

Essence of Some Trace Elements in Seminal Fluid and Their Role in Infertility

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Abstract: This study is aimed to compare the levels of zinc, copper, iron and magnesium in the seminal plasma of infertile group and control group, to find out relationships in male infertile group between these elements and both seminal characteristics, serum reproductive male hormones (Luteinizing hormone, Follicle stimulating hormone, and testosterone), and also to understand the biological significance of such elements in male infertility. Seventy two infertile male subjects, without any treatment who had regular unprotected intercourse for at least 12 months without conception, aged 20-50 years, were selected from the central infertility center Al Shifa hospital, Gaza, Palestine. Seventy two known fertile males selected as control group from general population (their wives had given birth to a child within one year). Semen samples analyzed according to WHO criteria and seminal plasma trace elements were analyzed by atomic absorption spectrophotometry. Serum samples for endocrine studies were measured by (Enzyme Linked Immunosorbent Assay) ELISA. Our results showed that the mean values of zinc (68.9 mg/L) and magnesium (67.1mg/L) were significantly lower in infertile men compared to controls zinc (122mg/L) and magnesium (120 mg/L). Almost all studied seminal parameters (sperm count, forward motility, weak motile and non-motile) were significantly lower in infertile group compared to controls ($P < 0.001$, $P < 0.001$, $P < 0.05$, $P < 0.001$, respectively) as well as hormonal parameters (LH and Testosterone) in the infertile group were significantly lower compared to the control group ($p < 0.05$). There was no significance difference between means of FSH levels ($p > 0.05$). Correlation analysis in infertile group showed that while seminal plasma zinc and magnesium levels are directly proportional to sperm count ($r = 0.376$, $P = 0.001$ and $r = 0.293$, $P = 0.013$ respectively), and testosterone ($r = 0.293$, $P = 0.012$ and $r = 0.324$, $P = 0.003$ respectively), it is indirectly proportional to seminal volume ($r = -0.251$, $P = 0.034$ and $r = -0.369$, $P = 0.001$ respectively). The results obtained in our study indicate that both zinc and magnesium are important for both spermatogenesis and steroid genesis.

Key words: Trace elements, Male Infertility, Semen Composition,

Introduction

World health organization defined infertility as failure of conceiving a child for at least 12 month of unprotected intercourse (1). Infertility has been shown to have a high prevalence worldwide (affects one in six). It has been reported that male factor infertility plays a role in approximately 30-55 % of infertile couples (2). However, despite advances in diagnostic methods in the field of andrology, there remains a significant subset of these sub fertile men who are classified as having unexplained male infertility (UMI). Male infertility has multiple causes and the commonest single defined cause is sperm dysfunction (2). However, reports has referred to the worsening of this problem due to the deterioration of the human semen quality by as much as 3% per year, leading to fears that male reproductive problems may be on an increase (3). Despite the problem in assessing the prevalence of infertility in developing countries, between 8 - 12 % of couples around the world have difficulty conceiving a child at some point in their lives, affecting 50 - 80 million people (108). In Palestine infertility is a tragic and a costly problem, also it has both psychological and financial burden on the local population.

The etiologies of male infertility include gene mutations, aneuploidies, infectious diseases,

ejaculatory duct occlusion, varicocele, radiation, chemotherapy and erectile dysfunction (5). 90% of male infertility is due to low sperm count. The role of trace elements on the quality of human semen, and their mechanism of action have been a focus of study for many researchers (6). Essential trace elements to human health include iron, copper, selenium, manganese, chromium, molybdenum and iodine. Trace elements have been shown to play essential roles in major cellular activities i.e. antioxidants. Zinc is another essential trace element with many enzymatic functions, including antioxidant actions, and involved in chromatin scaffold proteins, DNA synthesis, protein synthesis and cell division (7).

Although there are some studies which demonstrate the significance of trace elements in male fertility, the biological role of these elements is not fully understood (8). Zinc was found to have high levels in semen from mammals, and zinc has been found to be critical to spermatogenesis. Deficiency of zinc is associated with hypogonadism and insufficient development of secondary sex characteristics in human (9). Also, deficiency of zinc was found to cause failure in spermatogenesis due to atrophy of the seminiferous tubules in the rat (10).

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However, high concentrations of zinc have been reported to depress oxygen uptake in the sperm cells (11), head-tail attachment/detachment and nuclear chromatin condensation/de-condensation is also influenced by seminal zinc (12). Also sperm motility has been suggested to be affected by zinc levels (13). The generation of oxidants, also described as reactive oxygen species (ROS), in the male reproductive tract has been a real concern because of their potential toxic effects, at high levels, on sperm quality and function (14,15). A number of reports indicate the significance of trace elements in male infertility and its effect on the level of antioxidants (16). ROS are needed for the regulation of normal sperm functions, such as sperm capacitation, the acrosome reaction, and sperm-oocyte fusion (17). Increased levels of metal ions in semen (19) or blood plasma (18) appear to be significantly and positively correlated with male infertility (20).

Spermatogenesis in mammals requires the action of a number of peptide and steroid hormones (sex hormones), each of which plays an important role in normal functioning of the seminiferous epithelium. Sex hormones are not critical only for regulation of male germ cell development, but also for proliferation and function of the somatic cell types required for proper development of the testis (21). Among the most common somatic cells that are affected by sex hormones are the interstitial steroidogenic leydig cells, whose primary function appears to be production of testosterone (22). The sertoli cells, whose direct contact with proliferating and differentiating germ cells within the seminiferous tubules makes them essential for providing both physical and nutritional support for spermatogenesis (23). FSH and LH are secreted by the anterior pituitary and act directly on the testis to stimulate somatic cell function in support of spermatogenesis (24). LH is known to act on leydig cells to produce testosterone while FSH acts on sertoli cells to promote spermatogenesis (23).

Materials and Methods

Subjects:

We studied two groups of subjects: a) Case group: this group consists of 72 infertile subjects aged 20-50 years with oligospermia (low sperm count) or/and asthenospermia. (poor sperm motility) b) Control group: this group consists of 72 fertile men aged 20-50 years their wives were pregnant or had delivery of a child within the previous 12 months. An individual of both groups has signed consent form before giving samples. The number of individuals was chosen to be able to carry out Pearson test analysis.

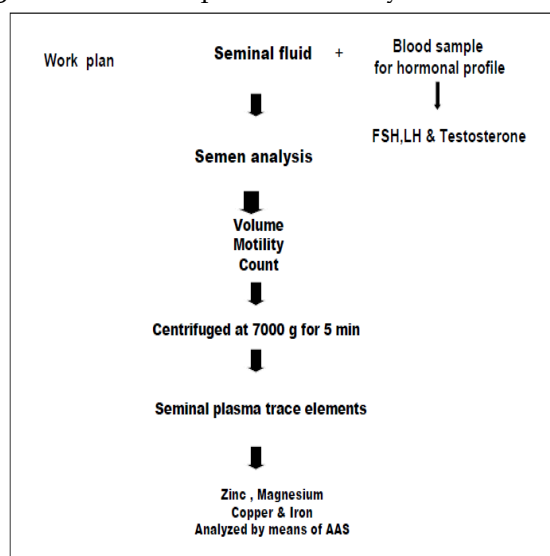
Sample Collection:

Seminal fluid collection and analysis were carried out in strict compliance with the WHO guidelines. The specimen is best collected by masturbation into a sterile container. Serum hormonal profiles of LH, FSH and testosterone as well as seminal plasma measurement of the trace elements zinc, copper, iron and magnesium were carried out.

Work plan:

The work plan is shown in Figure 1. The study population consists of seventy-two infertile men (case group) and seventy-two fertile men (control group) were included in this study. The case group was selected according to family history and the case group was not able to conceive at least for twelve months of unprotected sex and their wives had no medical problems. All men were non-smokers, consume no-alcohol and have normal dietary pattern of life. Seminal plasma trace elements (zinc, copper, iron and magnesium), seminal parameters and endocrine parameters were measured.

Figure.1: The work plan of the study



Results

Semen analysis has shown that sperm parameters excluding the volume of the case group were below the WHO reference range Table (1). Mean sperm count compared with the control group mean count was very much lower than the control group ($P < 0.001$). Forward progression percentage was lower in the case group than the control group ($P < 0.001$). Weak-motile percentage were lower in the case group than the control group ($P < 0.05$), non-motile percentage were lower in the case group than the control group ($P < 0.001$).

Table (1): Comparison between seminal parameters of case group (infertile men) with control group (fertile men)

| | Case group N=72 Mean±SD | Control group N=72 Mean±SD | P-value |
|------------------|-------------------------------|----------------------------------|-----------|
| Volume (ml) | 3.08±1.12 | 3.32±0.9 | >0.05 NS |
| Count (million) | 23.9±22 | 63.1±16.7 | <0.001*** |
| Forward motility | 22.8±14 | 51.3±5.36 | <0.001*** |
| Weak motile | 21.3±10.6 | 18.5±5.08 | <0.05* |
| Non-motile | 55.9±18.6 | 30.3±6.27 | <0.001*** |

The mean levels of FSH, LH and testosterone were normal in both groups, however LH level were significantly lower in the case group than in the control group (P<0.05), testosterone mean levels were significantly lower in the case group than the control group (P<0.05), while the mean levels of FSH in both groups were not significantly different (P>0.05). (Table 2).

Table (2): Comparison between serum endocrine parameters of case group (Infertile men) with control group (fertile men)

| | Case group N=72 Mean±SD | Control group N=72 Mean ±SD | P -value |
|--------------|-------------------------------|-----------------------------------|----------|
| LH | 4.52±1.45 | 5.05±1.23 | <0.05* |
| FSH | 5.33±0.6 | 5.37±0.65 | >0.05 NS |
| Testosterone | 5.09±1.03 | 5.4±0.9 | <0.05* |

Trace elements zinc, copper and magnesium levels were significantly lower in the patients than the control group (Table 3). Zinc concentration in seminal plasma were highly lower than the control group (P<0.001). Copper seminal plasma concentration in patients was lower than the concentration of the control group (P<0.05). Magnesium seminal ±plasma mean concentration in case group was much lower than the mean concentration of the control group (P<0.001). The mean concentration of iron was significantly higher in case group than the mean concentration of the control group (P<0.05).

Table (3): Comparison between seminal plasma trace elements in the case group with the control group

| | Case group N=72 Mean±SD | Control group N=72 Mean±SD | P-value |
|------------------|-------------------------------|----------------------------------|-----------|
| Zinc (mg/L) | 68.9±37.7 | 122±26.1 | <0.001*** |
| Magnesium (mg/L) | 67.1±36.4 | 120±28.2 | <0.001*** |

In the patient group, Zinc correlated inversely with semen volume (P<0.05), correlated directly with sperm concentration (P<0.001) and no relationship was found with the other seminal plasma parameters (P>0.05) Seminal plasma zinc was found to be positively correlated with testosterone levels of the case group (p< 0.05). Seminal plasma zinc was also positively correlated with testosterone levels of the case group (p < 0.05) (Table 4). The linear regression analysis between trace elements in seminal plasma of the case group showed a strong positive correlation between magnesium and zinc levels (P<0.001) (Table 4 and Figure 2).

Figure.2: Relationship between seminal plasma zinc and seminal plasma magnesium in case group n = 72.

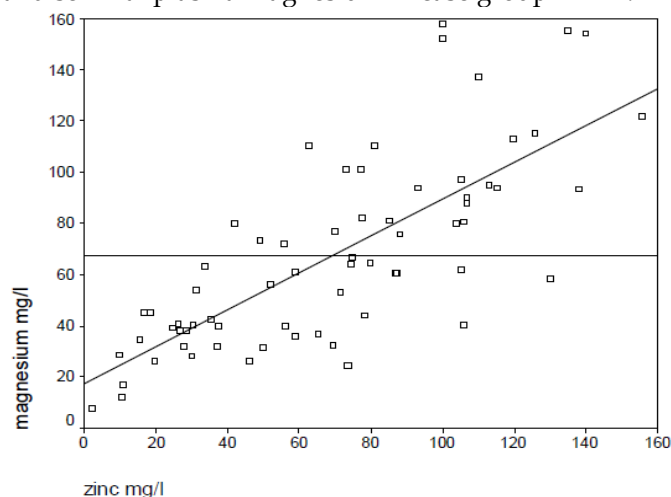


Table (4): linear regression analysis between zinc and magnesium seminal plasma level and seminal parameters in case group n = 72

| | □□(slope) | | r | | P-value | |
|------------------|-----------|-----------|---------|-----------|----------|-----------|
| | zinc | Magnesium | zinc | Magnesium | zinc | Magnesium |
| Volume | -8.482 | -12.04 | - 0.251 | -0.369 | 0.034* | 0.001** |
| Count (million) | 0.646 | 0.484 | 0.376 | 0.293 | 0.001*** | 0.013* |
| Forward motility | -0.488 | -0.290 | - 0.182 | -0.112 | 0.127 NS | 0.349 NS |
| Weak motile | 0.702 | 0.268 | 0.197 | 0.078 | 0.097 NS | 0.515 NS |
| Non-motile | 0.025 | 7.86 | 5.075 | 0.040 | 0.835 NS | 0.738 NS |
| LH | 5.472 | 9.577 | .211 | 0.383 | 0.075 NS | 0.001** |
| FSH | 4.532 | 6.55 | .074 | 0.110 | 0.538 NS | 0.355 NS |
| Testosterone | 10.694 | 11.37 | .293 | 0.324 | 0.012* | 0.006* |

Linear regression analysis between magnesium seminal plasma of infertile men and seminal parameters were studied. There were an inverse correlation between seminal plasma magnesium and semen volume ($P < 0.001$), positive correlation with semen count ($P < 0.01$), while there was no significant correlation with other semen parameters was found ($P > 0.05$) as shown in Table (4).

Linear regression analysis between seminal plasma magnesium and serum endocrine parameters in the case group showed a positive correlation between seminal plasma magnesium with LH and testosterone ($P < 0.001$) and ($P < 0.01$) respectively and no correlation found with FSH level ($P > 0.05$) as shown in Table (4).

Discussion

Abnormalities associated with trace elements can be due to specific efficiency from dietary inadequacies and imbalances, or abnormality secondary to other diseases. Both kinds of abnormality can be diagnosed by analysis of trace elements in body fluids or other tissues. However secondary changes, which occur as a result of diseases, are not exactly understood. Our study suggests that zinc is an essential trace element for male infertility. This supports early studies which conclude that zinc is an essential trace element required for normal spermatogenesis and steroidogenesis and zinc deficiency considered as one of the factors responsible for decreased testicular function in infertile male subjects (25).

Our results indicate that both zinc and magnesium co-exist either intracellular or extracellularly as shown by the positive correlation between them. Since magnesium is the second major intracellular action to potassium, the positive correlation found with zinc indicates that zinc is important intracellularly (i.e. in reproductive cells). Previous work has shown that increase in cAMP due to hormonal changes enhances that outlet of magnesium and its increase extracellularly. It is well known that FSH and LH act intracellularly by increasing the levels of cAMP. Therefore it is expected that FSH and LH promotes the export of magnesium, potassium and consequently zinc (26). Our study indicates that increase in seminal plasma magnesium or zinc reduces seminal plasma volume. Since deficiency in magnesium is considered a deficiency in potassium, it is expected that the outlet of either magnesium or zinc will lead to reduction in potassium therefore to loss of water in the seminal plasma therefore leading to reduction in the seminal plasma volume. We concur with the previous

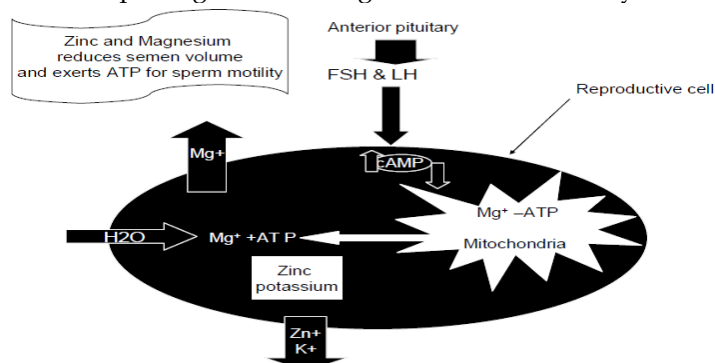
reports which found inverse association between zinc concentration in seminal plasma and semen volume more than 5ml ($P = 0.034$) and a positive correlation with hypo-viscosity of semen and conclude that zinc and calcium and physical analysis of ejaculate was also found to be clinically useful for evaluating the secretory activity of the seminal vesicles and prostate; abnormal coagulation, liquefaction, volume, viscosity and pH strongly suggest gland dysfunction (27).

Our results supports the hypothesis that zinc is essential for spermatogenesis and steroidogenesis, as shown by the positive correlation between the zinc concentration and the semen count and serum testosterone. Our results agrees with other studies which have found a positive association between seminal plasma zinc concentration with sperm count ($r = 0.33, p < 0.05$) and with sperm motility, positive association with serum testosterone levels of the case group (28). Another study which concordant with our study has shown that seminal plasma zinc concentration was significantly correlated with sperm density (7, 1, 112). Zinc is a trace element essential for normal functioning of the male reproductive system. Numerous biochemical mechanisms are zinc dependent, including more than 200 enzymes in the body (29).

Zinc deficiency is associated with decreased testosterone levels and sperm count, an adequate amount of zinc ensures proper semen motility and production. Zinc levels are generally lower in infertile men with diminished sperm count, and several studies found supplemental zinc may prove helpful in treating male infertility (30). In one trial, the effect of zinc supplementation on testosterone, dihydro testosterone and sperm count was studied. Thirty seven patients with idiopathic subfertility of more than five years duration and diminished sperm count received twenty four milligrams of elemental zinc from zinc sulfate for forty five to fifty days. The results were dramatic in the twenty two subjects with initially low testosterone levels; a significant increase in testosterone levels and sperm count (from eight to twenty millions/ml) was noted, along with nine resulting pregnancies (31). LH increases seminal magnesium levels. The lower levels of magnesium (Mg) in infertile population indicate that magnesium might play a role in male fertility. Despite the lack of direct analytical studies on the role of magnesium in infertility, a number of studies have shown that magnesium is essential for energy requiring cells such as muscle and heart cells (32). It binds to ATP in the mitochondria in great amounts. However when hormones such as

adrenaline binds to the cells it increases the levels of cAMP by activating Adenylate cyclase. The increase of cAMP will encourage the release of ATP-Mg from the mitochondria (33). This will disassociate leading to free of ATP and exporting magnesium outside the cells. LH, which acts on leydig cells during spermatogenesis, has been shown to increase the levels of cAMP (26). Therefore we assume that this will lead the export of magnesium into the seminal fluid (Figure 3). This could explain the direct relation between the increase in LH levels and magnesium levels, which our data has demonstrated. Therefore the increase in magnesium in seminal plasma improves fertility. It is worth mentioning that the increase in seminal plasma means free ATP in spermatocytes and good fertility. Since magnesium is an intracellular component (34) means that it moves against the water. Since the increase in LH increases magnesium in the seminal plasma, this will lead to lower amount of water in the seminal plasma. This explains the inverse relations between the levels of both magnesium and LH and seminal volume in our studied population.

Figure (3): Proposed illustration showing that FSH and LH lower the levels of seminal fluid volume by exporting zinc and magnesium extracellularly.



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