Magnesium and selected parameters of the non-enzymatic antioxidant and immune systems and oxidative stress intensity in the seminal plasma of fertile males

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Abstract. Introduction: The present study investigated associations between environmental exposure to magnesium (Mg) and the levels of oxidative stress parameters and selected cytokines, and the antioxidant defense system in the seminal plasma of fertile males. Materials and methods: The study population consisted of 57 healthy, non-smoking, fertile men from the southern region of Poland. Based on the median magnesium levels in seminal plasma, subjects were divided into two groups: those with low (Mg-L) and high (Mg-H) magnesium concentrations. Results: Differences were not observed between the Mg-L and Mg-H groups as regards semen volume, pH, count, motility, or morphology of sperm cells. In the Mg-H group, cholesterol levels were significantly higher (77%) compared with the Mg-L group; however, γ-glutamyltranspeptidase activity was significantly lower (21%). The values of total oxidant status were significantly different between the two groups, with 78% higher values observed in the Mg-H group compared with the Mg-L group, whereas malondialdehyde levels did not differ significantly. The values for total antioxidant capacity and uric acid levels were significantly lower in the Mg-H group compared with the Mg-L group (14% and 17%, respectively). However, levels of bilirubin, albumin, thiol groups and α-tocopherol were significantly higher in the Mg-H group (71%, 44%, 35% and 47%, respectively). Conclusion: No associations between Mg levels in the seminal plasma of fertile males and standard semen parameters were found. However, Mg levels may be associated with altered function of the non-enzymatic antioxidant system.

Key words: magnesium, semen, oxidative stress, cytokines, antioxidant defense system

Human semen, including the seminal plasma, contains high concentrations of calcium (Ca), magnesium (Mg), zinc (Zn), and copper (Cu). Abnormal levels of Ca and Mg and trace elements, particularly Zn and Cu, may influence spermatogenesis. Consequently, the production, maturation, motility, and fertilizing capacity of spermatozoa may be impaired [1].

Magnesium is an essential element for normal cell functioning. Mg serves as a cofactor in more than 300 enzymatic reactions. Other biochemical processes that require Mg include protein and DNA biosynthesis, anaerobic energy production, and the hydrolysis and transfer of phosphate groups [1-3]. Consequently, sperm survival depends on an optimal concentration of Mg. In the presence of Mg, sodium (Na) and potassium (K) ions stimulate ATPase. This hydrolyzing enzyme serves as the energy source for human spermatozoa. Because any alteration of the ATP pool affects sperm motility, Mg is believed to indirectly affect this semen quality parameter [4]. Moreover, Mg acts as a marker of the secretions of the seminal vesicles and is an intracellular calcium antagonist. However, the relationship between Mg and semen quality remains unclear [1].

The normal metabolic activity of spermatozoa results in reactive oxygen species (ROS) production [5]. Interestingly, the pro-inflammatory and immunoregulatory cytokines and chemokines, such as IL-1β, IL-6, IL-8, and IL-12, which are the natural components of seminal plasma, are believed to influence sperm cell metabolism, resulting in enhanced oxidative stress [6]. Additionally, IL-8 exerts its function by causing chemotraction of leukocytes to inflammatory sites, recruitment and activation of neutrophils, and is characterized by chemoattractant activity on basophils and T cells [7]. However, IL-12 creates an interconnection between innate and adaptive immunity, activating both NK cells and cytotoxic T lymphocytes [8].

Because the cytoplasmic membrane of spermatozoa contains a large amount of polyunsaturated fatty acids, sperm cells are especially susceptible to oxidative damage [9]. ROS generation can negatively affect motility and sperm-oocyte fusion, and cause mid-piece abnormalities [10]. Therefore, seminal plasma and spermatozoa contain ROS scavengers, antioxidant enzymes and the non-enzymatic antioxidants, such as α-tocopherol, ascorbic acid, glutathione, uric acid (UA), thiol groups, and albumin [5].

Although Mg is crucial for cell metabolism, little is known about its influence on sperm quality. We investigated the association between Mg concentration in the sperm of fertile males, using clinical and biochemical sperm quality markers and selected parameters of oxidative stress intensity and function of the immune (IL-1β, IL-6, IL-8, IL-12) and non-enzymatic antioxidant systems.

### Materials and Methods

#### Study population

The authors obtained the agreement of the Institutional Ethics Committee (permission number: KNW/0022/KB1/13/09) before launching the trial. The study population consisted of 57 healthy, non-smoking, fertile men from the southern region of Poland. The participants were drug tested, and no drug consumption (including antioxidant medications) was reported at the time of the study. All of the participants had normal semen specimens according to the WHO standards [9]. The subjects were divided into two groups based on the median of the values of Mg concentration in seminal plasma (MgS = 2.03 mmol/L):

1. a group with low environmental exposure to Mg (L-Mg) - Mg concentration in seminal plasma between 0.62 and 2.03 mmol/L; n = 29
2. a group with high environmental exposure to Mg (H-Mg) - Mg concentration in seminal plasma between 2.04 and 8.91 mmol/L; n = 28.

#### Sample collection

Semen was collected on the same day, in the morning, before the first meal. Semen samples (2-6 mL) were collected by masturbation at home or at the laboratory research facilities after at least three days of sexual abstinence (number of days elapsed since last ejaculation was recorded for each volunteer). Semen was collected into the precisely labeled sterile containers. Specimens produced at home were delivered to the laboratory within 60-90 minutes of collection.

#### Semen analysis

All of the semen specimens were analyzed according to WHO standards [11], including the assessment of seminal volume, sperm cell density, total sperm cell count, motility, and supravitral eosin staining (for the percentage of live spermatozoa). Sperm morphology was examined after Papanicolaou staining. After liquefaction, the semen samples (1.5 mL) were centrifuged at 6000 g for 10 minutes to separate the spermatozoa from the seminal plasma. The seminal plasma was transferred to fresh tubes and stored at −75°C until analysis. Additionally, a 10% spermatozoa lysate in bi-distilled water was made.
Determination of Mg

Mg levels were determined in seminal plasma using atomic absorption spectrometer (ICE 3300 Thermo Fischer, Waltham, MA, USA). For this method, 100 μL of seminal plasma were diluted with 4.9 mL of 0.2% solution of cesium chloride (CsCl) (Sigma-Aldrich, St Louis, MI, USA). Mg concentration in this solution was determined at a wavelength of 285.2 nm. The control material, TruLab N and TruLab P (DiaSys, Germany), was determined in each series of measurements after calibration curve preparation and at the end of the series. A standard curve was drawn using a standard solution of Mg at a concentration of 1 mg/mL (Merck, Darmstadt, Germany). Mg concentrations are expressed as mmol/L.

Determination of total oxidation status (TOS)

Total oxidation status was measured in seminal plasma according to Erel [12]. This assay is based on the oxidation of ferrous ions to ferric ions in the presence of various oxidant species in acidic medium. The color change to xylenol orange (POCH, Gliwice, Poland) by the ferric ions (CHEMPUR, Piekary Śląskie, Poland) was measured as the increase in absorbance at 560 nm. This method was conducted in an automated analyzer Perkin Elmer (Waltham, MA, USA) calibrated with hydrogen peroxide (POCH). Data are shown as μmol/L hydrogen peroxide.

Determination of malondialdehyde (MDA)

MDA, a product of lipid peroxidation, was measured fluorometrically as a 2-thiobarbituric acid-reactive substance (TBARS) in seminal plasma, according to Ohkawa [13] with modifications. Samples were mixed with 8.1% sodium dodecyl sulfate (Sigma-Aldrich), 20% acetic acid (POCH) and 0.8% 2-thiobarbituric acid (POCH). After vortexing, the samples were incubated for one hour at 95.0°C, and then butanol (POCH)-pyridine (Sigma-Aldrich) 15:1 (v/v) was added. The mixture was shaken for 10 minutes and then centrifuged. The butanol-pyridine layer was measured fluorometrically at 552 nm and 515 nm excitation (LS45 spectrofluorimeter (PerkinElmer, Waltham)). TBARS values are expressed as malondialdehyde (MDA) equivalents. Tetraethoxypropane (Sigma-Aldrich) was used as the standard. Concentrations are expressed as μmol/L seminal plasma.

Determination of lipofuscin (LPF)

The LPF concentration was determined in seminal plasma according to Jain [14]. Fluorescence was measured using an LS45 spectrofluorimeter (PerkinElmer, Waltham) at wavelengths of 360 nm (absorbance) and 440 nm (emission). Values are presented as relative units (relative fluorescence lipid extract, RF), where X corresponds to a fluorescence solution of 0.1 mg/mL quinidine sulfate (Sigma-Aldrich) in 0.1 N sulfuric acid (POCH).

Determination of total antioxidant capacity (TAC)

Total antioxidant capacity was measured according to Erel [15]. For this colorimetric method, radicals are generated and the antioxidant activity of seminal plasma reduces radical formation. The change in color of ABTS+ ions (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonate) (Sigma-Aldrich) is measured as the change in absorbance at 660 nm. This method was conducted in an automated PerkinElmer (USA) analyzer calibrated with Trolox (Sigma-Aldrich). Data are shown as mmol/L.

Determination of uric acid (UA), bilirubin and albumin

These parameters were determined in seminal plasma by colorimetric methods using biochemical analyzer (Hitachi, Tokyo, Japan). For uric acid and bilirubin, concentrations are provided in mg/dL, and albumin is expressed in g/mL.

Determination of protein sulfhydryl groups (PSH)

The PSH concentration in seminal plasma was determined as described by Koster [16] using 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich), which undergoes reduction by compounds containing sulfhydryl groups, yielding the yellow anion derivative 5-thio-2-nitrobenzoate, which was absorbed at a
wavelength of 412 nm using an automated analyzer (PerkinElmer, USA). The results are shown as μmol/g protein.

**Determination of α-tocopherol**

The concentration of α-tocopherol in seminal plasma was determined according to Shearer [17], using high-pressure liquid chromatography (HPLC) (Knauer, Berlin, Germany), coupled with UV/VIS. Data processing was performed with a EUROCHROM 2000. Concentrations are provided as μg/mL.

**Determination of cholesterol**

The cholesterol concentration in seminal plasma was determined using a biochemical analyzer (Hitachi, Japan). The action of hydrolytic cholesterol esterase on cholesterol ester produces free cholesterol and fatty acids. Hydrogen peroxide reacts with 4-aminophenazone and phenol in the presence of peroxidase, creating a colored compound. The color intensity of the resulting product is proportional to the concentration of cholesterol. Results are presented as mg/dL.

**Determination of gamma-glutamyltranspeptidase (GGTP) activity**

GGTP activity in seminal plasma was determined using a biochemical analyzer (Hitachi, Japan). GGTP moves the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzene released is proportional to the activity of GGTP. Results are presented as IU/L.

**Determination of alkaline phosphatase (ALP) activity**

ALP activity in seminal plasma was determined using a biochemical analyzer (Hitachi, Japan). In the presence of Mg and Zn ions, p-nitrophenyl phosphate is degraded by the enzyme to phosphate and p-nitrophenol. The amount of p-nitrophenol released is directly proportional to ALP activity, and can be measured photometrically. Results are presented as IU/L.

**Determination of alanine aminotransferase (ALT) activity**

ALT activity in seminal plasma was determined using a biochemical analyzer (Hitachi, Japan). ALT catalyzes the reaction of α-ketoglutarate and L-alanine in both directions. The resulting pyruvate and reduced nicotinamide adenine dinucleotide (NADH) are then substrates for lactate dehydrogenase (LDH) in the initial reaction. In the second reaction, NADH is oxidized to NAD. The decrease in the optical density of NADH, measured photometrically, is directly proportional to the concentration of pyruvate and thus to ALT. Results are presented as IU/L.

**Determination of aspartate aminotransferase (AST) activity**

AST activity in seminal plasma was determined using a biochemical analyzer (Hitachi, Japan). AST catalyzes the reaction of α-ketoglutarate and L-aspartate in both directions. The resulting oxaloacetate and NADH is then an indicator substrate for malate dehydrogenase (MDH). NADH is oxidized to NAD. The decrease in the optical density of NADH measured photometrically directly proportional to the concentration of oxaloacetate and thus, to AST. Results are presented as IU/L.

**Determination of cytokines**

IL-1β, IL-6, IL-8, IL-12 were detected in seminal plasma using a Bio-Plex 200 System (Bio-Rad Laboratories Inc., California, USA). The Bio-Plex system is based on three core elements. The first is a technology that uses fluorescently-dyed, magnetic microspheres (beads), each with a distinct color code to permit discrimination of individual tests within a multiplex suspension, and allows the simultaneous detection of diverse analyte molecules in a single well of a 96–well microplate. Moreover, the magnetic beads allow for magnetic separation during the washing steps. The second element is a dedicated flow cytometer with two lasers (a 532 nm Nd-Yag laser used to excite phycoerythrin in the assay and a 635 nm solid state laser used to excite the dyes inside the beads to determine their “color” or “region”) and associated optics to measure the different molecules bound to the surface of the beads. The third element is a
high-speed digital signal processor that efficiently manages the fluorescent output.

The principle of these bead-based assays is similar to sandwich immunoassays. The samples and standards were incubated with the coupled beads (antibodies directed against the desired cytokines were covalently coupled to internally dyed beads) in the wells of 96-well plates and washed. Next, the biotinylated detection antibodies specific for different cytokine epitopes were added. After incubation and washing, streptavidin (phycoerythrin solution) was added to bind biotinylated detection antibodies onto the beads. Next, the suspensions of washed beads were analyzed using the Bio-Plex System. Software presented data as both median fluorescence intensity as well as concentration (pg/ml).

**Statistical analysis**

The data were assimilated in MS Excel 2007. Statistical analysis was performed using Statistica 10.0 PL software. The results are presented as the means and standard deviations (SD) for normal distributions and as medians and interquartile ranges (IQR) for abnormal distributions. A Shapiro-Wilk’s test was used to verify normality, and Levene’s test was used to verify the homogeneity of variances. Statistical comparisons between groups were performed by a t-test, a t-test with a separate variance or the Mann-Whitney U test (non-parametric test). Spearman’s coefficient (r) for non-parametric correlations was calculated. Additionally, regression analysis was performed (R - multiple correlation coefficient, $R^2$ coefficient of determination, $\beta^*$ regression standardized coefficient). A value of $p<0.05$ was considered to be significant.

**Results**

The mean age of the Mg-L and Mg-H groups was not significantly different. Similarly, differences were not observed between groups as regards semen volume, pH, count, motility or morphology of the sperm cells (table 1).

In the Mg-H group, the cholesterol concentration was significantly higher (77%) compared with the Mg-L group. However, GGTP activity was significantly lower (21%). AST and ALT activity was not significantly different between the groups examined (table 2).

TOS values were significantly different between groups, with 78% higher values observed in the Mg-H group compared with the Mg-L group, whereas MDA concentrations did not differ significantly (table 2).

TAC values and UA levels were significantly lower in the Mg-H group compared with the Mg-L

| Table 1. Semen parameters and magnesium concentration in the study population divided into two groups (Mg-L, Mg-H) based on the median Mg concentration. |
|---------------------------------|-----------------|-----------------|-------------|-------------|---------------|
|                                | Mg-L group | Mg-H group | p value | relative difference |
|                                | Mean | SD | Mean | SD |          |               |
| Age                            |   33.0 | 5.96 |   31.5 | 5.56 | 0.330 | -5%           |
| Magnesium concentration in seminal plasma (mmol/L) | | | | |  |
|                                | 1.53 | 0.39 | 3.43 | 1.77 | $<0.001$ | 124%          |
| Volume                         | 4.47 | 2.31 | 3.87 | 0.97 | 0.207 | -14%          |
| pH                             | 7.57 | 0.07 | 7.56 | 0.06 | 0.511 | -0.2%         |
| Sperm cells count in 1mL (10^6/mL) | 63.6 | 40.0 | 75.7 | 59.6 | 0.368 | 19%           |
| Total sperm cell count (10^6)   | 273 | 190 | 276 | 201 | 0.945 | 1%            |
| Motile sperm cells after 1 hour (%) | 58.1 | 9.77 | 57.8 | 10.83 | 0.908 | -1%           |
| Progressively motile sperm cells after 1 hour (%) | 26.4 | 7.4 | 25.7 | 9.10 | 0.751 | -3%           |
| Motile sperm after 24 hours (%)  | 19.0 | 16.7 | 19.1 | 16.4 | 0.975 | 1%            |
| Progressively motile sperm after 24 hours (%) | 6.87 | 9.11 | 6.76 | 8.26 | 0.960 | -2%           |
| Normal morphology (%)           | 51.0 | 8.34 | 52.6 | 8.06 | 0.473 | 3%            |
Table 2. The biochemical parameters, indicators of oxidative stress, and the non-enzymatic antioxidant system parameters in seminal plasma of the study population divided into two groups (Mg-L, Mg-H) based on the median Mg concentration.

<table>
<thead>
<tr>
<th></th>
<th>Mg-L group n = 29</th>
<th>Mg-H group n = 28</th>
<th>p value</th>
<th>relative difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Cholesterol concentration (mg/dL)</td>
<td>25.6</td>
<td>10.5</td>
<td>45.4</td>
<td>20.7</td>
</tr>
<tr>
<td>GGTP activity (IU/L)</td>
<td>1935</td>
<td>489</td>
<td>1526</td>
<td>651</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>282</td>
<td>615</td>
<td>214</td>
<td>179</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>510</td>
<td>329</td>
<td>566</td>
<td>386</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>87</td>
<td>57.5</td>
<td>116</td>
<td>43.1</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td>6.13</td>
<td>11.86</td>
<td>10.9</td>
<td>11.5</td>
</tr>
<tr>
<td>MDA concentration (µmol/L)</td>
<td>2.47</td>
<td>0.94</td>
<td>2.18</td>
<td>0.75</td>
</tr>
<tr>
<td>Lipofuscin concentration (RF)</td>
<td>3.98</td>
<td>1.45</td>
<td>3.69</td>
<td>1.14</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>1.37</td>
<td>0.21</td>
<td>1.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Uric acid (mmol/L) (mg/dL)</td>
<td>4.79</td>
<td>1.51</td>
<td>3.98</td>
<td>1.23</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.08</td>
<td>0.05</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>0.40</td>
<td>0.10</td>
<td>0.57</td>
<td>0.16</td>
</tr>
<tr>
<td>PSH concentration (µmol/L)</td>
<td>183</td>
<td>80.1</td>
<td>247</td>
<td>100</td>
</tr>
<tr>
<td>α-tocopherol (µg/mL)</td>
<td>6.51</td>
<td>3.09</td>
<td>9.57</td>
<td>2.74</td>
</tr>
</tbody>
</table>

A Spearman correlation showed positive correlations between the concentration of Mg and TOS values (R = 0.49, p = 0.003), albumin concentration (R = 0.66, p<0.001), cholesterol concentration (R = 0.61, p<0.001), ALT activity (R = 0.4, p = 0.034), and IL-12 concentration (R = 0.41, p = 0.032). Negative correlations were observed between Mg concentration and TAC value (R = -0.41, p = 0.015), UA concentration (R = -0.3, p = 0.025), and GGTP activity (R = -0.28, p = 0.037).

Regression analysis showed that among the parameters associated with Mg concentration were: cholesterol concentration (β* = 0.50) and UA concentration (β* = -0.21) (R = 0.57, R² = 0.32, p<0.001).

Discussion

In the present study, no association was observed between Mg concentration and standard semen parameters such as volume, pH, count, motility, and morphology in fertile males. The majority of authors have reported consistent findings [18, 19]. However, Wong et al. [1] showed a weak, significant correlation between plasma Mg and

Table 3. Concentrations of cytokines in seminal plasma of the study population divided into two groups (Mg-L, Mg-H) based on the median Mg concentration.

<table>
<thead>
<tr>
<th></th>
<th>Mg-L group n = 29</th>
<th>Mg-H group n = 28</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median</td>
<td>IQR</td>
<td>median</td>
</tr>
<tr>
<td>IL-1β (pg/mL)</td>
<td>0.70</td>
<td>1.12</td>
<td>1.97</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>11.53</td>
<td>16.56</td>
<td>5.77</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>194.4</td>
<td>276.5</td>
<td>129.6</td>
</tr>
<tr>
<td>IL-12 (p70) (pg/mL)</td>
<td>1.87</td>
<td>2.92</td>
<td>3.50</td>
</tr>
</tbody>
</table>
sperm concentration, but not motility. Animal studies have shown that severely reduced magnesium in the diet causes decreases fertility potential in several different species, such as Drosophila melanogaster [20] and boar [21]. However, Zavaczki et al. [3] reported that magnesium-orotate treatment at a dose of 3,000 mg per day was not effective in improving sperm variables, or the pregnancy rates of female partners of treated males diagnosed with idiopathic infertility. These data indicate that the possible associations between Mg and standard semen quality parameters become significant only when severe hypomagnesemia occurs.

The transaminases play an important role in the transamination processes of sperm metabolism and could serve as indicators of the secretory activity of male accessory sex glands. The activities of AST and ALT have been reported to be consistently lower in static than in motile ejaculate of bulls [22]. Greater ALT activity coincided with increased semen quality in rabbits treated with isoflavones. Conversely, decreased AST and ALT activity coincided with decreased semen quality in rabbits treated with aluminium chloride [10, 23]. Accordingly, the positive correlation between Mg and ALT activity observed in the present study appears to be beneficial. However, some authors have concluded that spermatozoa with damaged cell membranes release high concentrations of AST and ALT into the seminal plasma and that plasma transaminase activity could be used as indices of sperm integrity and acrosomal damage [24]. Consistent with this hypothesis, negative correlations between ALT and AST activity and semen quality parameters have been observed in rabbits and poultry [24, 25]. Similar to transaminases, GGTP is a ubiquitous enzyme present in many tissues, including the testes and seminal vesicle. GGTP has been used as a marker for testicular growth and development [26]. The negative association between Mg concentration and ALT and GGTP activity is difficult to interpret and requires further investigation.

The unique properties of the sperm cell membrane, such as a high level of motility and fusion capability, depend mainly on its lipid content. The lipid content of sperm cell membrane is positively associated with sperm motility and viability. Sterols play the most important role in modifying sperm cell membrane properties, and it is generally accepted that cholesterol is the most effective sterol at modulating membrane properties [27, 28]. Cholesterol is believed to function as a membrane stabilizer to prevent the premature release of proteolytic enzymes from the acrosome. The process of spermatozoa maturation is associated with a decrease in the ratio of cholesterol to other sterols, such as desmosterol. Consistently, the highest concentration of cholesterol is present within immature and immotile sperm, which are collected from the testes or caput epididymis. A higher cholesterol-to-phospholipid ratio has been observed in patients with idiopathic infertility. These results confirm that cholesterol depletion appears to be crucial for normal sperm function [27]. However, other authors have postulated that cholesterol plays an important role in promoting sperm membrane permeability and fluidity in bulls, stallions, and rams. Conversely, a lower cholesterol content was associated with low sperm motility in boars [28]. The discrepancies between the above-mentioned results may be due to evolutionary differences between the species studied and the different methods of semen collection and sample preparation. The higher seminal plasma cholesterol concentration in the Mg-H group may be due to its increased leakage from sperm cell membranes. Positive correlations between concentrations of Mg and cholesterol and ALT activity suggest that higher levels of Mg may result in decreased membrane integrity. However, it is difficult to propose possible mechanisms. The strong association between levels of Mg and cholesterol is also confirmed by the regression analysis.

ROS has been proposed to be one of the potential causes of infertility in men. Because spermatozoan cytoplasm disposes of the relatively low activity of antioxidant enzymes, non-enzymatic antioxidants may play a most important role in protecting spermatozoa against ROS [9]. In the present study, all of the antioxidants examined, including bilirubin, albumin, thiol groups, and uric acid, were observed in seminal plasma. Bilirubin is an effective superoxide scavenger and inhibits the formation of oxidized, low-density lipoprotein. The antioxidant capacity of bilirubin is even more powerful than vitamins C and E. Bilirubin biosynthesis depends on heme oxygenase (HO) activity [29]. HO activity in seminal plasma correlated positively with sperm concentration and motility, and negatively with the percentage of abnormal sperm [30]. The increased
Magnesium in the seminal plasma of fertile males

Bilirubin concentrations in the Mg-H group indicate indirectly the possible influence of Mg on HO activity.

The antioxidant properties of albumin have been attributed to the thiol groups of cysteine. Thiol groups are involved in thiol exchange reactions and act as ROS scavengers. Moreover, albumin sequesters pro-oxidant molecules and redox-active metals [31]. Similar to bilirubin, higher levels of albumin and thiol groups were observed in the Mg-H group compared with the Mg-L group. Only UA concentration was lower in the Mg-H group. Also, the regression analysis showed a negative relation between Mg and UA levels. UA is a strong reducing agent and serves as a potent antioxidant. UA is synthesized by xanthine oxidase from xanthine and hypoxanthine. Lahnsteiner et al. [32] showed that UA is the primary antioxidant in brown trout semen. Our findings are in agreement with those of Lahnsteiner and coworkers. We found lower TAC values in the Mg-H group compared with the Mg-L group, despite higher concentrations of bilirubin, albumin, thiol groups and α-tocopherol. These data indicate that the lower antioxidant capacity of a small UA pool is not compensated for by higher levels of other antioxidants, which results in lower TAC values.

Thus, lower UA concentration in the Mg-H group may be one possible explanation for the simultaneously higher TOS values observed in the Mg-H group compared with the Mg-L group. The second possible explanation is that higher Mg concentrations may enhance sperm cell metabolism resulting in increased reactive oxygen species production. Moreover, a positive correlation was observed between Mg and IL-12, which is believed to have pro-inflammatory properties, and may contribute to the increased TOS values. However, the action of IL-12 is wider and is probably focused on Th1-type immune response stimulation [8]. Other pro-inflammatory cytokines investigated were not associated with Mg concentration. Hypomagnesemia has been postulated to induce oxidative stress and increase the release of pro-inflammatory cytokines [33, 34]. Our findings indicate that this relationship declines when the intake of Mg is within the normal range. However, a positive relation between Mg and TOS, which was observed in the present study, suggests that Mg supplementation should be introduced carefully but it might be not as beneficial to sperm quality as expected.

Conclusions

Mg concentration in the seminal plasma of fertile males is not associated with standard semen parameters. However, Mg may modify the non-enzymatic antioxidant system function.

The clinical significance of cholesterol level and activities of GTP, ALT, and AST in seminal plasma has not yet been established. However, associations between Mg, cholesterol level and ALT activity suggest that Mg concentration influences membrane integrity, possibly through the decreased concentration of UA which is believed to serve as a potent antioxidant.

Disclosure


References


