

# Using Zinc in Management of Subfertile Male Patients: a Clinical Trial

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## ABSTRACT

**Background:** The use of minerals in treatment of different diseases is as old as man himself. zinc is the most famous trace mineral related to male sexual function. Oligoasthenozoospermic subfertile patients were treated with zinc sulphate for three months.

**Objectives:** Aim of the research is to investigate the role of Zinc and if it affects the abnormalities of some semen parameters and to study the possible role of pharmaceutical preparations of zinc in amelioration of male subfertility as well as to assess the ability of Zinc to induce changes in the serum and semen zinc levels in addition to the levels of reproductive hormones (FSH and Testosterone).

**Type of the study:** The study is a single group pretest-posttest experimental prospective comparative self-control; clinical trial research.

**Methods:** The patients were tested before and after the treatment for semen analysis via Computerized Assisted Semen Analysis (CASA) dynamic analysis report I and II as well as for FSH and Testosterone hormonal levels, serum and semen zinc levels .

**Results:** Zinc administration induced a significant increase ( $p \leq 0.001$ ) in FSH, Testosterone, serum and semen

zinc level as well as in the total and progressive sperm motility percentages .

**Conclusions:** Zinc administration induced significant changes ( $p \leq 0.001$ ) towards improvement in the total and progressive sperm motility percentages in oligoasthenozoospermic patients by CASA dynamic analysis report I and II.

**Keywords:** Zinc, subfertile male, oligoasthenozoospermic

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Male infertility is a major health problem[1]. It is a frequent complex medical problem, since about 15% of couples worldwide fail to conceive and the male factor being involved in roughly 50% of cases. So that, it is very important to tackle male subfertility problem and trying to find solutions for it because it is a delicate and complex for several reasons. Secondly, the background of male infertility seems extremely heterogeneous including many environmental causes. Thirdly, in as many as 30% of individuals, the origin of infertility remains unknown[1]. Zinc is the most critical trace mineral for male sexual function[2], zinc serves to help in stabilization of the DNA-containing chromatin in the sperm cells[3], The prostatic secretion contain high level of zinc[4]. Zinc has been selected to be used in management of certain cases of male subfertility. Oligoasthenozoospermic patients included in the research were labelled for a long time as idiopathic cases of male subfertility with no definite clear underlying cause for their subfertility problem.

The gonads are the most rapidly growing tissues in the body, and vital enzymes involved in nucleic acid and protein synthesis are zinc metallo-enzymes. Zinc deficiency can cause severe damage to the testes such as atrophy of the testicular tubules and the inhibition of spermatid differentiation, gonads dysfunction, decreases

testicular weight, shrinkage of seminiferous tubules[5], Exposure to zinc can alleviate testis damage by stresses such as heavy metals, fluoride and heat, suggesting that the testes may harbour a Zn-incorporation system and that zinc itself may exert protective effect against testicular injury and play an essential role in the maintenance of testicular functions[6].

Hypogonadism can be caused by zinc deficiency[7,8,9]. A study confirms the relation between zinc deficiency, poor semen quality and male infertility[10]. In the male reproduction, zinc is important for normal testicular development, Testosterone synthesis and conversion to its active form 5 $\alpha$  dihydrotestosterone, spermatogenesis, stabilization of cell membrane and nuclear chromatin of spermatozoa and in sperm motility[11].

CASA (Computerized Assisted Semen Analysis)[12]. Dynamic parameter analysis report I, which include the details and percentages of sperms motility (progressive motile PR type A: the strongest and swim fast in a straight line. Progressive motile PR type B (non-linear motility): These also move forward but tend to travel in a curved or crooked motion, combined PR (A+B), non progressive motility (NP) type C: These have non-progressive motility because they do not move forward despite the fact that they move their tails (all other types of motility with an absence of progression: swimming in

small circles, the flagella force hardly displacing the head or when only a flagella beat can be observed), and lastly the immotile IM type D: These are immotile and fail to move at all[13].

CASA dynamic parameter analysis report II, which include some details of sperm velocities (Velocity is the rate of change of the position of an object), equivalent to a specification of its speed and direction of motion. Several sperm velocity parameters: curvilinear velocity (VCL), average path velocity (VAP) and straight-line velocity (VSL), were evaluated. Sperm velocities contribute to overall sperm mobility and are important characteristics of sperm function[14,15].

**Methods:** The study is a single group pretest-posttest experimental prospective comparative self-control; clinical trial research, it has been conducted in Sudan - Khartoum state at andrology clinic in the Reproductive Health Care Centre (RHCC), in the period from September till December 2013. Cases included were idiopathic oligoasthenozoospermia, after isolation of known causes and failure of the conventional reproductive therapeutic measures. Patients received zinc sulphate 110 mg (equal 25mg zinc) twice daily for three months. Blood samples were taken from the patient for serum level of zinc, FSH & Testosterone hormonal level assessed. Finally, a semen sample taken for seminal level of zinc, and for computerized assisted semen analysis (CASA). After three months the same samples were taken again for analysis and data collection. Semen samples were been obtained by masturbation into sterile containers after an abstinence period of 3-5 days to be sended for testing in the lab. Samples were allowed to liquefy at 37°C for 30 minutes and analysed for descriptive semen parameters within 1 h of collection. Analysis was performed according to the WHO criteria (WHO 2010), by CASA, the instrument used here is: wlyj-9000: weili color sperm analysis system (dynamic analysis, CASA-WeiLi,ISO9001-ISO13485), the system follows WHO strict criteria for motility detailed patterns[12]. CASA for Sperm count, motility (total and progressive: report I) and dynamic parameters: report II. Then the semen samples were centrifuged at 2000 rpm for 15 to 20 minutes and supernatants were collected in metal free sterile tubes and stored frozen to be used in future to evaluate zinc . Assessment of blood serum zinc level and semen zinc level done by preparing samples: 0.1 ml of sample was digested over night in 0.2 ml of concentrated nitric acid and 0.2 ml of perchloric acid. The mixture was diluted 40 times with deionised water. Determination of blood serum and semen zinc level done by flame atomic absorption spectrophotometer with the appropriate zinc hallow cathode lamp and wave length specific for zinc 213.8 nm, according to standard procedure[16]. Serum zinc normal range: N.R =0.66 - 1.1 mcg/ml, while semen zinc N.R=78-278 mg/L [17].

Assessment of FSH and Testosterone changes done by radioimmunoassay using special Bio kit. FSH (N.R= 2.1

-18.6  $\mu$ IU/ ml), while Testosterone (N.R =262-870 ng/dl). The procedure and normal range mentioned by the manufacturing company (CIS bio international) were followed.

**Statistical Analysis** done by Paired Student T-test through mean and standard deviation were used to demonstrate the significant differences between pre and post treatment values via determination of P value. Data were statistically analyzed by the software computerized program SPSS Version 16. Data were presented in form of tables[18,19].

#### Results:

**Table 1: The pre-post treatment values of FSH and Testosterone .**

Measurement group	Mean $\pm$ SD	
	FSH $\mu$ IU/ ml	Testosterone ng/dl
Pre-treatment measurement	15.90 $\pm$ 9.06	340.6 $\pm$ 133.213
Post-treatment measurement	* 20.07 $\pm$ 10.57	475.9 $\pm$ 127.915*

n=12 \* =  $p < 0.001$  (Significant differences)

**Table 2: The pre-post treatment values of serum zinc & semen zinc levels .**

Measurement group	Mean $\pm$ SD	
	Serum Zinc mcg/ml	Semen Zinc mg/L
Pre-treatment measurement	0.458 $\pm$ 0.111	168.6 $\pm$ 77.96
Post-treatment measurement	* 1.18925 $\pm$ 0.345	258.9 $\pm$ 57.94*

n=12 \* =  $p < 0.001$  (Significant differences).

**Table 3: The pre-post treatment values of general semen parameters (sperm count, total motility percentage and progressive motility percentage) .**

Measurement group	Mean $\pm$ SD		
	conc. (Mill/ml)	Total motility (%)	progressive motility (%)
Pre-treatment measurement	11.19 $\pm$ 6.01	15.68 $\pm$ 13.34	11.20 $\pm$ 12.31
Post-treatment measurement	13.82 $\pm$ 8.35 <sup>NS</sup>	37.65 $\pm$ 18.55*	32.94 $\pm$ 19.37*

n=12 \* =  $p < 0.001$  (Significant differences of motility) N.S = not significant.

**Table 4: The pre-post treatment details and percentages of sperm motility (CASA dynamic parameter analysis report I).**

Measurement group	Mean $\pm$ SD				
	PR(A)% Class A: Rapid progressive motility	PR(B)% Class B: Slow or sluggish	NP(C)% Class C: Non progressive motility	IM(D)% Class D: Im motile	PR(A+B)%
Pre-treatment measurement	7.07 $\pm$ 10.04	4.18 $\pm$ 6.82	4.47 $\pm$ 4.21	84.31 $\pm$ 13.34	11.20 $\pm$ 12.31
Post-treatment measurement	22.76 $\pm$ 16.17*	10.17 $\pm$ 6.35*	4.65 $\pm$ 3.91 N.S	62.39 $\pm$ 18.62*	32.94 $\pm$ 19.37*

n=12 \* =  $p < 0.001$  (Significant differences)

N.S = not significant

**Table 5: The pre-post values of sperm tracks and velocities (CASA dynamic parameter analysis report II).**

Measurement group	Mean $\pm$ SD		
	VCL ( $\mu$ m/s) Track velocity	VSL ( $\mu$ m/s) Progressive velocity	VAP ( $\mu$ m/s) Path velocity
Pre-treatment measurement	29.89 $\pm$ 20.08	17.65 $\pm$ 12.98	20.63 $\pm$ 14.57
Post-treatment measurement	39.94 $\pm$ 9.47 N.S	27.56 $\pm$ 7.56*	30.14 $\pm$ 7.69*

n=12 \* =  $p < 0.001$  (Significant differences) N.S = not significant

**Discussion:** Male infertility is a complex medical problem, and has multiple causes and consequences depending on the general health state, sexual history, life style of society and cultural background of people it affects[20]. Sperm parameters such as count and motility are reportedly key indices of male fertility; being fair markers of spermatogenesis and epididymal maturation of spermatocytes [21]. When a diagnostic evaluation reveals no specific diagnosis, apart from abnormal semen analysis to explain a man's infertility, it is said that he has idiopathic infertility. The incidence of idiopathic infertility reported in published retrospective

reviews from male infertility clinics has ranged from 5 to 66% with an average of 25%[22,23]. Idiopathic infertility, therefore, is often the "diagnosis" given to a large number of men seeking evaluation and treatment. Empiric therapy is the only available form of therapy for these patients. Therefore, empiric therapy might be offered to any infertile man for whom a specific treatment is unavailable or with whom a specific treatment has failed[24].

As shown in Table 1, pre-treatment mean level of FSH [N.R: 2.1 -18.6  $\mu$ U/ml] was 15.90  $\mu$ U/ml changed to 20.07  $\mu$ U/ml in the post-treatment mean level in patients treated with zinc, the changes were with statistically significant differences ( $p < 0.001$ ). The mean serum level of FSH among oligoasthenozoospermic patients who receive zinc treatment was within normal range in the pre-treatment values and increased slightly above the maximal upper normal FSH level in the post treatment values, as shown by the results in Table 1.

The treatment induced a significant change in FSH after zinc administration, this is in contrary to the finding of other studies[25,26], which showed no change in serum FSH level in sub-fertile patients after zinc therapy.

Table 3, shows that zinc therapy failed to improve the oligospermic (low sperm count) state in the patients after the end of three months (13.82 millions/ml), which is lesser than the minimum accepted sperm count number of normal semen analysis (20 millions/ml) stated by WHO manual human semen criteria[12], so that, this elevation can be explained by the persistence of low sperm count, that might not initiate the negative feedback loop required to inhibit the central release of FSH, its major role in the regulation of spermatogenesis through targeting sertoli cells, FSH binds with specific FSH receptors attached to the sertoli cells in the seminiferous tubules, this causes these cells to grow and secrete various spermatogenic substances. When the seminiferous tubules failed to produce sperm, secretion of FSH by the anterior pituitary gland increases manifestly. Inhibin hormone induce a negative feedback effect on the anterior pituitary secreted by the sertoli cells[27].

In table 1, a significant increase ( $p < 0.001$ ) in Testosterone was induced after therapy [Testosterone N.R: 262-870 ng/dl]. Testosterone 340.6 ng/dl changed to be 475.9 ng/dl, this is in agreement with the finding of a previous study[25], which occurred because testosterone synthesis is zinc dependent[28]. It is a documented fact by many studies that zinc deficiency leads to decreased serum Testosterone level[29,30]. Leydig cells synthesis of Testosterone was dependant on adequate dietary zinc and plasma Testosterone (T) and dihydrotestosterone (DHT) rose significantly after oral administration of zinc.

It is a vital element in spermatogenesis, through its direct association with testosterone metabolism and synthesis[31]. Zinc involved in Testosterone synthesis

and conversion to its active form: 5 $\alpha$ - dihydrotestosterone and also involved in spermatogenesis, stabilization of cell membrane and nuclear chromatin of spermatozoa and in sperm motility[11].

Additionally, zinc may increase, the conversion of androstenedione to testosterone in the periphery. Zinc also interferes with the metabolism of testosterone by decreasing its hepatic clearance and reducing hepatic 5 alpha- reductase activity[32]. Finally, zinc may increase not only the serum concentration of testosterone, but it may affect the action of androgens as well, since the DNA-binding domain of the androgen receptors is a cysteine-rich zinc-finger protein[33].

As shown in table 2, the treatment induces a significant increase ( $p < 0.001$ ) in serum zinc level, where pre-treatment measurement of serum zinc [N.R:0.66-1.1mcg/ml] has 0.458 mcg/ml changed to 1.189 mcg/ml in the post-treatment measurement. This finding comes in agreement with the other studies [26]. This is clearly attributed to the exogenous zinc supplementation.

In table 2, the treatment induces a significant increase ( $p < 0.001$ ) in semen zinc level in which pre-treatment measurement of semen zinc [N.R:78-278 mg/L] was 168.6 mg/L changed to 258.9 mg/L in the post-treatment measurement.

Current knowledge on the relationship between seminal zinc levels and different parameters of human semen is inconsistent[34]. Some authors reported significantly different seminal zinc levels between fertile and subfertile groups, indicating low seminal zinc levels in the sub-fertile populations[35]. while some others have shown that there is no difference between the two groups [36].

Table 3 shows that the sperm's count in oligoasthenozoospermic patients after zinc treatment has increased but not with a statistically significant value. Sperm's count percentage in pre treatment measures has been increased from 11.19 to 13.82 million/ml in the post treatment measure, this comes in agreement with researchers who found the same results, where they state that semen is normally rich in zinc, as contributed by prostatic secretions. Other studies show that the sperm's count was increased significantly in oligoasthenozoospermic patients after zinc administration [35].

Table 3 shows that, both total motility and progressive motility percentages of sperm motility measured in oligoasthenozoospermic patients stated by CASA dynamic parameter analysis report I after zinc administration for three months has increased ( $p < 0.001$ ) in a statistically significant value, while Table 4 shows an increasing progressive motility PR type A percentage, progressive motility PR type B percentage and progressive motility combined PR (A+B) percentage with a significant decrease ( $p < 0.001$ ) in the percentage of immotile spermatozoa percentage (IM% Class D) in a statistically significant value.

It is found that, total motility percentage in pre treatment measures has increased significantly from 15.68 to 37.65 %, while progressive motility percentages from 11.20 to 32.94% in the post treatment values (Table 3). According to the improvement in progressive motility percentages in the post treatment value (table 4), the partitions of progressive motility stated by CASA dynamic parameter analysis report II, has increased significantly as follows: progressive motility PR type A percentage has increased from 7.07 to 22.76%, progressive motility PR type B percentage has increased from 4.18 to 10.17% and progressive motility combined PR (A+B) percentage has increased ( $p < 0.001$ ) from 11.20 to 32.93% after zinc administration for three months in a statistically significant value.

As shown in Table 4, non progressive motility (NP) type C percentage after zinc administration for three months has increased from 4.47 to 4.65% but in non significant value. This percentage increase in the (NP) type C percentage strengthen the percentages of motility partitions in a positive way towards improving the motility parameters [37].

immotile spermatozoa percentage (IM% Class D) has dropped in a statistically significant value ( $p < 0.001$ ), from 84.31 % in the pretreatment measure to 62.39% in the post treatment (table 4), which is regarded as a good sign of general sperm motility improvement. These results can be explained in agreement with some studies which show that most of zinc in the seminal fluid is in binding state to ligands, from prostate such as metallothionein and prostasomes (prostasomes are present in the seminal fluid and adhering to the surface of the spermatozoa and it contains zinc dependent ATPase that involved in sperm motility) [38]. The treatment of infertile men with zinc sulphate improved motility and fertilizing capacity of sperms[39]. The finding of our current research agreed with a recent research which claims that elevation of seminal zinc concentration reflected clearly on motility status of sperms and that, the andrological variable sensitive to seminal plasma zinc variations is motility[34].

As with the current study's results in improving the sperm motility after zinc administration, Abasalt et al, found that zinc therapy reduces immotile sperm percentage through several mechanisms such as prevention of oxidative stress, apoptosis and sperm DNA fragmentation[40].

Reactive oxygen species (ROS) are needed for capacitation, the acrosome reaction, and ultimately fertilization, but their uncontrolled production is detrimental to cell function as they damage a variety of biomolecules such as lipids, amino acids, carbohydrates, protein, and DNA and adversely affect sperm function due to DNA damage reduced motility and defective membrane integrity[41,42]. Zinc is an acknowledged antioxidant factor that in addition to the fact that it is a core constituent of free radical scavenging enzymes such as: superoxide dismutase

(SOD) and a recognized protector of sulfhydryl groups, it is also thought to impair lipid peroxidation. Zinc administration has also shown to attenuate the testicular oxidative DNA damage, and this could be one of causes of motility improvement. Elevated reactive oxygen species (ROS) levels in the semen may be an etiologic factor for male infertility[43].

It is estimated that 25-40% of oligoasthenozoospermic patients possess high levels of semen ROS, whereas fertile men do not have high levels of semen ROS[44]. Spermatozoa are particularly susceptible to oxidative injury due to the abundance of plasma membrane polyunsaturated fatty acids.

Studies have shown that seminal antioxidant capacity is suppressed in infertile men with high ROS levels compared to men with normal levels of ROS. Many researches, on the other hand, showed significant improvements in sperm concentration after oral intake of different antioxidants as zinc[45]. A latest research, shows that antioxidants play an important role in protecting semen from ROS and it can improve basic sperm parameters in case of idiopathic oligoasthenozoospermia. A significant number of patients diagnosed with unexplained (Idiopathic) subfertility has remarkably high degrees of fragmented sperm DNA[46].

Effects of zinc treatment on sperm tracks and velocities measured in oligoasthenozoospermic patients stated by CASA dynamic parameter analysis report II: [As shown in table 5, both average path velocity (VAP), straight-line velocity (VSL) after zinc administration for three months increased in a statistically significant value ( $p < 0.001$ )]. The current study found that, (VSL) progressive velocity in pre treatment measures has been increased significantly from 17.65 to 27.56  $\mu\text{m/s}$ , while average path velocity (VAP) from 20.63 to 30.14  $\mu\text{m/s}$  in the post treatment values (Table 5). VCL (Track velocity) increased as well from 29.89 to 39.94  $\mu\text{m/s}$  in the post treatment values (Table 5) but insignificantly.

The three commonly reported CASA parameters as shown by figure 1, include curvilinear velocity (VCL), average path velocity (VAP) and straight line velocity (VSL). These CASA motility parameters have been modelled and refined mathematically to describe the best motion parameters of each spermatozoon as it travels through a microscopic field [47].

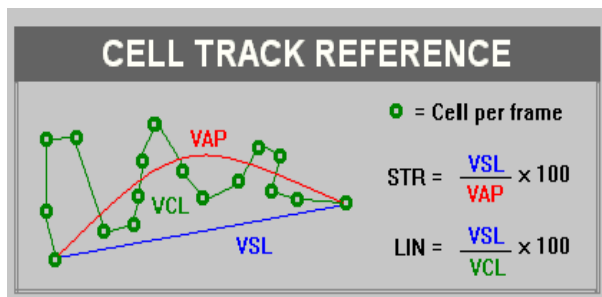


Figure 1: Sperm Tracks and Velocities

The two important movements of spermatozoa, the vigour movement (circular or whiplash) that could be represented by the VCL and the progression movement can be represented by VSL. VAP represents the general trajectory of the spermatozoa[48]. A research found that there is significant positive correlations were found between sperm velocities and sperm mobility[49], and as the motility parameters improved in our current research by zinc administration (Table 3 and 4) in oligoasthenozoospermic patients, so that we can conclude that sperm velocities improvement is a reflection for sperm motility improvement. Both the progressive linear motility (VSL) and the vigorous non-linear non- progressive motion (VCL) are of high importance[48], in our current study both increased and improved by the administration of zinc in oligoasthenozoospermic patients.

**Conclusions:** Zinc administration in oligoasthenozoospermic patients induce: A significant change in FSH and Testosterone, A significant increase in serum and semen zinc level. , Both total motility and progressive motility percentages of sperm motility measured show a statistically significant changes toward improvement.

**Declaration :** The research is a part of PhD thesis.

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