

## Selenium in human male reproductive organs

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**The objective of the study was to obtain information on the concentration and distribution of selenium throughout the human male reproductive tract. Material was removed at autopsy from 41 men who had died suddenly and unexpectedly. Semen samples were also provided from 184 men attending an andrology clinic for fertility investigation and from 32 healthy volunteers. Significant positive correlations in the selenium concentration were demonstrated between the different reproductive organs, the testis having the highest concentrations. No correlation was found between the concentration of selenium in the genital organs and liver, kidney or blood, suggesting that its uptake and/or biochemical activity in the reproductive organs may be controlled by similar mechanisms not shared by the other organs. No significant age-dependent changes could be detected in tissue selenium concentrations. In a group of men under fertility investigation, a significant positive correlation was obtained between seminal plasma concentrations of selenium and concentrations of spermatozoa in the same ejaculate. A significant positive correlation between concentrations of zinc and selenium in the same ejaculates indicated that selenium may arise largely from the prostate gland.**

*Key words:* human/male reproductive organs/selenium/seminal fluid/zinc

### Introduction

There is growing evidence to indicate that reactive oxygen species are involved in the peroxidative damage of human spermatozoa seen in many cases of male infertility (Aitken, 1994). These free radicals may arise from defective spermatozoa and from leukocytes (Aitken and West, 1990). To counteract the effects of reactive oxygen species, semen is believed to possess a number of antioxidant systems (Kovalski *et al.*, 1992). Glutathione peroxidase has been assumed to play a role in protecting cells from the harmful effects of toxic metabolites and free radicals by preventing lipid peroxidation of membranes (Alvarez and Storey, 1989). Selenium constitutes an essential component of this enzyme (Rotruck *et al.*, 1973).

Both low and high concentrations of seminal plasma selenium may be harmful to male fertility (Bleau *et al.*, 1984). A

reduction in selenium concentration could theoretically render spermatozoa more vulnerable to oxygen radicals. Reactive oxygen species are involved in the initiation of normal biochemical processes in human spermatozoa, such as hyper-activated motility and the acrosome reaction (see review by Aitken, 1994), which occur in the Fallopian tubes and uterus. Since a premature induction of these processes in the male reproductive tract would be detrimental for the fertilization process, it is likely that there exist mechanisms in the semen which hinder this taking place.

There is a paucity of information in the literature regarding selenium and human reproductive function, and therefore the main aim of the present work was to provide further data on the distribution of this metal throughout the human male reproductive tract. A previous article which was based on the same material reported the tissue concentrations of heavy metals, including lead and cadmium (Oldereid *et al.*, 1993).

### Materials and methods

#### Subjects

Organs were removed at necropsy from 41 men (median age 39 years, range 18–80 years) who had died suddenly and unexpectedly (between March 1988 and June 1988, and in September 1990). All had undergone routine autopsy at the Institute of Forensic Medicine, The National Hospital, University of Oslo. Subjects with known alcohol or drug abuse were excluded from the study. Tissue samples were collected from the liver, kidney, testes, prostate gland, epididymis and seminal vesicles within 2 days of death. In addition, blood samples were taken from each subject. After the organ samples were dissected free from fat and superficial connective tissue, they were stored at –20°C until the day of assay.

The occupations of the subjects were divided according to their potential level of exposure to environmental pollutants. High-risk occupations included men involved in industry and transport, low-risk occupations included office workers. No information was available regarding tobacco and alcohol consumption or dietary intake.

Human semen samples were obtained from men under fertility investigation ( $n = 196$ ) and healthy volunteers ( $n = 32$ ). Azoospermic men ( $n = 12$ ) were excluded from the study because the reason for their azoospermia was unknown. The seminal concentration of selenium was measured in all cases. Routine measures of sperm quality had been carried out on the same subjects. Seminal zinc measurements were carried out on a group of volunteers ( $n = 15$ ).

#### Sample preparation

##### Tissue samples

After thawing, tissue samples were freeze-dried in a standard laboratory system. Accurately weighed amounts of ~0.5 g of the dried tissue samples were digested with 5 ml concentrated sub-boiled nitric acid,

typically for 1 h at 85°C in Teflon tubes. They were then diluted with water to a volume of 25 ml.

#### Seminal fluid and whole blood

A 2.5 ml volume of sub-distilled 65% nitric acid was added to 2 ml of the sample in a polypropylene digestion tube. After degasification at room temperature overnight, the tube was heated at 95°C for 1 h in a laboratory oven. After cooling to room temperature, the solution was diluted to a fixed volume (13.7 ml).

#### Measurement of selenium and zinc

Selenium was measured by electrothermal atomic absorption spectrometry using a Perkin-Elmer Zeeman-based 5100 PC/HGA 600 system calibrated with matrix-matched standards under stabilized thermal platform atomizer conditions using palladium chemical modification.

Zinc was determined by inductively coupled argon plasma atomic emission spectrometry using a Perkin-Elmer Model Optima 3000 spectrometer calibrated with nitric acid matched under standard conditions.

The accuracy and precision of the measurements were assessed continuously by using Seronorm human serum and whole blood quality control materials (Sero Ltd, Asker, Norway), and the certified reference materials NIST SRM 1577 Bovine Liver (National Institute of Standards and Technology, Gaithersburg, MD, USA) and IAEA H-4 Animal Muscle (International Atomic Energy Agency, Vienna, Austria). The reproducibility (day-to-day variation) of the measurements for the elements was typically 4–6% and the average concentrations found were within  $\pm 10\%$  of the recommended values reported by the manufacturers.

#### Sperm analysis

Semen specimens were provided in the laboratory by masturbation after a recorded abstinence period of 3–5 days. After a liquefaction period of 30 min at room temperature, sperm counts were performed using a Makler Chamber. Samples of the ejaculates (5  $\mu$ l) were transferred to a Hamilton Thorn sperm motility analyser (HTM-C Motility Analyzer, Hamilton Thorn Research Inc., MA, USA). The motility parameter chosen in the subsequent analyses was the proportion of spermatozoa exhibiting any form of movement (% motile), and those with a forward velocity exceeding 25  $\mu$ m/s (% progressive).

The seminal fluid was separated from spermatozoa by centrifugation at 90 g for 10 min.

#### Statistics

Statistical analyses were performed using the SPSS computer program (SPSS for Windows, release 6.0, SPSS Inc., Chicago, IL, USA). The data were compared by Spearman's rank correlation analysis. Regression analysis were performed before and after logarithmic transformation. Values are given as means and medians with corresponding standard deviation and range. Non-parametric statistical tests (Mann-Whitney test or Kruskal-Wallis test) were used for comparisons of non-normally distributed data.

## Results

### Reproductive organs

The median selenium concentration in the various reproductive organs, liver and kidney are summarized in Table I. The highest selenium levels were recorded in the kidney, followed by the testis which contained approximately double the concentration of selenium found in the other reproductive organs. The mean selenium concentration in whole blood was  $1.26 \pm$

**Table I.** Selenium concentration ( $\mu$ g/g dry weight) in tissue samples of human reproductive organs, kidney and liver from 41 men

Organ	Mean	SD	Median	Range
Testis	2.6	0.8	2.6	1.6–6.3
Epididymis	1.2	0.4	1.1	0.2–1.9
Prostate	1.3	0.4	1.3	0.6–2.1
Seminal vesicle	1.5	0.5	1.5	0.6–2.6
Kidney	4.7	1.7	4.3	2.2–9.7
Liver	1.9	0.6	1.9	0.9–3.7

0.29  $\mu$ mol/l (median value 1.25  $\mu$ mol/l, range 0.74–2.04  $\mu$ mol/l).

Significant positive correlations in selenium concentration were demonstrated between all of the reproductive organs (Table II). No correlation was demonstrated between the concentration of selenium in these organs and those concentrations in the liver, kidney and blood.

Table III shows the different causes of death in the study group. Selenium concentrations in the seminal vesicles and kidney were significantly higher in men who had died after an accident than in those who had succumbed suddenly to an illness. Data were analysed with Mann-Whitney non-parametric test with  $P = 0.008$  and  $P = 0.023$ , respectively (data not shown). The same trend was also seen in the other tissues, but the differences were not significant.

The mean age of subjects undergoing autopsy was 42.9 years (median 40 years, range 18–80 years). No significant age-dependent changes were detected in the selenium concentrations in any of the tissues examined, as judged by Spearman's rank correlation analysis of the whole study group or by comparison of selenium concentrations in different age groups (<40 years, 40–60 years, >60 years) (data not shown).

Extremes of theoretical occupational exposure to metals or environmental pollutants, as predicted from the occupation of the subject, did not significantly influence the tissue concentrations of selenium.

### Semen analysis

Characteristics of semen specimens in men with suspected infertility are shown in Table IV, and mean selenium concentrations of seminal fluid in Table V. The selenium values in the infertile group and the control group were not significantly different when data were compared by Mann-Whitney non-parametric test ( $P = 0.16$ ). In the infertile subjects, significant positive correlations could be demonstrated between seminal plasma selenium concentration and sperm count ( $r_s = 0.49$ ,  $P < 0.001$ ) (Figure 1) and total sperm number ( $r_s = 0.44$ ,  $P < 0.001$ ). Using regression analysis, a significant positive relation was obtained between seminal selenium concentrations and sperm count (regression coefficient = 0.001,  $P < 0.001$ ,  $R^2 = 0.21$ ) and total sperm concentration (regression coefficient = 0.0003,  $P < 0.001$ ,  $R^2 = 0.15$ ). There was only a weak association between seminal plasma selenium and the age of the subject using regression analysis (regression coefficient = 0.06,  $P = 0.005$ ,  $R^2 = 0.04$ ).

A significant negative correlation was demonstrated between seminal selenium concentration and seminal volume ( $r_s =$

**Table II.** Correlation coefficients (Spearman rank) of selenium concentrations ( $\mu\text{g/g}$  dry weight) between human male reproductive organs and the liver and kidney

Epididymis	0.39 <sup>a</sup>				
Prostate	0.36 <sup>a</sup>	0.63 <sup>c</sup>			
Seminal vesicle	0.37 <sup>a</sup>	0.38 <sup>a</sup>	0.40 <sup>b</sup>		
Kidney	0.23	0.09	0.18	0.29	
Liver	-0.03	0.18	0.25	0.05	0.24
Organ	Testis	Epididymis	Prostate	Seminal vesicle	Kidney

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**Table III.** Characteristics of the subjects undergoing autopsy

Causes of death	No. (%) according to cause of death	Age (years) (median) <sup>a</sup>
Cardiovascular disease	10 (24)	63
Other disease	5 (12)	39
Accident/other violent cause	19 (46)	30
Intoxication	7 (17)	47

<sup>a</sup>Significant difference between groups:  $P = 0.03$  (Kruskal-Wallis non-parametric test).

**Table IV.** Characteristics of semen specimens ( $n = 184$ ) in men with suspected infertility

Parameter	$n$	Median	Range
Sperm count ( $\times 10^6/\text{ml}$ )	184	45.1	0.1-412
Motile spermatozoa (%)	183	43.0	0-96
Progressive spermatozoa (%)	181	14.0	0-57
Semen volume (ml)	184	4.4	1.5-9.9

**Table V.** Selenium concentration in seminal fluid ( $\mu\text{mol/l}$ ) in infertile men and in a control group

	$n$	Mean	SD	Median	Range
Infertile men	184	0.50	0.20	0.45	0.13-1.16
Control group	32	0.43	0.13	0.43	0.12-0.76

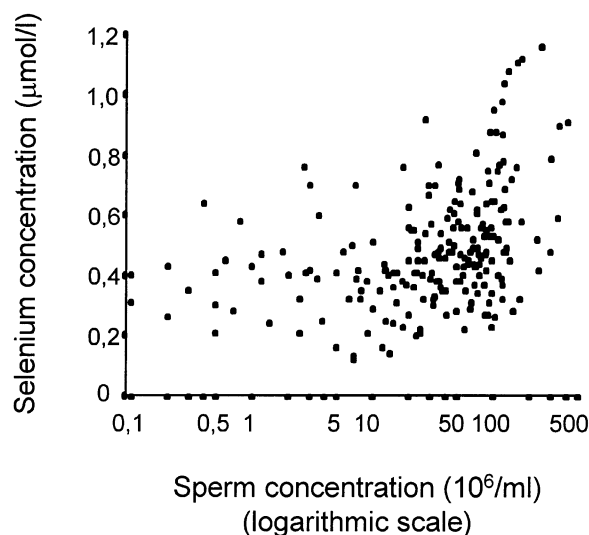
No significant differences between groups based on non-parametric test (Mann-Whitney).

-0.233,  $P < 0.001$ ), although regression analysis revealed only a weak association (regression coefficient = -0.03,  $P = 0.002$ ,  $R^2 = 0.05$ ). There was no significant correlation between selenium concentration and sperm motility parameters in the same ejaculate.

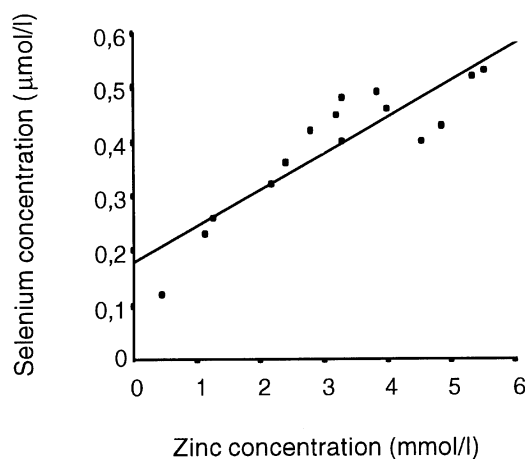
Figure 2 shows the highly significant correlation which was observed between selenium and zinc concentrations in seminal fluid in the semen samples from a random group of the volunteers ( $n = 15$ ). Regression analysis revealed a regression coefficient at 0.067 with 95% confidence interval 0.047 to 0.088,  $R^2 = 0.80$ , and  $P < 0.001$ .

### Discussion

In animals, selenium has been shown to be an essential element for normal male reproductive function. The best-characterized effect of selenium deficiency on mammalian spermatozoa is a loss of motility, breakage at the midpiece level and an increased



**Figure 1.** Relationship between selenium concentration ( $\mu\text{mol/l}$ ) in seminal plasma and sperm count ( $10^6/\text{ml}$ ) in infertile subjects. (Linear regression line,  $y = 0.401 + 0.0014x$ ).



**Figure 2.** Relationship between the concentrations of selenium ( $\mu\text{mol/l}$ ) and zinc ( $\text{mmol/l}$ ) in seminal fluid from a random group of volunteers. (Linear regression line,  $y = 0.176 + 0.0674x$ ).

incidence of sperm-shape abnormalities, mostly of the sperm head (Wallace *et al.*, 1983, Watanabe and Endo, 1991).

In the present study, selenium concentrations in the reproductive organs were found to be generally higher (30% and 15% in the case of testes and prostate tissues, respectively) than those reported for the same organs in a Finnish study (Saaranen *et al.*, 1986). This is in accordance with the blood levels of selenium which also appear to be lower in the Finnish population (see review by Thomassen and Aaseth, 1989), a

discrepancy which may be accounted for by differences in dietary intake.

The highest concentrations of selenium were found in the kidney, which confirms previous studies (Schroeder *et al.*, 1970), and may underscore the organ's capacity for detoxification. Selenium has been reported to exert a protective action against the toxic effects of heavy metals through undefined mechanisms (Wahba *et al.*, 1993). Because selenium has a tendency to form complexes with heavy metals, it has been suggested that this may constitute one of the ways of rendering these metals non-toxic (see review by Diplock *et al.*, 1986). Another protective function of selenium may be related to its antioxidation properties, protecting tissues from damage by free radicals (Sugawara and Sugawara, 1984).

Among the reproductive organs, the testis had the highest selenium concentration, which exceeded that of the liver. This high selenium concentration may imply a protective role of this trace element and its associated enzymes during spermatogenesis. In rats, the selenium concentration in the testis is regulated by a homeostatic mechanism which ensures a priority in the supply of selenium to the male gonads over other tissues (Behne *et al.*, 1982). Furthermore, the selenium requirement of the testis is increased during pubertal maturation, coincident with the beginning of spermatogenesis (Behne *et al.*, 1986). The high rates of mitosis and the various stages of meiosis in the seminiferous tubules may expose germinal cell chromosomes to the potentially damaging influence of free radicals in the local environment, thus creating a need for an effective antioxidant system.

The role of intracellular selenium in spermatozoa is still poorly understood (Iwanier and Zachara, 1995). Selenium appears to be present in spermatozoa (Saaranen *et al.*, 1989) and, during the secondary spermatocyte or early spermatid stage, is incorporated into the spermatozoa—at least in the rat—where selenium is found in the outer membrane of the sperm mitochondria in the form of a specific selenoprotein (Calvin *et al.*, 1987). In humans, in the terminal stages of sperm differentiation, a considerable portion of the sperm cytoplasm is discarded and the remnants are confined to the mid-piece (Aitken, 1994). As a consequence, these cells are not well endowed with the cytoplasmic defensive enzymes that protect most cell types from peroxidative damage. This, combined with the presence of high concentrations of unsaturated fatty acids in the plasma membrane, may therefore render spermatozoa particularly susceptible to oxidative stress (Aitken, 1994). Interestingly, there appears to be considerable variation in the degree of protection offered by components in seminal plasma against free radical damage of spermatozoa (Kovalski *et al.*, 1992).

The biological functions of selenium in mammals appears to be expressed through different biologically active compounds, including glutathione peroxidases (Behne *et al.*, 1996) and other selenoproteins in serum and tissues (Hill *et al.*, 1996). One role of glutathione peroxidase, which is present in both animal and human semen (Saaranen *et al.*, 1989), is to remove hydrogen peroxide and lipid peroxides and thus protect tissues, including spermatozoa, from peroxidative damage (Alvarez and Storey, 1989).

In studies of subfertile men, selenium supplementation raised the selenium concentration and glutathione peroxidase activities in seminal fluid (Iwanier and Zachara, 1995; Vézina *et al.*, 1996) indicating an equilibrium between the blood and seminal compartments. However, previous studies have demonstrated that the selenium concentration in seminal plasma is <50% of serum values (Saaranen *et al.*, 1986), suggesting a relative barrier at the level of the secretory epithelia in the male reproductive organs.

Reports on the relationships between sperm quality and selenium in seminal fluid are inconsistent. Our results are in agreement with those of three other studies (Bleau *et al.*, 1984; Noack-Füller *et al.*, 1993; Xu *et al.*, 1993), which reported that seminal plasma selenium concentrations were significantly and positively correlated with sperm density, though others were unable to show such a relationship (Behne *et al.*, 1988; Roy *et al.*, 1990). An explanation for this positive correlation between sperm count and seminal fluid selenium could be that selenium containing proteins from the outer surface of the spermatozoa become detached during centrifugation. On the other hand, approximately 75–85% of the total ejaculated content of selenium is from the seminal plasma fraction (Bleau *et al.*, 1984; Noack-Füller *et al.*, 1993). Indeed, the high values found in vasectomized men has suggested that most of the semen selenium originates from the accessory sex glands (Bleau *et al.*, 1984). The positive correlation between selenium and zinc concentrations in seminal plasma found in the present study suggests that the prostate may play a significant role in the secretion of selenium as zinc is an acknowledged marker of prostatic secretion—a correlation also shown in a previous study (Behne *et al.*, 1988). It is appropriate that selenium is in the prostate fraction as, during ejaculation, the prostate and epididymal contributions are the first to mix. This would ensure an interaction between selenium and the spermatozoon before it is confronted with the hostile vaginal environment.

The accessory sex glands and epididymides had comparable concentrations of selenium, approximately 50% of the testis level. The epididymis has a concentrating ability due to fluid resorption (Levine and Marsh, 1971), yet it has a selenium concentration less than half that of the testis. If the selenium in the testis was solely associated with sperm cells, a higher level in the epididymis would have been expected because of the accumulation of sperm in the epididymal tubule. It is therefore more likely that selenium exists primarily in the testis not only in spermatozoa, but also in other tissue compartments. Indeed, evidence has been presented for a specific selenium receptor in the rat testis (Gomez and Tappel, 1989), and in a previous study in the bull, selenium in the testis was concentrated in the Sertoli cells (Vanha-Perttula and Remes, 1989).

The significant correlations between the tissue levels of selenium in the reproductive organs suggest that the uptake and/or biochemical activity in these various tissues may be controlled by a similar mechanism. One possibility is that selenium concentration is regulated by androgen-dependent events.

Tissue selenium concentrations could not be correlated with potential occupational exposure to adverse environments. However, it must be emphasized that the allocation of the

subjects to such high- and low-risk groups was based on limited information about their occupations and the groups were limited in numbers of subjects. Future studies must involve the measurement of blood and seminal plasma concentrations of the elements in living subjects whose degree of exposure to industrial environments can be more accurately defined.

In conclusion, the current interest in the potential role of oxidative damage in sperm function has highlighted the importance of natural substances involved in protecting tissues and cells from free radical damage. In this regard, selenium may play a role in conferring protection in the reproductive organs and further studies on this trace element are therefore warranted.

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