Journal of Andrology, Vol. 16, No. 5, September/October 1995

Selenium Supplementation Enhances the Element Concentration in Blood and Seminal Fluid But Does Not Change the Spermatozoal Quality Characteristics in Subfertile Men

KRZYSZTOF IWANIER* AND BRONISLAW A. ZACHARA†

*From the *Gynecology and Obstetrics Ward, Municipal Hospital, Grudziądz, Poland; and the †Department of Biochemistry, University School of Medical Sciences, Bydgoszcz, Poland.*

ABSTRACT: The objective of this study was to evaluate the effect of selenium (Se) supplementation on Se concentration and glutathione peroxidase (GSH-Px) activity in blood components and seminal fluid and on spermatozoal quality characteristics in subfertile men. Thirty-three men were supplemented for 12 weeks with 200 μg Se/day in the form of yeast-rich Se (group I, $n = 16$) or sodium selenite (group II, $n = 17$).

Blood samples and sperm were collected at the start of the study and after 2, 4, 8, and 12 weeks following Se supplementation. Se concentration in whole blood and plasma and GSH-Px activity in red cells and plasma increased significantly during the study, but in the group supplemented with yeast-Se the effect was more pronounced. Se concentration in seminal fluid also increased in both groups, but the effect of yeast-Se was markedly higher than that of selenite. In both groups statistically significant correlations were found between Se concentration in plasma and seminal fluid. GSH-Px activity in seminal fluid in the yeast-Se group increased significantly and reached a plateau after 2 weeks, whereas in the selenite group the activity did not change throughout the whole study period. Weak correlations between Se concentrations and GSH-Px activities in seminal fluid were seen, but only in the yeast-Se group were the relations statistically significant. The subjects in both groups showed no response in sperm count, motility, and morphology.

In conclusion, we can ascertain that the supplementation of subfertile men with yeast-rich Se showed a more pronounced effect on Se concentrations and GSH-Px activities in blood components and seminal fluid than selenite did. Se supplementation did not improve the spermatozoal quality characteristics of sperm count, motility and, morphology.

Key words: Selenium, glutathione peroxidase, semen, spermatozoa, blood components, subfertility.

J Androl 1995;16:441–447

The biochemical composition of human semen has attracted the attention of various scientists because of its possible influence on fertility. Selenium (Se) is an essential element required for normal animal growth and reproduction (Smith and Picciano, 1986). Se has been demonstrated to be a constituent of spermatozoa and an essential element for spermatogenesis (Wu et al, 1973). The first change observed in sperm of Se-deficient mice and rats is abnormal development of the midpiece (Calvin et al, 1981; Wallace et al, 1983). Wu et al (1973) found that Se-deficient rats produced sperm with poor motility and a high percentage of broken tails. It has been shown that after i.p. injection of ^{75}Se-selenite to Se-deficient rats a considerable amount of radioactivity was found in the testes (Brown and Burk, 1973). The increase in ^{75}Se retention was due to incorporation of the element into the midpiece of spermatozoa. Calvin et al (1981) argued that almost all of the Se in spermatozoa is contained in the mitochondrial capsule, further defined as the outer membranes of sperm mitochondria. Several authors have shown that the concentration of Se in rat and bull seminal fluid was extremely high (Calvin et al, 1981; Behne et al, 1986; Kantola et al, 1988; Saaranen et al, 1989). In human seminal fluid the concentration of this element was markedly lower (Behne et al, 1986; Sławeta et al, 1988; Saaranen et al, 1989). Essentially all of the Se in rat spermatozoa is bound to a protein confined to the capsule that surrounds the sperm mitochondria (Calvin et al, 1987). This protein has recently been described in more detail and shown to contain three selenocysteine residues. For that reason the authors have called it "mitochondrial capsule selenoprotein" (Kleene, 1994). About 20 selenoproteins are now known to be present in animal tissues (Zachara, 1992); a few have been found in the seminal fluid (Pond et al, 1983; Calvin et al, 1987).

In 1973 the biochemical function of Se was demonstrated to reside in its involvement in the active site of the enzyme glutathione peroxidase (GSH-Px; EC 1.11.1.9) (Flohe et al, 1973; Rotruck et al, 1973). This enzyme is present in animal and human semen (Bartle et al, 1980;

Correspondence to: Bronislaw A. Zachara, MD, PhD, Department of Biochemistry, University School of Medical Sciences, 24 Karłowicza St., 85-092 Bydgoszcz, Poland.

Received for publication April 10, 1995; accepted for publication July 14, 1995.

Behne et al, 1986; Kantola et al, 1988; Slaweta et al, 1988; Saaranen et al, 1989). The main role of GSH-Px is to remove hydroperoxide and free fatty acid hydroperoxides and thus to protect the spermatozoa against peroxidative damage (Alvarez and Storey, 1989). Similar to Se concentration, the seminal fluid activity of GSH-Px varies markedly between species. It is very high in the bull, low in man and ram, and absent in boar and stallion (Saaranen et al, 1989). Saaranen et al (1989) have shown that the sperm Se level of infertile men is lower than that of men with normal reproductive function and that low sperm motility is associated with decreased sperm Se concentration. Quite recently MacPherson et al (1993) have shown Se supplementation to subfertile men to effect a significant increase in sperm motility that was not achieved by any of the other antioxidant micronutrients supplied. During the experiments the authors did not measure the effect of Se supplementation on seminal fluid Se concentration, GSH-Px activity, and sperm count and morphology. Therefore the aim of our study was to evaluate the effect of supplementation of two forms of Se on Se concentration and GSH-Px activity in seminal fluid and on spermatozoal quality characteristics of subfertile men.

Materials and Methods

Patients and Sample Preparations

Semen and venous blood samples were obtained from 9 fertile men and from 33 patients aged 19–38 years (mean age, 31 years) with suspected infertility. Semen samples were collected into acid-washed glass containers at the Andrology Outpatient Clinic by masturbation. The patients participating in the study gave written informed consent, and the study was approved by the local Ethics Commission. They had been advised to abstain from sexual activity for 4–5 days before the collection of the semen. After allowing about 30 minutes for liquefaction to occur, the ejaculates were analyzed for spermatozoal count, motility, and morphology. Microscopic analyses were performed within 2 hours of collection by an experienced biologist using standard World Health Organization criteria (WHO, 1992). Sperm counts were performed in a hemocytometer, and motility was analyzed visually. For morphology evaluation the smears were air-dried, fixed, and stained according to the Papanicolaou method (WHO, 1992).

The seminal fluid was separated from spermatozoa by centrifugation at 4°C at 600 × *g* for 10 minutes. Venous blood samples were obtained at the same time in heparinized acid-washed glass tubes. Blood was centrifuged at 4°C at 3,000 × *g* for 10 minutes, plasma was harvested, and red blood cells were washed two times in an excess of physiological saline solution.

Se Supplementation

Two forms of Se were administered daily for 12 weeks. One group (16 subjects) was supplemented with 200 μg Se/day in the form of Se-rich-yeast, whereas the other group (17 subjects) was given 200 μg Se/day of sodium selenite mixed with baker's yeast. Semen and blood samples were collected prior to the start of supplementation and after 2, 4, 8, and 12 weeks of Se administration.

Assay Methods

Se concentration was assayed in whole blood, plasma, and seminal fluid by the fluorometric method of Watkinson (1966) using 2,3-diaminonaphthalene as the complexing reagent. The 4,5-benzpiazselenol formed in the reaction was quantitatively extracted with cyclohexane. Fluorescence measurements were made using a Hitachi F-4010 spectrofluorometer. The Se concentrations in all studied materials were expressed as ng/ml. The accuracy of the method was monitored by measurement of certified reference materials produced by Seronorm, Nycomed, Oslo, Norway (whole blood, batch no. 904 and serum, batch no. 105) and by participation in the interlaboratory comparison trials (Nève et al, 1992). The average concentrations found for Se were between 96% and 104%. The intraassay coefficient of variation for whole blood, plasma ($n = 8$), and seminal fluid ($n = 4$) was <4%.

GSH-Px activity was measured in red cell hemolysates, plasma, and seminal fluid using the method of Paglia and Valentine (1967), with *t*-butyl hydroperoxide as substrate. The reaction was carried out in a spectrophotometer fitted with a constant temperature cell housing. The method was based on a NADPH-coupled reaction whereby oxidized glutathione (GSSG) produced by GSH-Px and hydroperoxide was reduced by exogenous glutathione reductase and NADPH. Enzyme activity was expressed in units, each representing the oxidation of 1 μmol NADPH per minute per gram of hemoglobin (Hb) (U/g Hb) or per ml of plasma and seminal fluid (mU/ml).

The intraassay coefficient of variation for the examined materials was <3%. Hemoglobin concentration in the hemolysates was measured by the cyanmethemoglobin method.

Statistical Analysis

All statistical analyses, including the determination of mean ± standard deviation (SD; as all data are expressed), the Student's *t*-test, and the simple linear regression analysis were carried out using the computer program Statgraphics. A value of $P < 0.05$ was taken as significant.

Results

Se Concentrations

The mean Se concentrations in whole blood, plasma, and seminal fluid taken from nine fertile men were 92.3 ± 10.5, 71.1 ± 12.2, and 45.4 ± 16.5 ng/ml, respectively (1.17 ± 0.13, 0.90 ± 0.15, and 0.57 ± 0.21 nmol/ml, respectively; conversion factor to SI units, 0.0127). The mean initial Se concentrations in whole blood, plasma, and seminal plasma taken from 33 men, shown in Figure

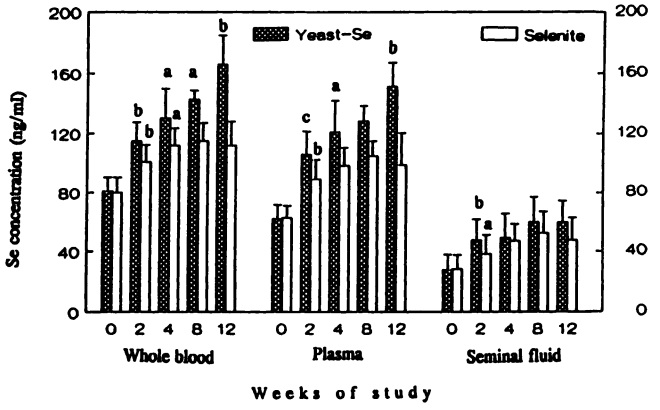

FIG. 1. Influence of Se supplementation in subfertile men on Se concentration in whole blood, plasma, and seminal fluid. Se was administered for 12 weeks at 200 μg/day in the form of yeast-Se or selenite. Data are shown as means ± SD. Differences are compared to the data from a previous period of study: **a**, $P < 0.05$; **b**, $P < 0.001$; **c**, $P < 0.0001$.

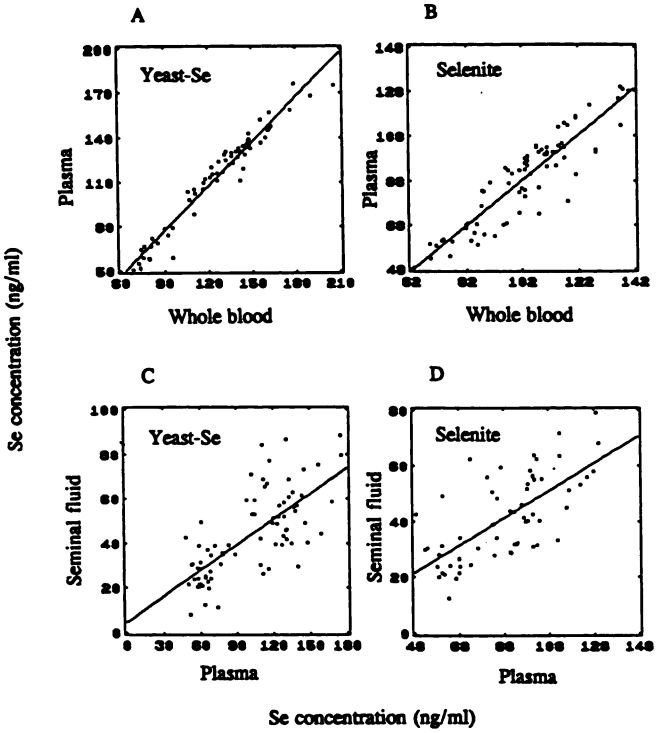

FIG. 2. Relations between whole blood and plasma Se concentrations in subfertile men supplemented with 200 μg Se/day in the form of yeast-Se (**A**) or selenite (**B**) and between plasma and seminal fluid Se concentrations in yeast-Se (**C**) or selenite treated groups (**D**).

1, were 80.4 ± 9.7, 62.4 ± 8.8, and 28.0 ± 9.5 ng/ml, respectively. The Se concentrations in the material studied from subfertile men were significantly lower ($0.005 < P < 0.02$) compared to fertile subjects. The seminal plasma Se levels in subfertile men were less than half of those in blood plasma. In the group supplemented with yeast-Se, the whole blood and plasma Se concentrations increased linearly and significantly throughout the whole study period. After 12 weeks of supplementation with yeast-Se, the element levels in whole blood were 2 times, and in plasma 2.4 times higher than the initial values ($P < 0.0001$). In the selenite-treated group, however, the whole blood and plasma levels both reached a plateau after almost 4 weeks ($0.0001 < P < 0.001$) and remained unchanged in the next 8 weeks. In seminal fluid, in both groups, the Se concentration reached a plateau after 2 weeks ($0.001 < P < 0.03$). In the later period there was a tendency to increment but the differences were statistically nonsignificant.

Strong, highly significant correlations between Se concentrations in whole blood and plasma of the yeast-Se group ($r = 0.974$; $P < 0.0001$) and the selenite-treated group ($r = 0.905$; $P < 0.0001$) were found when the data from all periods of the study were combined (Fig. 2A and B, respectively). Weaker but also highly significant correlations were found between Se concentrations in plasma and seminal fluid of the yeast-Se ($r = 0.721$; $P < 0.0001$) and the selenite group ($r = 0.686$; $P < 0.0001$) (Fig. 2C and D, respectively).

GSH-Px Activities

GSH-Px activities of red cells, plasma, and seminal fluid are presented in Figure 3. Similar to Se concentrations, the mean initial activities of the enzyme in seminal fluid (95.7 mU/ml) are more than two times lower when compared to blood plasma (207 mU/ml). As expected, the GSH-Px activities in the blood components increased following Se supplementation. In the red cells the increase was not observed until after 8 weeks in the yeast-Se group and 12 weeks in the selenite-treated group. In plasma, however, in both groups a highly significant increase was observed after 4 weeks ($0.0001 < P < 0.001$). At this time in both groups the activities reached a plateau and were 1.3 times higher than the initial values. Seminal fluid GSH-Px activity responded differently than plasma GSH-Px following Se supplementation. In the yeast-Se-treated group the activity reached a plateau 2 weeks ($P < 0.01$) after starting the supplementation and remained unchanged until the termination of the experiment. Surprisingly, no significant response in GSH-Px activity was observed in seminal fluid in the selenite-supplemented group. There is no apparent explanation for this phenomenon.

Because GSH-Px is a Se-containing enzyme, a positive correlation was observed between whole blood Se concentration and red cell GSH-Px activity. In the yeast-Se group, $r = 0.620$ ($P < 0.0001$), and the in selenite group, $r = 0.356$ ($P < 0.002$) (Fig. 4A and B, respectively). Significant correlations were also observed in plasma between Se concentration and GSH-Px activity in both groups. In the yeast-Se group, the values were: $r = 0.623$

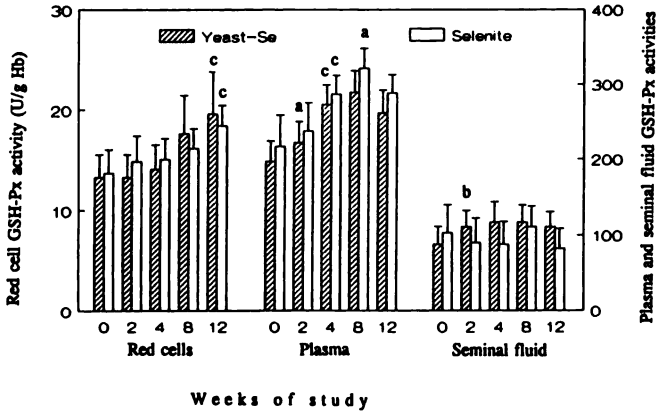

FIG. 3. GSH-Px activities in red cells (U/g Hb), plasma, and seminal fluid (mU/ml) in subfertile men supplemented with yeast-Se and selenite. Data represent means ± SD. Differences are compared to the values from a previous period of study: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$.

and $P < 0.0001$ (Fig. 4C), and in the selenite group, $r = 0.580$ and $P < 0.0001$ (Fig. 4D). Weaker correlations existed in seminal fluid between Se concentrations and GSH-Px activities. In the yeast-Se-treated group, $r = 0.399$ ($P < 0.001$), whereas in the selenite-supplemented group, $r = 0.227$ (P was nonsignificant) (Fig. 5A and B, respectively). By contrast, a very weak relationship existed between the plasma and seminal fluid GSH-Px activities (r

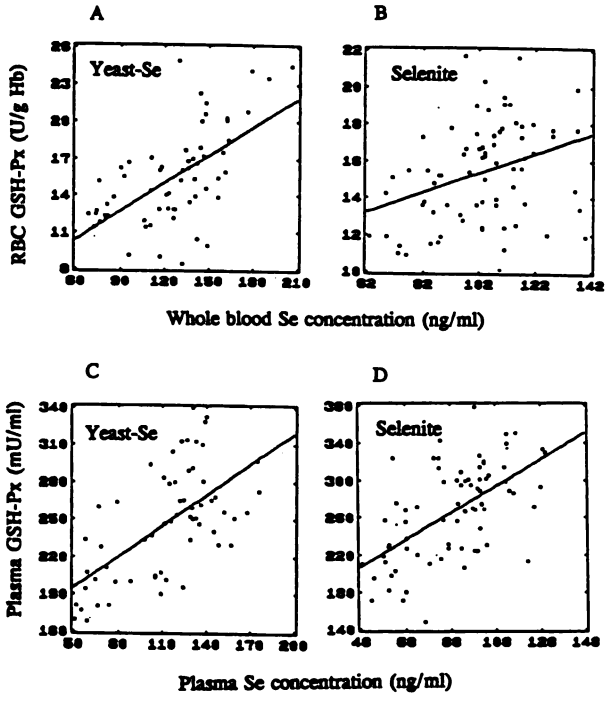

FIG. 4. Relations between whole blood Se concentrations and red cell GSH-Px activities in the yeast-Se (A), selenite-treated groups (B), and between Se concentrations and GSH-Px activities in plasma in the yeast-Se (C) or selenite-treated groups (D).

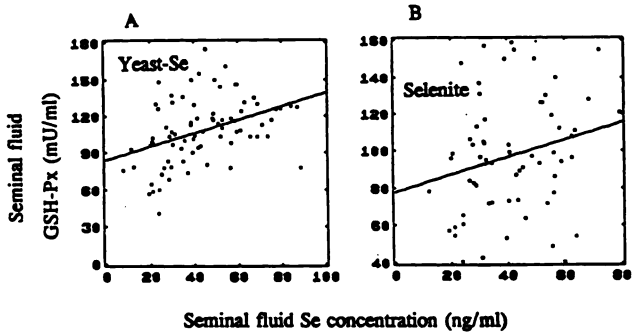

FIG. 5. Relations between Se concentrations and GSH-Px activities in seminal fluid in subfertile men supplemented with yeast-Se (A) or selenite (B).

= 0.088; $P = 0.44$ in the yeast-Se subjects and $r = 0.131$; $P = 0.28$ in the selenite-treated patients).

Microscopic Examination of Semen

Selenium supplementation, in both organic and inorganic form, did not improve the semen quality parameters examined (Fig. 6). At the beginning of the study the mean sperm density of all patients was 24.3 × 10^6/ml, and at the termination the value was 23.8 × 10^6/ml. The low motility of spermatozoa found before Se supplementation (30.5%) was at the same level after 12 weeks of the experiment (30.2%). The forward progression of spermatozoa was very low at the start of the study (12.3%) and remained unchanged during Se treatment (11.3%). The mean values of morphologically normal spermatozoa of both groups taken together before and after Se supplementation were 75.2% and 75.0%, respectively. No significant correlations between Se concentrations and GSH-Px activities in seminal fluid, nor between these two parameters and the semen quality parameters studied, were observed.

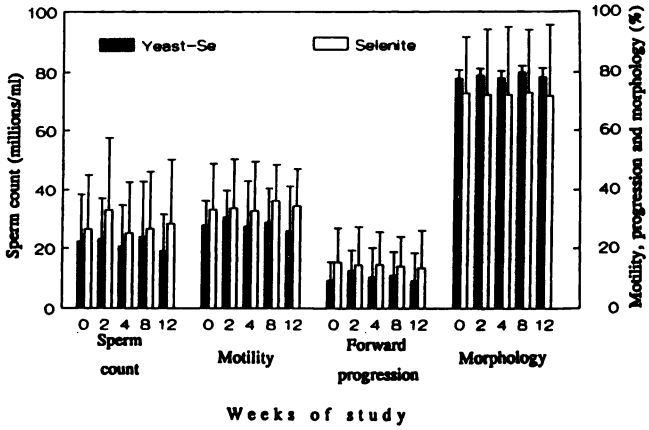

FIG. 6. Sperm count (× 10^6/ml), motility (%), forward progression (%), and morphology (%) of semen taken from subfertile men supplemented with yeast-Se or selenite. Data represent means ± SD.

Discussion

In several studies it has been shown that Se supplementation significantly increases blood Se concentrations and GSH-Px activities in several species of animals and in humans (Bartle et al, 1980; Butler et al, 1991; Johnsson and Westermarck, 1993). The results show that organic Se (selenomethionine [SeMet] or yeast-rich Se [which contains 50% SeMet or more]) was more effective than inorganic forms (selenite or selenate) in elevating Se concentrations in the blood components (Butler et al, 1991; Johnsson and Westermarck, 1993; Thomson et al, 1989). Our present study confirms these observations. Yeast-Se raised the Se levels in whole blood and plasma as well as in seminal fluid more rapidly than inorganic Se. Although the reason for the discrepancy is not clear, it may well be that SeMet is better absorbed and more efficiently retained than selenite (Butler et al, 1991).

The increase of GSH-Px activity in plasma and red cells of supplemented patients observed in this study indicates that Se bioavailability in the diet depends not only on intestinal absorption but also on the conversion of the absorbed Se into a biologically active form (Butler et al, 1991). Our results confirmed the observation of other authors, who have shown that the GSH-Px activity in plasma increased more rapidly compared with red cells (Thomson et al, 1989; Zachara et al, 1993). In both groups the plateau of plasma GSH-Px activity was reached 4 weeks after the start of Se supplementation, and the activity was higher by 30%–40% compared to the baseline. These results are comparable to those of Thomson et al (1989), who have shown that a plateau of plasma GSH-Px activity of selenite- and SeMet-supplemented subjects was 33%–50% higher than the initial values. Thus plasma Se and GSH-Px are the sensitive index of short-term Se status, whereas red cell Se and GSH-Px give a long-term index of Se status because of the long life span of these cells (Thomson and Robinson, 1980). The correlations between Se concentrations and GSH-Px activities of blood components found in this study are in accord with the results of other authors (Thomson and Robinson, 1980).

Although zinc levels in human semen and semen fractions have been investigated extensively, there are few publications concerning Se concentration in human semen. According to our knowledge, only one publication deals with the effect of Se supplementation on human semen (MacPherson et al, 1993).

The initial Se concentration in seminal fluid of our patients (28.0 ng/ml) was rather low and comparable only to Finish subjects with suspected infertility (28.8–34.4 ng/ml) (Saaranen et al, 1986, 1987, 1989). Behne et al (1988) have shown that in German subjects with suspected infertility, Se concentrations in seminal plasma and in semen were 38.3 and 49.6 ng/g (wet), respectively. Se concentrations in seminal fluid published so far by other authors were 2.15 to 2.55 times higher than our results (Pleban and Mei, 1983; Bleau et al, 1984; Takasaki et al, 1987; Roy et al, 1990). Low levels of Se in the seminal fluid of our patients are probably due to dietary factors, because the Se content in food in Poland is extremely low (Zachara and Wąsowicz, 1994).

In the literature there are contradictory results concerning the relationship between sperm or seminal fluid Se concentrations and spermatozoal quality characteristics. Bleau et al (1984) found a significant correlation between semen Se concentration and sperm count. They showed that sperm motility was maximal at as Se level in sperm ranging between 50 and 69 ng/ml; below and above this range motility was decreased, and the incidence of asthenospermia was high. A follow-up study 4.5–5 years after the initial assay of Se revealed that low Se levels (<35 ng/ml) were associated with male infertility. Saaranen et al. (1989) have shown that in the sperm of teratoasthenozoospermic men, the Se concentration was significantly lower than in normozoospermic men, and that sperm Se levels of infertile men were lower than in men with normal reproductive function. Takasaki et al (1987), however, did not find any significant difference in sperm and seminal fluid Se concentrations between fertile and infertile groups. Roy et al (1990) demonstrated that in normozoospermic, oligozoospermic, and asthenozoospermic samples no correlation could be found between semen Se level and sperm count or motility. Behne et al (1988), who are well-known experts in Se metabolism in reproductive tissues, also did not find any correlations between Se concentration in seminal fluid and sperm count, motility, viability, speed, and morphology. Our results fully support their observations. Quite recently MacPherson et al (1993) observed that Se supplementation to subfertile males (100 μg/day for 3 months) increased the blood plasma Se concentration significantly but did not change the sperm density. They have, however, observed significantly increased sperm motility, by almost 90%. The reasons for the above presented differences are not known.

In animal studies, Bartle et al (1980) injected dairy bulls with increasing doses of selenite. Subsequent injections increased blood and semen Se concentrations and GSH-Px activities. Semen from Se-treated bulls was significantly higher in Se than was the semen from untreated animals throughout the whole sampling period. The Se concentration was 10 times higher in the semen than in blood in both treated and untreated bulls. It is noteworthy that in their study the GSH-Px activity in seminal fluid increased significantly within 48 hours after the lowest dose (5 mg/90 kg body weight) of Se injection, and the activity almost tripled during this time. Although increasing the dose of injected Se from 5 to 40 mg/90 kg enhanced

the Se concentration and GSH-Px activity in seminal fluid, it did not significantly improve semen production and spermatozoal quality (motility and morphology) just after collection of the samples as well as after freezing–thawing process. On the other hand, Siegel et al (1980) demonstrated that bull sperm treated *in vitro* with 1 μg/ml selenite–Se after 24 hours significantly ($P < 0.05$) stimulated motility. Higher doses, however, reduced the motility, so that at 5 μg/ml the motility values were significantly lower than those of the controls (65.5 vs. 100.0; $P < 0.01$). The lack of response of seminal fluid GSH-Px activity following selenite supplementation in our study is thus obscure. Brown et al (1977) did not find any relationship between GSH-Px activity and sperm density of the bovine semen. They have shown, however, that there is a positive correlation of GSH-Px activity calculated per ejaculate volume. This suggests that the enzyme is low in spermatozoa in ejaculated bovine semen and high in seminal plasma. Synthesis of the majority of the GSH-Px present in seminal fluid probably occurs in the accessory sex glands (Bartle et al, 1980). It is believed that GSH-Px present in semen is the major enzyme reactive with lipid hydroperoxides produced by spermatozoa (Alvarez and Storey, 1989). By also reducing H_2O_2 to H_2O, GSH-Px plays a critical role in the prevention of oxidative damage to spermatozoa (Brown et al, 1977).

GSH-Px is highly specific for reduced glutathione (GSH) (Zakowski and Tappel, 1978). The relatively high level of GSH inside the cell is necessary to keep the enzyme in the active form. The GSH concentration outside the cells (plasma, seminal fluid, etc.) is very low (micromolar concentrations compared to milimolar concentrations in mammalian cells) (Meister and Anderson, 1983). Maddipati and Marnett (1987) have shown that the apparent K_m values for human plasma GSH-Px are 10-fold lower than the corresponding values for erythrocyte GSH-Px with H_2O_2 as substrate and as much as 30-fold lower than other hydroperoxides. Low K_m values make the GSH-Px a very effective hydroperoxide scavenger, even at a relatively low concentration of GSH.

The role of Se in sperm is still poorly understood. It has been shown that this element is incorporated in some proteins (Calvin et al, 1981, 1987; Kleene, 1994) in which GSH-Px plays an important role in protecting the cell membrane from oxidative damage (Brown et al, 1977; Behne et al, 1986; Alvarez and Storey, 1989). Although organic Se as well as inorganic Se caused Se increment in seminal fluid, they did not improve the spermatozoal quality parameters that we studied.

Acknowledgments

We thank Prof. Florian Ryszka, head of the Department of Applied Pharmacology and Drug Technology, Silesian University of Medical Sciences, Poland for supplying us with yeast-rich-Se and sodium selenite used in this study. This work is a PhD thesis submitted by K.I. to the Medical Faculty, University School of Medical Sciences in Bydgoszcz.

References

Alvarez J, Storey BT. Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. *Gamete Res* 1989;23:77–90.

Bartle JL, Senger PL, Hillers JK. Influence of injected selenium in dairy bulls on blood and serum selenium, glutathione peroxidase and seminal quality. *Biol Reprod* 1980;23:1007–1013.

Behne D, Duk M, Elger W. Selenium content and glutathione peroxidase activity in the testis of the maturating rat. *J Nutr* 1986;116:1442–1447.

Behne D, Gessner H, Wolters G, Brotherton J. Selenium, rubidium and zinc in human semen and semen fractions. *Int J Androl* 1988;11:415–423.

Bleau G, Lemarbre J, Faucher G, Roberts KD, Chapdelaine A. Semen selenium and human fertility. *Fertil Steril* 1984;42:890–894.

Brown DG, Burk RF. Selenium retention in tissues and sperm of rats fed a Torula yeast diet. *J Nutr* 1973;102:102–108.

Brown DV, Senger PL, Stone SL, Froseth JA, Becker WC. Glutathione peroxidase in bovine semen. *J Reprod Fertil* 1977;50:117–118.

Butler JA, Thomson CD, Whanger PD, Robinson MF. Selenium distribution in blood fractions of New Zealand women taking organic or inorganic selenium. *Am J Clin Nutr* 1991;53:748–754.

Calvin HI, Cooper GW, Wallace EW. Evidence that selenium in rat sperm is associated with a cysteine-rich structural protein of the mitochondrial capsule. *Gamete Res* 1981;4:139–149.

Calvin HI, Grosshans K, Musicant-Shikora SR, Turner SI. A developmental study of rat sperm and testis selenoproteins. *J Reprod Fertil* 1987;81:1–11.

Flohe L, Gunzler WA, Schock HH. Glutathione peroxidase: a selenoenzyme. *FEBS Lett* 1973;32:132–134.

Johnsson E, Westermarck T. Studies of selenium supplementation with inorganic and combined inorganic-organic Se in humans. *J Trace Elem Electrolytes Health Dis* 1993;7:113–114.

Kantola M, Saaranen M, Vanha-Perttula T. Selenium and glutathione peroxidase in seminal plasma of men and bulls. *J Reprod Fertil* 1988;83:785–794.

Kleene KC. The mitochondrial capsule selenoprotein—a structural protein in the mitochondrial capsule of mammalian sperm. In: Burk RF, ed. *Selenium in Biology and Human Health.* New York: Springer-Verlag; 1994:134–149.

MacPherson A, Scott R, Yates R. The effect of selenium supplementation in sub-fertile males. In: Anke M, Meissner D, Mills CF, eds. *Trace Elements in Man and Animals—TEMA 8.* Gersdorf, Germany: Verlag Media Touristik; 1993:566–570.

Maddipati KR, Marnett LJ. Characterization of the major hydroperoxide-reducing activity of human plasma: purification and properties of a selenium-dependent glutathione peroxidase. *J Biol Chem* 1987;262:17398–17403.

Meister A, Anderson ME. Glutathione. *Annu Rev Biochem* 1983;52:711–760.

Nève J, Thomassen Y, Van Damme M. Cooperative study on measurement of concentration of selenium in freeze-dried (human whole) blood. *Pure Appl Chem* 1992;64:765–780.

Paglia DE, Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158–169.

Pleban PA, Mei D-S. Trace elements in human seminal plasma and spermatozoa. *Clin Chim Acta* 1983;133:43–50.

Pond FR, Tripp MJ, Wu ASH, Whanger PD, Schmitz JA. Incorporation of selenium-75 into semen and reproductive tissues of bulls and rams. *J Reprod Fertil* 1983;69:411–418.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973;179:588–590.

Roy AC, Karunanithy R, Ratnam SS. Lack of correlation of selenium level in human semen with sperm count/motility. *Arch Androl* 1990; 25:59–62.

Saaranen M, Suistomaa U, Kantola M, Remes E, Vanha-Perttula T. Selenium in reproductive organs, seminal fluid and serum of men and bulls. *Hum Reprod* 1986;1:61–64.

Saaranen M, Suistomaa U, Kantola M, Saarikoski S, Vanha-Perttula T. Lead, magnesium and zinc in human seminal fluid: comparison with semen parameters and fertility. *Hum Reprod* 1987;2:475–479.

Saaranen M, Suistomaa U, Vanha-Perttula T. Semen selenium content and sperm mitochondrial volume in human and some animal species. *Hum Reprod* 1989;4:504–508.

Siegel RB, Murray FA, Julien WE, Moxon AL, Conrad HR. Effect of *in vitro* selenium supplementation on bovine sperm motility. *Theriogenology* 1980;13:357–367.

Slaweta R, Wąsowicz W, Laskowska T. Selenium content, glutathione peroxidase activity, and lipid peroxide level in fresh bull semen and its relationship to motility of spermatozoa after freezing-thawing. *J Vet Med A* 1988;35:455–460.

Smith AM, Picciano MF. Evidence for increased selenium requirement for the rat during pregnancy and lactation. *J Nutr* 1986;11:1068–1079.

Takasaki N, Tonami H, Simizu A, Ueno N, Ogita T, Okada S, Miyazaki S, Deguti Y, Ogata A. Semen selenium and infertility. *Bull Osaka Med School* 1987;33:87–96.

Thomson C.D., Robinson M.F.: Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand. Am. J. Clin. Nutr. 1980;33:303–323.

Thomson CD, Robinson MF, Whanger PD. Selenium (Se) and glutathione peroxidase (GSH-Px) in blood components of New Zealand women during long-term supplementation with selenite or selenomethionine (Semet). In: Wendel A, ed. *Selenium in Biology and Medicine.* Barlin: Springer Verlag; 1989:254–257.

Wallace E, Calvin HI, Cooper GW. Progressive effects observed in mouse sperm during course of three generations of selenium deficiency. *Gamete Res.* 1983;4:377–387.

Watkinson JH. Fluorometric determination of selenium in biological material with 2,3-diaminonaphthalene. *Anal Chem* 1966;38:92–97.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.* Cambridge, UK: Cambridge University Press; 1992.

Wu SH, Oldfield JE, Whanger PD, Weswig PH. Effects of selenium, vitamin E and antioxidants on testicular function in rats. *Biol Reprod* 1973;8:625–629.

Zachara BA. Mammalian selenoproteins. *J Trace Elem Electrolytes Health Dis* 1992;6:137–151.

Zachara BA, Trafikowska U, Labedzka H, Mikolajczak J. Effect of dietary Se intake on blood Se levels and glutathione peroxidase activities in lambs. *Small Rum Res* 1993;9:331–340.

Zachara BA, Wąsowicz W. Selenium concentration in blood components of Polish subpopulation and in some other countries (in Polish). In: Kabata-Pendias A, Szteke B, eds. *Arsenium and Selenium in the Environment: Ecological and Methodological Problems.* Warsaw: Polish Academy of Sciences; 1994:157–166.

Zakowski JJ, Tappel AL. Purification and properties of rat liver mitochondrial glutathione peroxidase. *Biochim Biophys Acta* 1978;526: 65–76.