Impact of zinc supplementation on male infertility: a systematic review

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Abstract

Introduction: According to the World Health Organization (WHO), infertility is defined as a failure in fertilization without any use of a contraceptive method for at least 12 consecutive months, with the individual being of reproductive age and having an active sexual life, where male infertility has been assuming a prominent position. This condition is considered to be of high importance in the contemporary world. Objectives: To systematically review the literature on the impact of oral zinc supplementation on male infertility and to assess whether its intake exerts a therapeutic effect, based on the evaluation of seminal parameters, according to manuals published by the WHO. Methodology: A systematic review was carried out by means of a search, in the English and Portuguese languages, for original articles in the Pubmed, Science Direct, Lilacs and Scielo scientific databases. Results: The intervention groups ranged from 18 to 77 individuals and the control groups ranged from 8 to 113 individuals. Regarding age, the average age of the participants in the studies was 31 years. The daily dose of zinc administered in the trials varied from 66mg to 500mg and its use time ranged from three to six months. In the end, 87.5% of the studies showed positive results on the sperm parameters evaluated after the intervention. Conclusion: The results were mainly attributed to the protective effect that the trace element provided, reducing the lipid peroxidation index of plasma membranes and improving DNA integrity, thus increasing the fertilization capacity.

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Introduction

Most couples demonstrate a desire to have children, especially with the passing of the years and the achievement of maturity, when it becomes one of the most prevalent desires in adult individuals. However, not every couple can spontaneously achieve pregnancy and some will require treatment to reach this goal. Different approaches will be necessary, depending if the case is one of infertility or sterility.[1]

According to the World Health Organization (WHO),[2] infertility is defined as a failure to achieve fertilization without use of any contraceptive method for at least 12 consecutive months, when the individual is of reproductive age and has an active sex life.

The terms infertility and sterility are often confused and interpreted as being equal. Infertility is characterized as a situation that can be reversible, where individuals who are in this state can become fertile again. In contrast, sterility is a permanent condition.[3]

According to Bhongade,[4] male infertility has been assuming an increasingly prominent position, since it is already considered of high importance in the contemporary world and involves psychological aspects that need to be taken into account.[4]

A male factor check is obligatorily carried out during the first visit due to its usually simple, non-invasive and relatively low-cost nature. Findings in the literature note that, in general, one in every six couples has difficulties in having children and that male infertility is a primary or associated cause in half of these cases. This explains why the evaluation of the male factor is mandatory and should consist of a clinical history, physical examination, sperm analysis and endocrine profile.[5]

After performing the spermogram test, different results regarding the seminal parameters are obtained. A specific nomenclature exists for each situation, which only classifies the quality of the semen and does not suggest any biological etiology.[6] These terms are used to describe samples with values outside the reference range and therefore possibly coming from a different population. Much of semen classification refers to a single parameter; however, normozoospermia refers to three sperm parameters considered normal: count, motility, and morphology. Thus, deviations from the reference interval for each parameter can be described individually (Table 1).[6]

One of the factors that can jeopardize the quality of spermatozoa, and consequently cause changes in the spermogram, is the accumulation of free radicals (reactive oxygen species - ROS). These are molecules derived from highly reactive oxygen that are characterized by the presence of unpaired electrons in the valence shell. They play an important role in homeostasis and cell signaling, and are produced in small amounts by sperm cells. Free radicals provide beneficial functional effects, ranging from sperm capacitation and regulation of the maturation state to the increase of cell signaling pathways.[8]

However, high levels of ROS can exert negative effects on sperm function, resulting in male infertility. Due to increased DNA damage and lipid peroxidation, these effects are visible in the presence of exaggerated levels of free radicals in seminal plasma.[9] However, free radicals are counterbalanced by antioxidants that help maintain homeostasis at the ideal reduction potential for adequate sperm function.[10]

In addition, there is the seminal fluid, which is a compound rich in antioxidant substrates that nourish and protect the sperm, arranged in enzymatic and non-enzymatic antioxidant systems. The enzymatic system is composed of natural sperm enzymes and originates from the prostate (superoxide dismutase, glutathione peroxidase and catalase), unlike the non-enzymatic system which is composed of several components that originate in food.[11,12]

Table 1. Nomenclature of seminal parameters and their definitions

Term	Description
Aspermia	Without semen (no or retrograde ejaculation)
Asthenozoospermia	Percentage of spermatozoa with progressive motility below the lower reference limit
Asthenoteratozoospermia	Percentages of progressively motile and morphologically normal sperm below lowest thresholds
Azoospermia	No spermatozoa in the ejaculate (given as a limit of quantification for the valuation method employed)
Cryptozoospermia	Sperm absent in fresh preparations but observed in centrifuged pellet
Hemospermia	Presence of erythrocytes in the ejaculate
Leucospermia	Presence of leukocytes above the threshold value in the ejaculate
Necrozoospermia	Low percentage of live sperm and high percentage of immobile sperm in the ejaculate
Normozoospermia	Total number of sperm and % of sperm progressively mobile and morphologically normal, the same or above lower reference limits
Oligoasthenozoospermia	Total number of sperm and % of sperm progressively moving below the lower reference limits
Oligoasthenoteratozoospermia	Reported total number of sperm and % of both progressively motile and morphologically normal sperm below reference limits lower
Oligoteratozoospermia	Total number of spermatozoa and % of morphologically normal spermatozoa below the lower reference limits
Oligozoospermia	Total number of sperm below lower reference limits
Teratozoospermia	% of morphologically normal sperm below lower reference limits

Source: Nomenclature related to semen quality, WHO Manual.[7]

In a situation where the amounts of ROS produced increase excessively, or where the antioxidant function fails, the balance between oxidation and reduction is disrupted, resulting in oxidative stress. Therefore, sperm cells contain very low levels of enzymatic antioxidants, which are insufficient to achieve protection against high concentrations of ROS.[13]

In recent decades, several advances have occurred in the area of male infertility, such as sperm function tests, analyses of oxidative stress and sperm DNA fragmentation, providing a better understanding of the true male reproductive potential.[14]

Oxidative stress is still considered an essential factor that can exert considerable influence over the reproductive outcome. Finding its cause and treating it by reducing ROS and/or therapy with antioxidants is an interesting strategy for a possible reversal of this condition. However, no clear consensus has been reached on the actual effectiveness of this possible therapy.[15]

Although ROS are necessary for the normal physiological function of sperm, out-of-balance oxidative stress can cause increased susceptibility to DNA damage, potentially leading not only to infertility, but also to miscarriage or genetically inherited mutations that cause deficiencies.[16]

Therefore, zinc is essential for testicular steroidogenesis, testicular development, synthesis and secretion of luteinizing and follicle-stimulating hormones, testosterone synthesis, gonadal differentiation, sperm formation and maturation, acrosomal reaction, acrosin activity and fertilization.[17,18]

Zinc's function is vital to reproductive potential. It is believed that seminal zinc derives almost exclusively from prostatic secretions. Motility and structural integrity of spermatozoa are significantly influenced by the mineral since zinc deficiency has been associated with male sterility and subfertility. It is one of the most important antioxidant elements in seminal plasma fluid and appears to protect sperm from bacteria and chromosomal damage.[19]

This study aims to systematically review the literature on the impact of oral zinc supplementation on male infertility, in order to assess whether zinc intake exerts a therapeutic effect based on the evaluation of seminal parameters.

Methodology

Searches for articles were carried out in two bibliographic databases, namely: PubMed (National Library of Medicine) and Science Direct, and duplicate references were excluded. The descriptors used comprised combinations in English of: "supplementation", "effects", "male infertility", "zinc sulfate", and "seminal parameters". During the search, some filters were applied, such as research article, male, in addition to the or/and operators for combining the terms used.

This review included research articles published in English on interventions with oral supplementation of zinc sulfate in infertile men proven through collection from spermogram and analysis of seminal parameters. No time limit was established regarding the year of publication of the articles, onlyoriginal studies, both cross-sectional and longitudinal, were included. As exclusion criteria, review articles as well as animal and in vitro studies were not considered. Studies that did not apply zinc or use the analysis of seminal parameters as a method to evaluate results according to the manual for collection, processing, and analysis of human semen by the World Health Organization were also excluded from this review.

The selection of articles initially involved an analysis of the titles and abstracts of the works. Studies not excluded during this phase were read in full, so that they could be fully evaluated as to their fulfillment of the eligibility criteria. After this procedure, eight studies, published from 1998 to 2014, were selected for the present review.

Results

Table 2 presents data collected from the eight articles included in the review, which were published from 1998 to 2014. The studies were carried out in different countries, 37.5% of them in the Netherlands,[20,23,24], 25% in Iraq,[25,26] 25% in Kuwait[11,22] and 12.5% in Iran,[21] and examined groups of patients attending infertility clinics. Of the eight, seven are transverse and one is longitudinal.

All of studies followed the precepts of the Human Semen Collection, Processing and Analysis Manual published by the World Health Organization, although they differ with regard to the version of the manual used. Of the total, 50% of the studies used the 1992 version,[20,22-24] 37.5% used the 1999 version[11,21,26] and 12.5% used the 2010 version.[25] All the authors used atomic absorption spectrophotometry to determine the amount of seminal zinc.

A food frequency questionnaire for assessing the approximate intake of dietary zinc was not applied to research participants, except in the case of Wong and colleagues,[20] who used the determination of foods rich in zinc as a criterion for the exclusion of participants. The other works did not report the use of this criterion as a methodology.

The target audience consisted of fertile and infertile men. The intervention sample groups ranged from 18 to 77 individuals, and the control groups ranged from 8 to 113 individuals. The average age of the participants in the studies was 31 years. All the studies began by carrying out a survey of the pathological conditions of the participants in the infertility clinics, seeking to select men with a proven infertility factor, through the evaluation of sperm parameters, who were in a position to gain significant benefits from the intervention. The studies excluded those already on medication or supplementation with antioxidants, patients with endocrinopathies and varicocele, alcoholics, men with infertile partners and smokers (with the exception of Raigani and colleagues,[21] who included fertile and infertile smokers in their sample). In those studies that also used fertile men as a sample, the selection was based on proven fathers or those with a pregnant partner and diagnosis of normozoospernia by a spermogram test.

The daily dosage of zinc sulfate administered between the studies ranged from 66mg to 500mg and use time ranged from three to six months.

Table 2. Summary of the main results obtained after the intervention with oral zinc sulfate supplementation

Author, year and country	Sample group	Daily dose of Zn and period	P value	Results of sperm parameters
Omu et al., 1998[22] (Kuwait)	n= 97 (I: 49 e C: 48)	500 mg 3 months	P<0,05	Positive effects (↑count and ↑ motility)
Wong et al., 2002[20] (Netherlands)	n= 71 (I: 23 e C: 48)	66 mg 6 months	P<0,05	Positive effects (↑count)
Ebisch et al., 2003[23] (Netherlands)	n= 190 (I: 77 e C: 113)	66 mg 6 months	P>0,05	No significant changes
Ebisch et al., 2006[24] (Netherlands)	n= 40 (I: 18 e C: 22)	132 mg 6 months	P<0,05	Positive effects (↑count)
Omu et al., 2008[11] (Kuwait)	n= 45 (I: 11 e C: 8)	400 mg 3 months	P<0,05	Positive effects (↑ motility, ↑ PM integrity, ↓ DNA fragmentation)
Hadwan et al., 2012[25] (Iraq)	n= 74 (I: 37 e C: 37)	440 mg 3 months	P<0,05	Positive effects (↑ motility and ↑ morphology)
Raigani et al., 2014[21] (Iran)	n= 42 (I: 24 e C:18)	440 mg 4 months	P= 0,05	Positive effects (↑ chromatin integrity)
Hadwan et al., 2014[26] (Iraq)	n= 120 I: 60 e C: 60	440 mg 3 months	P<0,05	Positive effects (↑count, ↑ motility and ↑ morphology)

Legend: *I: intervention, *C: control, *NR: not reported, *Zn: zinc, *PM: plasma membrane.

The study by Omu and colleagues[22] used medical records and tests to select 100 infertile patients with a diagnosis of proven asthenozoospermia based on the criterion of impaired total motility of at least 40% . The patients belonged to the Kuwait Maternity Andrology Clinic and were randomly divided into two groups, half of which took zinc supplementation of two capsules a day of 250mg each for three months, while the other half served as a control. At the end of the period, semen samples were collected again and compared with the control group. The result of zinc therapy significantly improved sperm parameters in relation to count and progressive motility. In addition, 11 pregnancies were recorded in the intervention group.

The participants in the study by Wong and colleagues[20] were 108 fertile individuals and 203 subfertile individuals recruited from two infertility clinics in the Netherlands. They were carefully selected and randomly distributed into eight groups, of which six were intervention and two control. Two of the intervention groups (one composed of infertile individuals and the other of fertile ones) received a zinc supplement, containing a daily dose of 66mg, for six months. At the end of the period, they were evaluated again and their results were compared with the base values recorded at the beginning. The result was that the sperm count increased significantly in the infertile group, while the count increased slightly in the fertile group but not in a statistically significant way.

The study by Ebisch and colleagues[23] examined 113 fertile individuals and 77 subfertile individuals with polymorphism in the gene of the enzyme methylenetetrahydrofolate reductase (MTHFR). They were patients from two infertility clinics in the Netherlands and were randomly assigned to four groups, in which the zinc sulfate group received 66mg daily for six months. Other groups received placebos or folic acid (5mg) combined with zinc. In the final evaluation, after the intervention, the samples were collected and analyzed. The results showed that the intervention group that received only zinc did not present statistically significant differences.

In the next study by Ebisch and colleagues,[24] 49 fertile men and 40 with idiopathic infertility were recruited from the same sites as in the previous study and were randomized into four groups, where two (fertile and infertile) groups received a dose of 132 mg of zinc sulfate for six months, while placebos were applied to the other two groups. After the study period, the men were reassessed based on their seminal parameters, which showed a significant increase in the count. Other sperm parameters were not evaluated in this study.

In the study by Omu and colleagues,[11] 45 men with asthenozoospermia and normal sperm concentration were recruited from the same infertility clinic as the previous study in Kuwait. After randomization, the participants were split into four groups, namely: group A with zinc only (n=11) 200mg twice a day; group B (n=12) zinc 200mg + vitamin E 10mg twice a day; group C (n=14) zinc 200mg + vitamin E 10mg + vitamin C 5mg twice a day, all for three months; and group D (n=8) served as a control. For inclusion in the review, only the result sof the group that solely used zinc were taken into consideration, although there was no difference in the outcome measures between zinc by itself and zinc with other antioxidants. Concentration and morphology improved, but not statistically enough compared to the control group. However, motility increased twice as much and membrane integrity improved, decreasing sperm DNA fragmentation.

The study by Hadwan and colleagues[25] included 37 fertile patients and 37 with asthenozoospermia from the Hilla Maternity Infertility Clinic, in Iraq. Only the subfertile group was treated with 220mg zinc sulfate capsules twice a day for three months, while the fertile group was allocated as control. After this period, the intervention group was reassessed and there was an improvement in all sperm parameters, but only progressive motility and morphology, as well as seminal volume, presented statistically significant differences.

The study by Raigani and colleagues[21] used 83 patients diagnosed with oligoasthenozoospermia collected from an infertility clinic in the city of Tehran, in Iran, who were randomly distributed into four groups with different supplements (G1: folic acid; G2: folic acid and zinc; G3: zinc; G4: placebo). Doses of folic acid and zinc sulfate were 10mg and 440mg, respectively, and all groups received the supplement for four months. Only information related to the isolated zinc intervention was included in this review. After analysis, the results showed an increase in concentration. However, the results were not statistically significant for this and the other sperm parameters between the intervention groups, when compared with the samples collected at the beginning of the study and the control group. Even so, a significant increase in integrity chromatin took place.

In the subsequent work by Hadwan and colleagues,[26] the authors evaluated 120 men, half of whom were fertile and the other half subfertile (asthenozoospermics), who were patients of the same infertility clinic as their previous study in Iraq. They used practically the same methodology, dosage, time and evaluation of results. This time the results reported were an increase in all sperm parameters (concentration, motility, and morphology).

Discussion

Male subfertility is a condition where the individual has a low sperm quality, usually of multifactorial etiology arising from genetic and environmental factors to which individuals are exposed, linked to imbalances between oxidative stress and antioxidant substances. In recent years, several studies have investigated interventions that include the supplementation of antioxidant substances with the aim of reversing the intense lipid peroxidation, and consequently, the DNA fragmentation of spermatozoa of some groups of infertile men.[20]

The main instrument used to support the studies were the WHO manuals, which underwent three changes in their reference values over the course of twenty years. Ongoing updates of their reference values confound the evidence surrounding the potential impact of antioxidants. This disadvantage is most evident in the inclusion criteria used by the studies in patients who were considered to have abnormal sperm quality before the 2021[7] update to the WHO reference values and were labeled as “normal” after its implementation. However, several studies have reported improvements in basic semen parameters after oral ingestion of zinc, alone or in combination with other associated micronutrients.

With the exception of Wong and colleagues,[20] who used the factor as a selection criterion for their sample, the vast majority (87.5%) of the studies did not investigate the participants' dietary factors. The use of this methodology leads to clearer and more reliable results. After all, the causes of possible nutrient deficiencies, especially those with antioxidant characteristics, could provide an idea of the possible etiology of infertility.

Regarding the results, 87.5% of the studies showed positive results in relation to the sperm parameters evaluated after intervention, even with different aspects. The only exception was the work of Raigani and colleagues,[21] who did not find significant results for the parameters of concentration, motility and morphology between the sample from the control group and the intervention group but found a significant increase in sperm chromatin integrity. Despite same dosage and administration time as Hadwan and colleagues,[25,26] Raigani did not verify improvement in sperm parameters of motility and morphology, as Hadwan observed in his works. This may be explained by the methodology used and the population assessed.

However, according to Raigani and colleagues,[21] the association of zinc with folic acid allows significant improvements in the parameters of morphology and sperm count, since zinc can potentially contribute to improvements in the parameters of evaluation of semen quality, while folic acid prevents the occurrence of aneuploidy (change in the number of chromosomes) and thus future errors in cell division after fertilization of a normal ovum[30].

On the other hand, Ebisch and colleagues[23] came to different conclusions in their two studies. The first study, in which Wong[20] participated, included individuals with polymorphism in the methylenetetrahydrofolate reductase (MTHFR) enzyme gene, a condition that disrupts the folic acid metabolism, which affects protein synthesis and, consequently, cell division. In this same study, the results do not show significant differences between the groups, unlike Wong and colleagues[20] who used the same dosage and duration and obtained positive parameters in the intervention group in the study he led. This outcome reinforces the idea that, unlike genetic factors, nutritional factors are changeable.[20]

In their next work, in 2006, Ebish and colleagues,[24] doubled the dosage (from 66mg to 132mg) over the same six months and used another sample model, this time defining criteria that excluded a possible genetic cause of infertility. They found improved parameters in sperm concentration per milliliter of semen.

Although this improvement in sperm count does not always result in sperm concentrations greater than the reference value of 20 million cells/ml, the increase observed in research suggests a beneficial effect on the quantitative aspect of spermatogenesis. This conclusion is supported by non-randomized controlled studies showing that oral zinc supplementation improves sperm concentration in males with idiopathic asthenozoospermia or oligozoospermia.[27,28]

The two works by Omu and colleagues[11,22] selected asthenozoospermic individuals and used dosages of 500mg and 400mg administered over three months. In both cases, a beneficial impact was recorded. In the first study, conducted in 1998,[22] the intervention improved concentration and motility. In the second study,[11] conducted ten years later, the researchers found increased motility, improvement in membrane integrity and a lower rate of fragmentation of sperm DNA.

All authors cited in this review used zinc dosages well above the tolerable intake levels (Tolerable Upper Intake Level - UL) for the sex and life cycle of the evaluated individuals, which is only 40mg daily for men over 19 years old, according to the Dietary Reference Intakes (DRI)[29]. Despite not being administered an extended period, this fact may be linked to the dropout rate of participants in some studies, due to reports of strong gastrointestinal discomfort described only by Omu and colleagues,[22] and Wong and colleagues.[20]

However, according to the Lifestyle Medicine approach, it appears that six pillars underlie the therapeutic use of lifestyle, which, consequently, will interfere with fertility. These are: adequate nutrition; regular practice of physical activity; quality of sleep; avoidance of exposure to toxic substances; control of stress; and positive personal relationships. These factors are correlated with increased oxidative stress, which in turn directly influences spermatogenesis.[31,32]

The presence of fatty acids and substances that promote the unbalanced generation of reactive oxygen species in the body directly influences spermatogenesis. This fact negatively contributes to the oxidative balance in the testicular cell environment,[33] such that adoption of the “prudent pattern”, i.e., a high consumption of fruits, vegetables, fish and whole grains, results in a lower rate of fragmentation of sperm DNA; while adoption of the “traditional Dutch diet” pattern, i.e., high consumption of potatoes, meat and whole grains associated with low consumption of alcoholic beverages and sweets, has positive effects on sperm concentration, noting that in both evaluated patterns have foods with antioxidant characteristics.

Another study[34] with 336 individuals diagnosed with infertility compared the influence of a dietary pattern rich in antioxidants (“prudent”) with a dietary pattern rich in saturated fatty acids and hypercaloric ("western") on sperm concentration, testosterone levels and sperm index of DNA fragmentation. The work reported positive results with the adoption of the "prudent" dietary pattern, while the opposite was registered for individuals with the "western" pattern.

Therefore, it appears that semen analyzes should always be interpreted with caution. After all, data can be easily influenced by intraindividual biological fluctuations and reflected in sperm parameters, in addition to possible limitations and inaccuracies of the methods used.[35,36]

Conclusion

In view of the evidence gathered from this systematic literature review, following the methodologies described above, one can conclude that interventions with zinc supplementation have a significant impact on the improvement of sperm parameters for concentration, motility, and morphology. This result is due to the protective effect provided by the trace element, which reduces the lipid peroxidation index of the plasmatic membranes and, consequently, improves the integrity of the DNA, thus increasing the fertilization capacity of these spermatozoa.

Although this review only investigated the variable of a single supplement in the condition of male subfertility, other studies have found superior beneficial effects obtained through zinc supplementation associated with other micronutrients, a fact that opens the door to conducting more clinical studies aimed at supporting the evidence already elucidated and improving therapy techniques.

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