to 90% abnormal spermatozoa as estimated by repeated determinations of the same set of specimen preparations by 3 or 4 technicians.

At the time of semen sampling, venous blood samples obtained after overnight fasting were drawn into heparin-

ized tubes and after centrifugation at 2000 *g*, blood plasma was collected and stored at - 20°C until further assay. Blood and seminal plasma specimens of each participant were analyzed for Ca, Mg, Zn, and Cu concentrations. Total Ca and Mg concentrations were determined with colorimetric end point assay kits (Sigma, Chemical Corporation, St Louis, Miss, USA, #587M for Ca and #595M for Mg) on 5 μl samples in duplicate following the instructions of the supplier. Certified standards (Sigma) were used as references.

Zinc was determined by flame atomic absorption spectrophotometer (AAS) (Perkin Elmer 4100; Norwalk, Connecticut, USA). The lower limit of detection was 0.1 μM and the response was linear up to at least 30 μM. The inter-assay CV for the determination of Zn in plasma was 4.5% at mean concentration of 12.4 μM ($n = 143$), while the intra-assay CV was 3.4% at 11.8 μM ($n = 10$). The inter-assay CVs for Zn in seminal plasma were 7.0% and 7.6%, at 11.1 and 21.6 μM, respectively ($n = 13$), while the intra-assay CVs, determined in five separate assays of four repeats each, ranged from 1.8% to 7.1%, and from 2.5% to 6.9% for the lower and higher concentration of Zn in these specimens. Copper concentrations in blood and seminal plasma were also measured with an atomic absorption spectrophotometer (AAS).

2.3. Statistics

Statistical analyses were performed with the Astute in Excel® 5.0 program. The Mann-Whitney *U* test was used to evaluate the significance of differences in characteristics and blood and seminal plasma concentrations of trace elements between fertile and subfertile males. Spearman's rank correlation coefficients were calculated to determine associations between Ca, Mg, Zn, and Cu concentrations, and semen parameters. Differences were considered significant at $P < 0.05$.

3. Results

Table 1 shows the population characteristics, semen parameters, and the concentrations of Ca, Mg, Zn, and Cu concentrations in blood and seminal plasma of the subfertile and fertile group. The sperm concentration, motility, and normal morphology were significantly ($P < 0.05$) lower in subfertile males than in fertile males. The concentrations of Ca, Mg, Zn, and Cu in both compartments were not significantly different between the subfertile and fertile groups. Therefore, the data from these two groups were pooled for further analysis.

Calcium, Mg, and Zn were significantly higher in semen than in blood plasma, with a semen-to-plasma ratio of 69.7 for Zn, 3.7 for Mg, and 2.7 for Ca. The Cu concentration was significantly higher in blood plasma, with a semen-to-plasma ratio of 0.4. No significant correlation was found between blood and seminal plasma concentrations of these elements except for Cu concentrations ($r_s = 0.65$, $P < 0.0001$).

Table 1
Population characteristics and blood and seminal plasma concentrations of Ca, Mg, Zn, and Cu. Semen parameters are given as median (5th-95th percentile); otherwise as mean (SD)

	Subfertile males (n = 103)	Fertile males (n = 107)
Age (years)	34.3 (3.9)	34.2 (4.2)
Abstinence period (d)	3.5 (1.6)	3.9 (1.9)
Sperm count ($\times 10^6$/mL)	8 (1–85)	75 (15–175)*
Motility (%)	30 (5–70)	58 (26–75)*
Abnormal morphology (%)	80 (57–93)	62 (37–82)*
Blood plasma		
Calcium (mM)	2.3 (0.3)	2.2 (0.2)
Magnesium (mM)	1.0 (0.11)	1.0 (0.2)
Zinc (μM)	20.1 (3.0)	20.8 (3.4)
Copper (μM)	13.5 (5.7)	15.5 (9.0)
Seminal plasma		
Calcium (mM)	6.1 (1.6)	6.0 (1.4)
Magnesium (mM)	3.6 (1.1)	3.7 (1.0)
Zinc (mM)	1.4 (0.7)	1.4 (0.7)
Copper (μM)	5.5 (3.9)	5.9 (3.7)

*$P < 0.05$

When studying the correlations between the elements Ca, Mg, Zn, and Cu, and the semen analysis parameters, weak correlations were found between plasma Zn concentrations and sperm concentration ($r_s = 0.18$; $P < 0.01$), sperm motility ($r_s = 0.15$; $P < 0.05$), and abnormal sperm morphology ($r_s = 0.13$; $P = 0.07$) for the fertile and subfertile group taken together. Zinc concentrations in seminal plasma correlated with sperm count ($r_s = 0.17$; $P < 0.05$). Seminal plasma Mg concentrations correlated with sperm concentration ($r_s = 0.16$; $P < 0.05$) and Cu concentrations in blood plasma with motility ($r_s = 0.25$; $P < 0.01$). Calcium concentrations in blood and seminal plasma were not correlated with any semen analysis parameters.

Table 2 shows the correlation matrix of the Ca, Mg, Zn, and Cu concentrations in blood and seminal plasma. Significant correlations were found between blood plasma Mg and blood plasma Ca ($r_s = 0.48$, $P < 0.0001$) and blood plasma Cu ($r_s = 0.21$, $P < 0.05$). Calcium and Cu concentrations in blood were negatively correlated ($r_s = - 0.41$, $P < 0.0001$). In semen, strong correlations were demonstrated between Zn and Ca, and between Zn and Mg ($r_s = 0.79$, $P < 0.0001$; $r_s = 0.73$, $P < 0.0001$, respectively; Figs. 1,2), while Ca and Mg also correlated significantly ($r_s = 0.85$, $P < 0.001$; Fig. 3).

4. Discussion

In this study we could not establish differences between fertile and subfertile males in the concentrations of the elements Ca, Mg, Zn, and Cu in blood and seminal plasma.

Table 2
Correlation coefficients between calcium (Ca), magnesium (Mg), zinc (Zn), and copper (Cu) concentrations in blood and seminal plasma after pooling

	Blood				Seminal plasma			
	Ca	Mg	Zn	Cu	Ca	Mg	Zn	Cu
Blood:								
Ca	—	0.48**	−0.02	−0.41**	−0.08			
Mg		—	0.04	0.21*		0.06		
Zn			—	−0.09			0.003	
Cu				—				0.65**
Seminal plasma:								
Ca					—	0.85**	0.79**	0.14
Mg						—	0.73**	0.13
Zn							—	0.05
Cu								—

*$P < 0.05$
**$P < 0.0001$

This finding confirms previous reports [7,8,23]. Pandy [24] however, observed lower concentrations of Ca, Mg, and Zn in the semen of subfertile males, i.e. 82% have subnormal quantities of one or more of these elements.

The highest concentration in seminal plasma in our study was that of Ca, followed by Mg and Zn. The mean Ca, Mg, and Zn concentrations in seminal plasma were significantly higher than in blood plasma. Copper concentrations, however, were higher in blood plasma than in seminal plasma. These findings are in accordance with the study of Omu et al. [23].

No correlation could be established between blood and seminal plasma of these elements, except for Cu. Ca, Mg, and Zn play an essential role in many enzymes and proteins and thus in cellular homeostasis. As a consequence, these elements are strictly regulated, a process in which the blood-seminal plasma barrier plays a pivotal role. However, the precise mechanism by which these elements are transferred from the circulating blood into the seminal plasma is unclear.

Zinc concentrations in semen obtained in this study were comparable with those reported elsewhere in the literature [23,25]. We observed a significant correlation between blood plasma Zn concentrations and sperm concentration, motility, and morphology, and between seminal plasma Zn concentrations and sperm concentration. The latter correlation has also been described in the studies of Saaranen et al. [18] and Madding et al. [26]. Results of other studies, however, indicate that high semen zinc concentrations are related to decreased sperm motility in infertile men [27,28]. Clinical studies with adult males experimentally deprived of zinc show that Leydig cell synthesis of testosterone is decreased due to a decreased activity of the Zn-dependent metalloenzyme 5α-reductase, which is responsible for the conversion of testosterone (T) to the biologically active form, 5α-dihydrotestosterone (DHT) [29–31]. Zinc also has a fundamental role in the antimicrobial activity of the seminal plasma [32], sperm nuclear chromatin condensation in men [13,14], and acrosin activity.

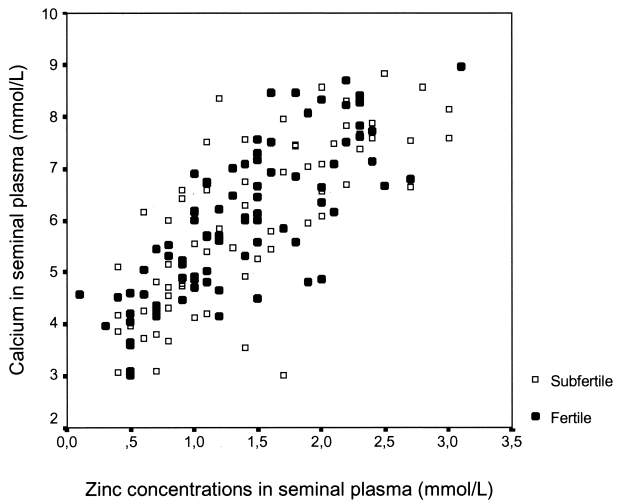

Fig. 1. Correlation between Zn and Ca concentrations in seminal plasma of fertile and subfertile males.

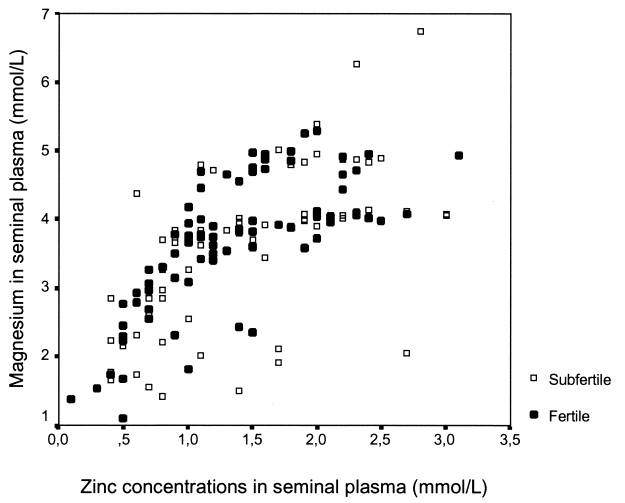

Fig. 2. Correlation between Zn and Mg concentrations in seminal plasma of fertile and subfertile males

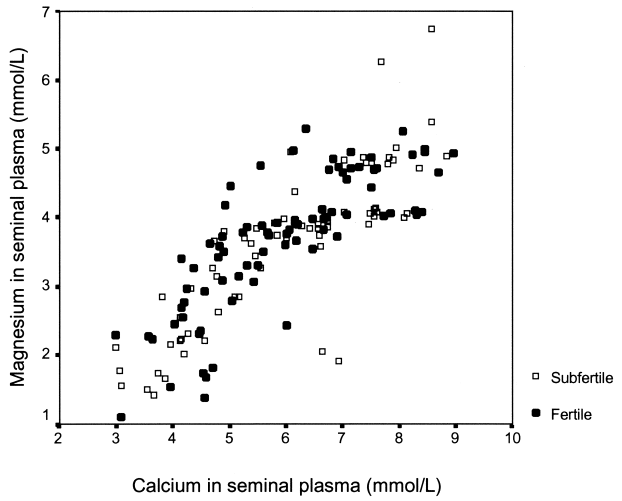

Fig. 3. Correlation between Ca and Mg concentrations in seminal plasma of fertile and subfertile males .

Our study shows a significant correlation between Mg in seminal plasma and sperm concentration, but not motility, while other studies did not find any correlation between Mg and semen parameters [25,33]. The role of magnesium in spermatozoa quality is as yet not clear. Depletion of intracellular Mg^{2+} is known to affect all functions dependent on this ion, including glycolysis, protein synthesis, respiration, and reproduction [34].

We could find neither beneficial nor adverse associations between calcium and sperm motility. Studies on the relationship between calcium and semen parameters are controversial. Prien et al. [5] showed that Ca^{2+} in semen decreased significantly in men with decreased sperm motility (<60%), while total Ca was not different compared to men with normal motility. Morton et al. [6] have demonstrated that men with decreased sperm motility in the epididymal plasma have significantly lower seminal Ca^{2+} concentrations, while Hong et al. [2] showed that Ca^{2+} has an adverse effect on the motility of mature spermatozoa in ejaculated semen. Nishida et al. [35] have demonstrated that in vitro exposure of human sperm to low Ca enhances fertilizing ability. Our finding that Ca did not effect sperm motility could be explained by the fact that we measured total and not ionized Ca.

Our study demonstrated a weak but significant positive correlation (rs = 0.25) between blood Cu concentrations and sperm motility. This finding is in agreement with data from Jockenhovel et al. [36] who also demonstrated a weak, though significant correlation between Cu concentrations and sperm count ($r = 0.32$), motility ($r = 0.23$), and normal morphology ($r = 0.21$). Recently, Ackerman et al. [37], however, demonstrated an adverse effect of high concentrations of Cu on sperm morphology in impala living in the Kruger National Park, South Africa. Copper concentrations determined in our study were within the normal physiologic range and it is known that Cu is an essential trace element that plays an important role in several enzymes such as superoxide dismutase. Human spermatozoa are particularly susceptible to peroxidative damage because they contain high concentrations of polyunsaturated fatty acids and also possess a significant ability to generate reactive oxygen species (ROS), mainly superoxide anion and hydrogen peroxide. Superoxide dismutase protects human spermatozoa from this peroxidative damage.

Finally, we found a strong positive correlation between Ca, Mg, and Zn, which confirmed the data of Sørensen et al. who showed a correlation coefficient of 0.79 between seminal plasma Ca and Zn, and 0.86 in the case seminal plasma Mg and Zn concentrations. Mutual interaction exists between the elements assessed in our study. Surprisingly, we found a positive correlation between Mg and Ca while it has been described that Mg acts as a physiologic Ca antagonist [38]. High concentrations of dietary Zn induce metallothionein synthesis in liver, kidney, and intestine, which inhibits the uptake and transfer of copper from the intestine into the blood, because Cu binds metallothionein with greater affinity than Zn [39].

In conclusion, although calcium, magnesium, zinc and copper play an essential role in spermatogenesis and fertility, the determination of these elements in blood and seminal plasma did not discriminate on the basis of fertility in this group of men.

Acknowledgments

We are indebted to all participants, the staff of the fertility clinic, and technicians of the Central Clinical Chemistry, Chemical Endocrinology, and Fertility Laboratories of the University Medical Centre Nijmegen for expert technical assistance; to Mr. J. Eygensteyn from the Central Instrumentation Department, Faculty of Science, to Mr. A.M.H.W. Fransen, gynecologist at the Canisius Wilhelmina Hospital Nijmegen; to all the midwifes in Nijmegen and surrounding areas for their assistance in recruitment; and to Mrs. Nelleke J. Hamel, for her assistance in data collection.

This study was financially supported by the Zorg Onderzoek Nederland/Dutch ‘Praeventiefonds’ (grant no. 28. 2877), The Hague, The Netherlands.

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