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Research Article

# Effects of Zinc Sulfate on Subfertility Related to Male Factors: A Prospective Double-Blind, Randomized, Placebo-Controlled Clinical Trial

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## Abstract

**Background:** Different studies found that zinc is necessary for sexual maturity, growth and fertility. But there are no distinct studies that clarify the role of zinc supplements on semen parameters.
**Objectives:** The current study aimed to evaluate the zinc supplement therapeutic effects on semen samples of infertile males.
**Patients and Methods:** The study comprised one-hundred-twenty sub fertile males. The study was a double-blinded placebo-controlled clinical trial. The subjects were randomly allocated to treatment with zinc supplement (n = 60) or placebo (n = 60) groups. Subjects in both groups were given 10 mL, three times daily. In order to determine the sperm concentration, Motility and morphology, standardized semen and blood samples were obtained before and after treatment, according to the World Health Organization (WHO) guidelines; semen morphology according to strict criteria, and blood and semen zinc concentration also were measured. Effects of the two interventions were evaluated in sub fertile males.
**Results:** Sub fertile males demonstrated a significant increase ($8.8 \pm 7.4 \times 10^6$ cells/mL to $17.2 \pm 13.5 \times 10^6$ cells/mL) in concentration and normal sperm in zinc group versus the placebo group. Blood serum zinc concentration increased in the interventional group significantly (P = 0.000), and also semen plasma zinc concentration increased significantly (P = 0.000).
**Conclusions:** Normal sperm percentage and total sperm concentration increased after zinc sulfate treatment. The beneficial effect of zinc and all results of the current study opened new way to medical purposes and public health researches.

*Keywords:* Intervention, Zinc Sulfate, Semen Parameters, Male Fertility

## 1. Background

One of the concerns of society and especially physicians is infertile couples (1-4). In the recent two decades, the perception of male reproduction function and the importance of male factors in infertility have significantly developed (5, 6). In the past, the main focus was on females and male factors which were considered as fairly common factors in infertility (7). These days, it is known that the exclusive reason of 20% of infertilities is because of male disorders, and in 20 - 40% of other couples who fail to reproduce, male factors are the important confounding (8).

Different studies found that zinc is necessary for sexual maturity, growth and fertility (8). Therefore, zinc deficiency had significant effect on male infertility (9). Other studies showed that zinc is also very effective on ejaculation semen volume (10). However, some studies showed that high rate of zinc interferes the stimulation of sperm and sperm receptor (11). Of course, it is not clear whether zinc deficiency is a risk factor for male factor sub fertility or not (7).

## 2. Objectives

The current study aimed to evaluate the zinc supplement therapeutic effects on semen samples of infertile males.

## 3. Patients and Methods

### 3.1. Protocol

From March 2015 to November 2015, one-hundred-twenty sub fertile males were selected to participate in the current study, based on the used protocol (12). The study was conducted as a prospective double-blind clinical trial, included infertile couples who referred to infertility center of Akbarabadi hospital and the sperm rates in their spermogram samples were 5 - 20 million cells/mL, and according to failure of female to conceive after one year regular and unprotected intercourse, male partner was regarded as sub fertile. After selecting males for the study, result of physical examination, sperm concentration and complete endocrine screening were part of the selection criteria. Males who had chromatically fertility disorder (Y chromosome deletions), were excluded and males with idiopathic sub fertility were asked to join the study. Sperm concentration was selected as a basic selection criterion since it is highly correlated with Motility and morphology, and it mostly predicts the fertility.

Before semen analysis, a questionnaire was distributed to obtain information on smoking habits, alcohol use, use or abuse of other substances and drugs, and a history of orchitis, testicular trauma, sexually transmitted diseases, varicocele, surgery for inguinal hernia and cryptorchidism. Exclusion criteria were use of zinc three months before recruitment.

The institutional review board and the medical ethical committee of the Iran University of Medical Science approved the study protocol (IR.IUMS.REC.1394.26155), and all subjects gave written informed consent before participation.

Before and three months after the study, entries prepared acceptable semen samples obtained by the subjects in containers; all samples were delivered to the laboratory within hours after production. The samples should be obtained by masturbation.

Then, semen samples were put in temperature room for analysis for 30 minutes. By microscopic examination, sperm count, percentage of motile sperm and sperm with normal morphology were objectively evaluated. The evaluation of sperm count and percentage of motile sperm was according to the guidelines of the World Health Organization (WHO) (13). Sperm morphology was evaluated according to the criteria offered by Kruger (14).

Semen samples were centrifuged at 600 g for 10 minutes. After centrifugation, deionized water diluted 10-fold supernatants. Atomic absorption spectroscopy measured the zinc level (Perkin Elmer model 2380). Following that, the rate of zinc in blood and semen in these patients was measured. In the current study males were divided into groups A and B by block randomized sampling. Group A were treated by a solution of 0.5% zinc, 30 mL, three times daily in separated doses, and group B received placebo syrup. Containers of zinc solution and placebo were similar, and all of them had zinc syrup label. Zinc solution and placebo solution were put in the same box separated with A and B labels. The secretary of infertility unit did not know about the box content and patients by showing their groups label could receive the medicine.

At the time of semen sampling, overnight fasting venous blood samples were drawn for measurement of zinc and follicle-stimulating hormone (FSH). Radio assays were used to measure zinc seminal plasma samples (dual count solid phase boil radioassay, DIAGNOSTIC Products Corporation, Los Angeles, CA). Zinc was measured by flame atomic absorption spectrophotometry. Serum testosterone was measured as described elsewhere (15).

After first and second months of intervention, subjects were called to know about the side effects which might happen during this time.

To create similar groups, sub fertile males were assigned according to a simple computer schedule into two groups to receive zinc sulfate or placebo.

Solutions were coded from 1 to 120 according to the randomization list by hospital pharmacy.

Each code was given to one participant to receive one container of solution that according to their group called participates took zinc sulfate (0.5) or placebo. Participants were asked to take 10 mL of the bottle three times daily for three months.

After three months investigation on blood and semen and sperm samples was again repeated. Patients' dissatisfaction for inclusion and exclusion from the project was the executive limitation which was acted out by providing a complete description, in order to solve the problem.

### 3.2. Statistical Analysis

In this study, subjects were briefed how to do. In the case of cancelation by a person, all treatment steps were done for him and no action and cost out of therapy was forced. All of the data about the patients were protected by the researcher. All stages of the study were based on the principles of Helsinki convention and the study was approved by ethics committee of Iran University of Medical Sciences, Tehran, Iran.

## 4. Results

One-hundred-twenty sub fertile males were enrolled in the trial; seven subjects in the zinc group withdrew because of adverse gastrointestinal side effects, and three subjects in the zinc group and four subjects in the placebo group withdrew because of lack of motivation (Figure 1).

Table 1 shows the baseline characteristics of the two groups after randomization. At baseline, there was no difference between the two groups. Pre-intervention concentrations of zinc in blood and seminal plasma were similar

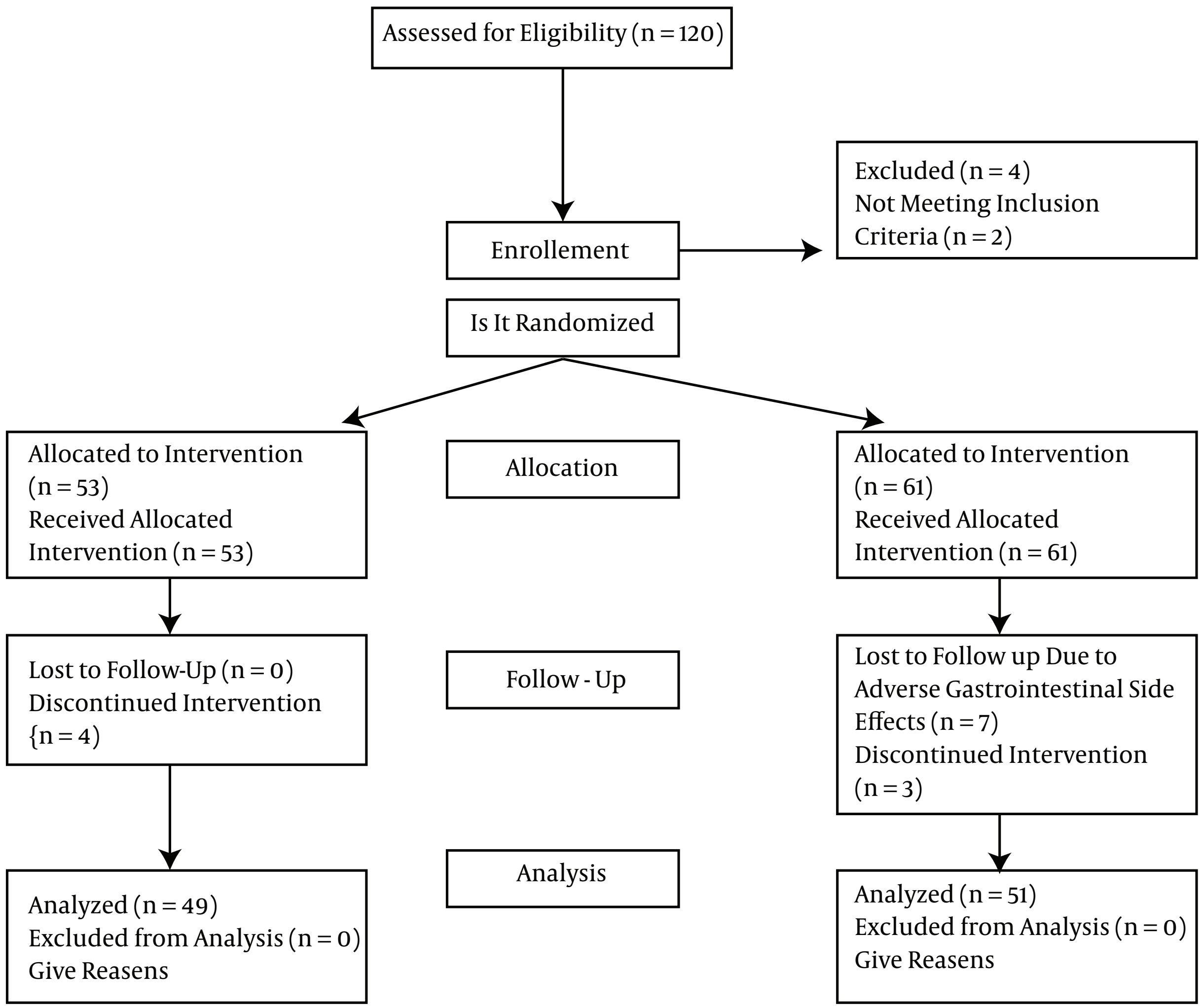


**Figure 1.** Flowchart of the Study

and within the lower normal limit ranges in both groups (Tables 1 and 2).

After 12 weeks of intervention by zinc and placebo, the median sperm concentration significantly increased from $8.8 \pm 7.4 \times 10^6$ cells/mL to $17.2 \pm 13.5 \times 10^6$ cells/mL in the zinc group ($P > 0.05$) (Table 3).

Sperm normal morphology increased significantly (P=0.008) but abnormal morphology decreased slightly and not significantly (P >0.05) (Table 3).

Blood serum zinc concentration increased significantly in the interventional group ($62.4 \pm 17.2$ microg/dL vs. $81.7 \pm 16.8$ microg/dL; P = 0.000), semen plasma zinc concentration increased significantly ($14410 \pm 9460$ mg/dL vs. $1990 \pm 9370$ mg/dL; P = 0.000), both of the parameters increased in the placebo group slightly with no significance.

## 5. Discussion

Throughout the last decades, studies over the cognition of male fertility and male factors influencing infertility advanced progressively.

Most of male infertility factors are reversible by drug therapy to provide a normal fertilization.

An abundant number of evidence demonstrate that zinc level in human semen plays an important role in sperm physiological functions as well as decrement in sperm quality and increment of fertilization probability.

In the current study, there was a significant increase in sperm concentrations in sub fertile males received zinc

**Table 1.** Baseline Characteristics

| Value | Zinc (n = 51) Pre- Intervention (SD) | Placebo(n = 49) Pre-Intervention (SD) | P Value |
|---|---|---|---|
| **Age, (year)** | 32.3±6.2 | 32.2±5.9 | 0.457 |
| **FSH** | 5.7±1.9 | 6.3±1.9 | 0.091 |
| **Abstinence period(day)** | 3.3±1.4 | 3.6±1.3 | 0.348 |
| **Sperm concentration ($\times 10^6$)** | 8.8 ± 7.4 | 8.7±7.3 | 0.926 |
| **Motility (%)** | 24.2±13.4 | 24.3±13.4 | 0.927 |
| **Morphology normal, No. (%)** | 2.1±1.5 | 2.1±1.4 | 0.996 |
| **Morphology abnormal, No. (%)** | 57.5±11.1 | 61.1±11.5 | 0.117 |

Abbreviation: FSH, follicle-stimulating hormone.

sulfate (30 mg) for 12 weeks. Zinc stabilizes cell membrane and sperm nuclear chromatin in seminal plasma.

This observation suggests a useful effect on the quantitative aspect of spermogenasis. However, the improvement of the result was not mainly expected. Nonrandomized controlled studies showed that in sub fertile males with idiopathic oligozoospermia; oral zinc supplementation improves sperm concentration (16, 17). However, Landau et al. (18) presented that using 10 mg supplementation of folic acid daily for one month had no advantage on sperm concentration in 40 normospermic and oligozoospermic males.

According to in vivo and in vitro studies, zinc deficiency changes the absorption and metabolism of dietary folate in animal (19-21). Folic acid and zinc are essential to transfer RNA and DNA synthesis, but the underlying mechanisms by which these micronutrients affect spermatogenesis are not known (22).

In contrast to enormous attention to male infertility, zinc concentrations in blood and seminal plasma were investigated in a small number of studies. In the current study, the blood and semen zinc concentrations were not compared between fertile and sub fertile males; Chia et al. (23) found that seminal plasma zinc concentrations differed significantly between fertile and sub fertile males. However, the study showed that Zinc administration affected the zinc group. This may be explained by zinc deficiency.

On the contrary, Wong et al. (24) displayed that zinc administration leads to no increment in blood and seminal plasma. It was described by unresponsiveness of blood physiologic zinc concentration to zinc consumption. And also, prostate as the main source of zinc excretion with high concentrations might prevent significant rise in the levels of zinc in seminal plasma substantially to zinc administration.

As a general rule, better effect can be observed on absorption, transport, and metabolic processes. Therefore, it was hypothesized that by lower doses of sulfate more beneficial effects can be achieved (25, 26).

Wong et al. (24) studied the effect of both zinc and folic acid supplement on semen variables in fertile and sub fertile males. They showed the 74% increase in total normal sperm count and in abnormal spermatozoa, it increased 4%. A similar attitude was observed in fertile males. There is no significant difference between fertility and subfertility about folate and zinc in blood and seminal plasma in pre-intervention.

Another study showed that oxidative damage was made by zinc that is a reason to make poor sperm. Therefore, measuring the seminal Zn of sub fertile or idiopathic infertile males is necessary and can be helpful in fertility assessment. Fertile males showed significantly higher seminal Zn levels than that of any infertile group (P= 0.001) (27).

On the other hand, using one semen sample to diagnose the fertile from sub fertile males may have bias. It is expected that this confounder could affect each group in the same rate; therefore, the effects may be larger than calculated by the current findings.

The above matter was further evaluated by reclassifying males according to their baseline sperm concentration. The data clearly showed stronger effects on sperm concentration in the zinc group (from $8.8 \pm 7.4 \times 10^6$ cells/mL to $17.2 \pm 13.5 \times 10^6$ cells/mL).

Total amount of zinc in human semen is high and it is essential for spermatogenesis; nevertheless, there is a controversy in the amount of zinc impression on semen and the sperm quality.

Some studies elaborated non-significant differences between the content of zinc in fertile and infertile males. Nonetheless, Chia et al. witnessed a significant discrepancy (23).

Some studies reported a relationship between high level of zinc and increase in sperm parameter, including: the numbers, mobility ad morphology.

According to Ashok et al. (28) semen zinc level had a significant effect over sperm morphology. Zanaty et al. (29), showed a direct relationship between viscosity of semen and semen zinc level. Weak sperm mobility and production was related to lower level of zinc in semen plasma in infertile males (30). As various relevancies were shown in different investigations, a number of studies did not find any relationship between semen plasma zinc level and

**Table 2.** Pre-Intervention and Post-Intervention of Zinc Concentration in Blood and Seminal Plasma of Sub Fertile Males

| Value | Zinc (51) | | | Placebo (49) | | |
|---|---|---|---|---|---|---|
| | Pre-Intervention (SD) | Post-Intervention (SD) | P Value | Pre-Intervention (SD) | Post-Intervention (SD) | P Value |
| Plasma zinc concentration, microg/ dL | 62.4 ± 17.2 | 81.7 ± 16.8 | 0.000 | 67.7 ± 16.8 | 68.3 ± 16.5 | 0.345 |
| Semen zinc concentration, mg/dL | 14410 ± 9460 | 1990 ± 9370 | 0.000 | 12650 ± 8590 | 12830 ± 8710 | 0.164 |

**Table 3.** Semen Quality in the Zinc and Placebo Groups

| Value | Zinc (51) | | | Placebo(49) | | |
|---|---|---|---|---|---|---|
| | Pre-intervention (SD) | Post-intervention (SD) | P Value | Pre-intervention (SD) | Post-intervention (SD) | P Value |
| Sperm concentration ($\times 10^6$) | 8.8 ± 7.4 | 17.2 ± 13.5 | 0.000 | 8.7 ± 7.3 | 9.8 ± 8.9 | 0.149 |
| Motility (%) | 24.2 ± 13.4 | 25.5 ± 11.1 | 0.129 | 24.3 ± 13.4 | 24.7 ± 12.5 | 0.609 |
| Morphology Normal, No. (%) | 2.1 ± 1.5 | 2.3 ± 1.4 | 0.008 | 2.1 ± 1.4 | 2.0 ± 1.3 | 0.659 |
| Morphology abnormal, No. (%) | 57.5 ± 11.1 | 56.8 ± 10.8 | 0.182 | 61.1 ± 11.5 | 67.6 ± 16.8 | 0.234 |

sperm quality.

Multifactorial disorders are intervened in male subfertility. The current study showed the importance of zinc in spermatogenesis. Nutritional factors can be changed by increasing intake, but not genetic factors. Therefore, there was a high rate of pregnancy after administrating zinc, because of improvement in sperm concentration. Before a wide-scale implementation of combined zinc administration, it was recommend conducting a larger randomized, placebo-controlled study on the efficacy and safety of these nutraceuticals. Nevertheless, the current study findings suggested new doors of future fertility research and treatment.

Evidently, regarding non-established results in this field, it is assumed that a larger sample size with more carful assessment and longer patient follow-up in multiple centers could lead to more accurate results. Semen analyses should be precisely explained because data may have biases by intra-individual biological fluctuations in semen variables, limitations and inaccuracies of the methods used and intra-observer mutability.

## Footnotes

**Authors' Contribution:** Study concept and design: Fatemeh Sharifzadeh and Sara Norouzi; acquisition of data: Fatemeh Sharifzadeh, Sara Norouzi and Mahnaz Ashrafi; analysis and interpretation of data: Sara Norouzi; drafting of the manuscript: Sara Norouzi; critical revision of the manuscript for important intellectual content: Soheila Aminimoghaddam; statistical analysis: Jalil Joohpayezadeh; administrative, technical and material support: Sara Norouzi; study supervision: Fatemeh Sharifzadeh.

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