

# The effect of oral selenium supplementation on human sperm motility

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**Objectives** To determine whether the decline in selenium intake and selenium status in men in the West of Scotland might be a contributory factor to male subfertility.

**Patients and methods** Two semen samples were collected from patients attending a subfertility clinic and those patients with samples showing reduced motility were invited to participate in an ethically approved double-blind clinically controlled trial with informed consent. Sixty-nine patients were recruited and received either placebo, selenium alone or selenium plus vitamins A, C and E daily for 3 months. A further semen sample was collected at the end of the trial. Plasma selenium status was determined at the beginning and end of the trial period, as was total sperm density and motility.

**Results** Plasma selenium concentrations were significantly ( $P < 0.001$ ) higher in both selenium-treated groups than in controls. No significant effect of treatment on sperm density was recorded. Sperm motility increased in both selenium-treated groups, in contrast to a slight decline in the placebo group, but the

difference was not significant. However, as the provision of additional vitamins had no effect on any variable measured it was considered justified to combine the two selenium-treated groups and compare them with the placebo treatment. On this basis, selenium treatment significantly ( $P < 0.002$ ) increased plasma selenium concentrations and sperm motility ( $P = 0.023$ ) but sperm density was again unaffected. Five men (11%) achieved paternity in the treatment group, in contrast to none in the placebo group.

**Conclusion** This trial confirms the result of an earlier study, that selenium supplementation in subfertile men with low selenium status can improve sperm motility and the chance of successful conception. However, not all patients responded; 56% showed a positive response to treatment. The low selenium status of patients not supplemented again highlights the inadequate provision of this essential element in the Scottish diet.

**Keywords** Sub-fertile men, sperm motility, sperm density, plasma selenium, selenium supplementation

## Introduction

The suggestion that sperm counts are falling in the Western world is topical, but at the same time may be questionable. There are doubts as to the accuracy and significance of many statements relative to this topic [1]. A literature survey suggesting that sperm counts are decreasing indicated that the percentage of individuals with high sperm counts appears to have declined over the past 50 years, while in the same period the number of subjects who have subnormal sperm counts appears to be increasing [2]. It has been suggested that this analysis of the published data might not reflect the true picture and that any apparent fall may well be related to a reduction in the reference values [3].

Infertility may, in married couples, be as high as 15%

in Western society [4]. Factors which are known to cause reduced fertility in men include chemicals with an oestrogenic effect which may enter the food chain. These include polychlorinated biphenyls, chlorinated hydrocarbons and phyto-oestrogens in plants, e.g. soya products [5,6]. It has long been recognized that chemotherapeutic agents such as cyclophosphamide cause low sperm counts and even azoospermia [7]. Not only will chemicals affect sperm production but oestrogens may be responsible for an increase in carcinoma of the testicle and congenital urethral abnormalities such as hypospadias [8]. Little attention has been paid to the potential effects of dietary deficiencies in human male subfertility.

A previous report from the present authors confirmed a reduction in the selenium content of the diet and in the blood concentration in males in the West of Scotland [9]. The administration of selenium to subfertile patients induced a statistically significant rise in sperm motility

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[10]. The present communication reports an extension of the original study in a randomized double-blind and ethically controlled trial when subfertile patients were given selenium in an attempt to improve sperm motility.

In the earlier study on selenium supplementation of subfertile men there was a significant response in sperm motility but there was no effect on sperm count [10]. The original study revealed that supplementation with vitamins A, C and/or E did not significantly influence either variable. The present follow-up study to confirm the validity of these findings commenced in 1994 and continued for 2 years, during which 69 patients were randomly allocated to one of three treatments, i.e. placebo, selenium or selenium plus vitamins in the ratio 1:1:2. This protocol was chosen for two reasons; first, as we had already observed a significant response to selenium it was considered unjustified to withhold the opportunity of selenium supplementation from the maximum number of patients; and second, it was felt that in the light of the well recognized poor intake of vitamins in the Scottish diet, every opportunity should be taken to discover whether the lack of response to vitamin supplementation previously recorded would be confirmed. This was felt to be particularly pertinent for vitamin E, as it is closely linked to selenium metabolism and has also long been claimed to influence mammalian reproduction.

### Patients and methods

Men attending the Combined Subfertility Clinic in Glasgow Royal Infirmary were routinely examined and blood LH, FSH, prolactin and testosterone measured. Each man was requested to produce two semen samples, obtained after a 4-day abstinence, at a convenient time within 6 weeks of the first appointment. On review at the clinic, the patient was interviewed and assessed in the light of the results of the above tests, by the urologist (R.S.) in the team. The gynaecologist (R.Y.) similarly assessed the female partners of the men. Further follow-up, including testicular biopsy and laparoscopy when indicated, was organized at that time.

Those men in whom the major problem of concern was the reduced motility of sperm were then, after a full explanation and informed consent, invited to participate in an ethically approved controlled trial involving selenium (L-selenomethionine 100 µg per day) or selenium combined with vitamins A (1 mg), C (10 mg) and E (15 mg) supplements against a placebo (the groups designated 1, 2 and 3, respectively) As the patients entered the trial they were randomly allocated to one of the three treatments, which had in turn been randomized within each block of four numbers and 'blinded' using a numeric code. The treatments were given as one tablet taken at night for 3 months. Two weeks after completing the

course of tablets, the man was asked to produce a semen sample and 2 weeks later was invited to attend the clinic, when blood selenium was further assessed and the most recent semen analysis considered. Blood samples were obtained in heparinized tubes, centrifuged, the plasma aspirated into vials and stored at  $-20^{\circ}\text{C}$  until analysed for selenium, using a continuous-flow PS Analytical hydride generator and a PS Analytical Excalibur atomic fluorescence spectrophotometer (PS Analytical Ltd, Orpington, Kent, UK). The accuracy of the measurement was verified using standard reference samples.

Sperm counts were determined by diluting a seminal fluid sample 1:10 with seminal diluting fluid (25 g sodium bicarbonate and 5 mL formaldehyde made up to 500 mL with distilled water) and mixing thoroughly. After 10 min some of the diluted sample was transferred to an 'Improved Neubauer' counting chamber and the number of sperm counted under the microscope. Sperm motility was determined by placing one drop of seminal fluid onto a cleaned microscope slide and covering with a clean  $24 \times 24$  mm coverslip. This was then left for 2–3 min to allow the fluid to stabilize before reading under the microscope. The result was taken as the mean of the estimates of two observers. Where a difference between the results was  $>10\%$  then both were repeated. Controls of normal spermatozoa morphology were not assessed with each analysis. The results were assessed using ANOVA on both a between-treatment and within-treatment comparison, the latter to ensure that the effect of between-patient variation was removed.

### Results

Sixty-four men completed the study (mean age 33.3, sd 0.64 years); five did not or would not take the tablets, one of whom had been allocated to group 1 and four to group 2. The effects of the treatments on plasma selenium, sperm count and sperm motility are detailed in Table 1. After treatment, the plasma selenium concentration was significantly higher in groups 1 and 2 ( $P < 0.001$ ) than in group 3. There were no significant differences among the three groups in sperm count, although the treatment in group 1 appeared to cause a 22% increase, in contrast to little or no effect in groups 2 and 3; large individual variations in response prevented the effect being significant. While selenium treatment increased sperm motility in both treated groups (by 40 and 34% for groups 1 and 2, respectively) in contrast to a slight decline in the controls ( $-15\%$ ), the effect was not significant ( $P < 0.068$ ). The inclusion of vitamins in group 2 had no significant effect on any of the variables measured, although it did appear to reduce the increase in plasma selenium and to have had no effect on sperm count, in contrast to the 20% increase in group 1. As

**Table 1** The effect of treatment on mean (SEM) plasma selenium concentration, sperm count and sperm motility in placebo-treated men compared with selenium supplementation only and Se+vitamins, and in placebo compared with the combined selenium treatments

Variable	Placebo (n = 18)	Se only (n = 16)	Se + vitamins (n = 30)	P	All Se-treated (n = 46)	P
Mean age (years)	32.9 (1.5)	32.6 (1.1)	33.9 (0.9)	0.639	33.4 (0.69)	0.646
Plasma Se (µg/L)						
Before	79.5 (4.7)	82.5 (3.3)	80.2 (2.1)	0.832	81.0 (1.8)	0.709
After	69.8 (3.0)	132.3 (14.1)	105.0 (6.3)	0.001	114.1 (6.8)	0.002
Sperm count (millions/mL)						
Before	24.7 (5.8)	39.8 (6.7)	36.6 (5.8)	0.238	36.6 (4.39)	0.275
After	27.5 (10.0)	48.7 (8.8)	34.0 (6.3)	0.243	38.5 (5.22)	0.416
Sperm motility (%)						
Before	18.1 (4.3)	21.6 (4.1)	20.1 (2.6)	0.814	20.6 (2.21)	0.571
After	15.3 (4.1)	30.2 (5.7)	27.0 (3.7)	0.068	28.2 (3.10)	0.023

The numbers of men assessed after treatment for plasma selenium mean values were 7, 7, 14 and 21 for untreated, Se only, Se+vitamins and combined Se and Se+vitamins treatments, respectively.

the inclusion of vitamins in group 2 had no effect, thereby confirming the findings of the earlier study, the results were re-analysed, combining groups 1 and 2, compared with controls (Table 1). Plasma selenium concentration was again significantly increased by selenium treatment ( $P < 0.002$ ) while sperm count was unaffected, as before. There was no difference in pretreatment sperm motility between the groups, but after treatment, that in the Se-supplemented groups was significantly ( $P < 0.023$ ) higher than that of group 3. The data were also analysed on a within-treatment basis to remove the effect of between-treatment variation; the results are presented in Table 2. On this basis, both selenium treatments significantly increased plasma selenium concentrations but had no effect on sperm count, and although again increasing sperm motilities by  $\approx 40\%$ , this remained statistically insignificant. Combining both selenium treatments gave significant increases in plasma

selenium concentration and sperm motility, but not in sperm count. Not all the patients responded to selenium, so the response in sperm motility in the 56% who did was correspondingly greater than the mean. Three patients in group 3 (placebo) did have a positive response in sperm motility, ranging from a doubling in two to a sixfold improvement in the other. No confirmed pregnancies have been reported from partners of those in the placebo group. In group 1 and 2, five men have confirmed paternity (11%).

## Discussion

Selenium is a common and essential constituent of Western diets. Biochemically the metal functions in a variety of seleno-enzymes, e.g. glutathione peroxidase. A specific selenoprotein has been identified in rat sperm [11,12]. Behne *et al.* [12] showed that in induced selenium

**Table 2** The effect of treatment on plasma selenium, sperm count and sperm motility as measured on a within-treatment basis by comparing values of each variable before and after treatment

Variable	Placebo	SEM	P	Se only	SEM	P	Se + vit	SEM	P	All Se-treated	SEM	P
Plasma Se (µg/L)												
Number	7			6			14			20		
Before	77.7	4.08	0.346	83.5	8.86	0.015	80.4	3.40	0.001	81.4	3.69	<0.001
After	69.8			133.3			105.1			113.6		
Sperm count (millions/mL)												
Number	17			15			26			41		
Before	25.9	5.85	0.887	39.8	5.78	0.446	37.3	4.52	0.716	38.2	3.54	0.871
After	27.5			48.7			34.0			39.4		
Sperm motility (%)												
Number	17			15			26			41		
Before	18.1	3.16	0.673	21.1	3.61	0.209	19.5	2.32	0.108	20.1	1.94	0.039
After	15.3			30.2			27.0			28.2		

deficiency in rats, supplementation with the element resulted in the selenium being selectively incorporated into the sperm selenoprotein at the expense of other selenoenzyme systems. Vitamin E (like selenium) is recognized as being an antioxidant and over the years there have been innumerable reports advocating its use in the treatment of subfertile men. However, the results have been equivocal and vitamin E therapy has not become established as a dependable method of treating male subfertility in general, and more specifically as a method of coping with reduced sperm motility. Recent evidence suggests that supplementation with vitamin E in much higher doses than used in this trial may prove more effective [13,14].

In agricultural practice, selenium is widely used where there is a natural deficiency of the element in soils; selenium is not applied to pastures to improve the quantity or the quality of the crop, but as an adjunctive route whereby animals such as sheep or cattle may increase their normal intake of the element. Another method of increasing the intake of selenium by animals is direct addition to their diet in animal mineral mixes. It can also be administered orally as a bolus, or parenterally. Such additions have prevented outbreaks of white-muscle disease in sheep and cattle, hepatosis dietetica in pigs and exudative diathesis in poultry.

Apart from the general conditions mentioned, selenium is associated with reduced or impaired reproduction throughout the animal kingdom [15]. In 1978, the mean dietary intake of selenium in the UK was 60 µg per day and half of this intake was derived from cereal sources, mainly North American and predominantly Canadian wheat. The mean intake from such sources of 60 µg per day was just below the recommended daily allowance (RDA), at that time 70 µg per day for men. The change to Europe as the main source of cereals for bread making has been shown to have been accompanied by a steady decline in the dietary intake of selenium, from 60 µg per day in 1978, to 43 µg daily in 1985 and 30 µg daily in 1990 [9].

Most population studies of subfertile men have tended to concentrate on possible toxic factors in the diet, environment, or workplace as being potential causative factors. It has been suggested that smoking and an increased coffee intake, along with unspecified environmental factors, may be important in causing azoospermia [16]. In a 20-year study of subfertile men in Paris, Auger *et al.* [17] commented that there had been a decline in sperm concentration and motility, with a decline in fertility, which paralleled an increase in testicular cancer. These authors postulated changes in diet and life-style, and implied that water, or some other environmental factor, may have been creating a pollution problem.

Benvold [18] noted a statistically significant deterioration in sperm morphology in a 20-year study in Norway

but did not comment about changes in motility or possible causative factors. Gold *et al.* [19] commented that 20 million workers in the USA were exposed to a variety of toxic elements which were not necessarily reproductive toxins. However, they also pointed out that >90% of 104 000 chemical/physical agents used commonly in the workplace had not been tested for their effects on the reproductive system. There are 400 known chemicals which have been tested and shown to have an effect on reproduction. The only environmental factors postulated as producing toxic effects are the heavy metals, including lead, cadmium, arsenic and mercury [20]. These authors also indicated a range of agricultural chemicals as potential toxic agents.

Abou-Shakra *et al.* [21] reported that in four different populations there was no significant difference in the levels of selenium in seminal plasma. In view of this finding, these authors suggested that trace elements, including selenium, will, if they influence fertility status, produce their effects on individual sperm. An apparent correlation between the selenium content of seminal plasma and sperm density [22] confirms a similar observation that the selenium level in a whole semen sample parallels the sperm count [23].

Bedwal and Bahugana [24] showed that total selenium levels increased in the gonad during the maturation of male gonads and the metal appeared to be situated in the mitochondrial capsule in the mid-piece of the sperm. Sperm motility appeared to be greatest when the selenium level in semen was 50–69 ng/mL [25]; when the selenium level is <36 ng/mL it has been shown that there is likely to be male subfertility. The linkage between fertility status and selenium levels in semen and fluid indicates that 40–70 ng/mL would appear to be the optimal range to give maximum fertility. Above such levels, i.e. >80 ng/mL, there is reported to be an increased likelihood of abortion/miscarriage.

There are good reasons, based on the presence of a sperm-specific selenoprotein, to suspect that selenium deficiency may be a factor in reducing reproductive capability in humans. The present authors have indicated that blood selenium levels in Western Scotland have been steadily declining since 1985. In this prospective double-blind study of subfertile men comparing selenium supplementation with a placebo, there was a statistically significant increase in sperm motility 3 months after the initiation of treatment in those treated with selenium. In the original report [10], we further confirmed the potential value of selenium medication in that after the withdrawal of the element in those subjects who had initially received it, sperm motility returned to pretreatment levels.

The present study confirmed a significant improvement in sperm motility in a larger sample of men taking

selenium supplements. The observed increase in plasma selenium to levels within the normal range (i.e. >90 µg/L [26,27]) in those taking a selenium supplement is in marked contrast to the subnormal selenium levels in the control group, which further highlights the inadequate provision of selenium in the normal diet in this area. Interestingly, five of those receiving the supplement within the sample have now successfully produced a pregnancy.

In the light of the recent report [28] of early miscarriage in women with a low selenium status in Wales, it is possible that a higher pregnancy rate would have been achieved if both partners had been taking supplements. The optimum duration and level of selenium therapy which will ensure a rapid and sustained improvement in the motility of sperm remains to be determined.

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