


# The Relationship between Body Mass Index, Metal Elements, and Antioxidant Capacity of Semen on The Human Sperm Chromatin

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## Abstract

**Background:** Sperm chromatin abnormalities are defects in nuclear maturation and DNA integrity. These defects originated from defective spermatogenesis due to a lack of DNA repair during chromatin remodeling. Changes in semen elements can cause damage to chromatin. There is little information about the relationship between changes in trace metal elements and total antioxidant capacity (TAC) with sperm chromatin damage. The present study was conducted to determine the relationship between Selenium (Se), Iron (Fe), Zinc (Zn), Copper (Cu) and the TAC of semen with the status of human sperm chromatin.

**Materials and Methods:** In this experimental study, semen samples (n=30) were collected from healthy men referred to Kermanshah Motazadi Hospital and stored in liquid nitrogen; after thawing and centrifugation, sperm were separated. The atomic absorption method was used to measure the concentration of metal elements. The TAC was evaluated using the ferric-reducing antioxidant capacity of the plasma method. Furthermore, the integrity of sperm chromatin was measured using the sperm DNA fragmentation (SDF) method.

**Results:** The status of sperm chromatin had a non-significant correlation with body mass index (BMI,  $P=0.25$ ,  $r=0.21$ ) and a non-significant negative correlation with sperm count ( $P=0.71$ ,  $r=-0.71$ ) and motility ( $P=0.75$ ,  $r=0.61$ ). In addition, there was no significant relationship between sperm chromatin and the TAC of semen ( $P=0.92$ ,  $r=0.01$ ). Additionally, there was no significant correlation between Se, Zn, or Cu concentration ( $P>0.05$ ) and Fe concentration, which had a partially positive relationship with the chromatin state of sperm ( $P=0.24$ ,  $r=0.20$ ).

**Conclusion:** The trace metal elements in the seminal fluid did not play a significant role in the status of sperm chromatin.

**Keywords:** Antioxidant Capacity, DNA Fragmentation, Infertility, Semen, Sperm

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## Introduction

Infertility is the inability of a couple to get pregnant with regular and unprotected intercourse after one year (1). Despite the development of medical science, infertility is still a significant problem; about 10-15% of couples have this problem. Infertility is divided into two primary and secondary groups; the prevalence of primary infertility is higher in most parts of the world. The causes of infertility vary from one population to another and are affected by socio-cultural differences and the prevalence of sexual transmission. In developed industrialized countries, about 15% of couples experience primary or secondary

infertility, and almost half will never succeed in having the desired number of children (2, 3).

Disturbance in sperm production and function and damage in the process of spermatogenesis is one of the most common causes of male infertility. In addition, oxidative stress in the reproductive system affects the ability of sperm to fertilize. Furthermore, there is mounting proof that sperm function is considerably compromised by an increase in reactive oxygen species (ROS). Through pathways including the generation of DNA damage, apoptosis, and peroxidative damage to the sperm plasma membrane, these deficiencies have led to male infertility.

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To guarantee proper physiological activity and avoid pathological harm to the spermatozoa, ROS must be kept at the proper levels (4). Trauma and some therapeutic drugs disrupt sperm production and damage its DNA, leading to male infertility (3, 5). In addition, sperm exposure to environmental toxins and pollutants, drugs, ultraviolet rays, smoking, febrile illness, varicocele, and aging are also essential factors affecting sperm chromatin quality (6).

There is a certain amount of DNA breakage in men's sperm, and its level varies from one sperm to another. The integrity of sperm DNA affects the couple's fertility (7) and helps to predict the probability of pregnancy and its successful outcome. In addition, factors such as the concentration of metal elements can affect sperm chromatin damage (8). Metal elements such as Fe, Zn, Cu, and Se are present in very low amounts in the body, and due to their activity at the molecular level, they play an important role in the reproductive process of men (9).

Zn is the most abundant element in human tissues after Fe, and the World Health Organization (WHO) estimates that one-third of the world's population has Zn deficiency. Zn is secreted from the prostate gland, and its amount in seminal plasma usually indicates the secretory function of the prostate. A decrease in semen Zn concentration may be due to insufficient intake, decreased absorption, increased losses, or increased sperm excretion (9). Fe and its compounds are not toxic to humans and animals. Its numerous disorders can appear due to pathological conditions or long-term consumption of high doses of Fe in the regulating mechanism of its absorption. Fe has an essential role in vital biochemical activities (10), and its increase can negatively affect the morphology of sperm. It can also induce lipid peroxidation and inhibit sperm motility. These metals have negative health effects on people, including male infertility. Male fertility is severely impacted by exposure to harmful contaminants such as heavy metals, according to studies done in a variety of *in vitro* and *in vivo* models (11, 12).

Fe may mediate the effects of oxidative damage and play an essential role in spermatogenesis and male infertility. At the same time, Fe's absorption and metabolic function depend on the impact of many other elements, especially the antagonistic activity of cadmium, manganese, lead, Zn, and Cu. Therefore, although Fe and its compounds are not toxic to humans and animals, its overload can increase sperm DNA damage (13). Fe and Cu are necessary to maintain the function of living organisms. Excessive absorption of them causes oxidative damage to testicular tissue (14).

The increase of these ions can decrease the number and viability of sperm. Cu sulphate reduces the concentration of FSH, LH, and testosterone through physiological mechanisms and dysfunction of the gonads (15). Zn is present in sperm and seminal plasma, and its concentration is significantly higher than in the other body fluids. It directly affects the formation, motility, and morphology

of sperm and may help fertility through its positive effect on spermatogenesis (16). Zn plays an important role in capacitation and may be a regulatory factor in spermatogenesis process. Its deficiency may lead to degenerative changes in the spermatogenesis lineage (17). This element affects the stability of sperm, also plays a role in forming free oxygen radicals, and can regulate the capacity and reaction of acrosomes (18). However, there is little information about the role of Zn in human semen.

Se has several important functions in human health and is an essential element for the normal function of sperm and the process of spermatogenesis. Its administration to infertile patients significantly increases sperm motility. It has a protective effect against oxidative damage to human sperm DNA (19). Antioxidants are compounds that can protect biological systems against the harmful effects of ROS. The increase in ROS production because of oxidative stress causes a decrease in the fertility capacity of sperm, as a result, damage to the cell membrane, motility, and morphological abnormalities such as twisted tail, and damage to the spermatozoa (20).

Oxidative stress in human sperm is associated with reduced its motility, viability, and weakness in sperm-to-ovum integration (21). Considering the concentration of metal elements influences the integrity of the sperm structure, on the other hand, the results of previous research have been contradictory in this connection. The present study was conducted to determine the relationship between Selenium (Se), Iron (Fe), Zinc (Zn), Copper (Cu) and the TAC of semen with the status of human sperm chromatin.

## Materials and Methods

This experimental study was done after the approval of the Medical Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1399.659) and obtaining informed consent and providing sufficient explanations to the patients, refers to 30 semen samples from men with normozoospermia. It was carried out at the infertility clinic of Motazadi Hospital in 2021. Inclusion criteria include age group 20-50 years, not having systemic diseases such as diabetes, blood pressure, heart and liver diseases. The participants in this study were anatomically healthy and did not intake any diet supplementation during the last three months, had both testicles, no history of smoking or drugs or intense exercise, without any special drugs, and refrained from sexual intercourse 2-3 days before giving the sample. Exclusion criteria included the absence of any of the items included in the study.

## Semen preparation

Semen samples were collected in the clinic by self-ejaculation in sterile containers with wide openings. After liquefaction: their concentration, volume, and morphology were determined and centrifuged at 1500 rpm for 10 minutes to separate seminal plasma and sperm. Then, the supernatant was separated and divided into two parts, and

stored in a cryotube in liquid nitrogen. In addition, the sediment containing sperm, after washing twice with PBS (pH=7.4), was re-centrifuged at 400 rpm for 5 minutes and used for DNA fragmentation assay.

An atomic absorption device (VGA 77; Varian Inc., Mulgrave, Australia) measured the level of metal ions Fe, Zn, Cu, and Se elements of the samples. Samples were prepared by the Zanao et al. method (22). 200 µl of the sample was mixed with 800 µl of 2% nitric acid preparation solution and 5% Triton-X-100. Finally, 20 µl of the obtained solution and 10 µl of the moderator solution containing palladium 0.05% and magnesium nitrate 0.03% were injected into the device. The obtained result was expressed in mg/ml units.

**FRAP assay**

The ferric reducing antioxidant power (FRAP) method is used to determine the reducing ability of biological samples (total antioxidant capacity/TAC). In this method, colourless ferric complex (Fe<sup>3+</sup> 2, 4, 6-tripyridyltriazine [TPTZ]) is reduced into a violent blue ferrous complex which indicates the reducing properties. Working FRAP reagent was prepared by mixing 2.5 ml TPTZ dissolved in HCl (40 mM), 2.5 ml ferric chloride (20 mM in water) and 25 ml acetate buffer (300 mM, pH=3.6). Then the mixture was heated to 37°C for 10 minutes before use. Next 200 µl of samples were added to 1.5 ml of working FRAP solution and placed in a water bath (37°C) for 30 minutes. The absorption at 593 nm was measured and recorded by spectrophotometer (Jenway 3620D, England). Standard solutions were used at concentrations of 0, 125, 250, 500, and 1000 µM from FeSO<sub>4</sub>·7H<sub>2</sub>O. Each sample was repeated three times (23).

**DNA fragmentation test**

The sperm DNA fragmentation (SDF) amount was checked with SDF kits manufactured by Jina Teb Company (Tehran, Iran). The sediment of the cellular part was washed with PBS and diluted with PBS to the amount of 5-10 million sperm per ml. Then the prepared samples were transferred into gel microtubes and kept in liquid nitrogen until the test was performed. Microtubes containing gel were placed at 95-100 degrees for 5 minutes until complete melting, and 50 µl of the sample was added to the microtube containing the gel and mixed completely. Then 20 µL was taken, placed on a special slide, and immediately covered with a coverslip. The prepared slide was incubated for 4 minutes in the temperature was kept at 2-8 degrees Celsius in the refrigerator.

Then the coverslip was slowly removed from the slide, and solution A of the kit was added to it and placed in the dark at room temperature for 7 minutes. Solution A was slowly drained, and solution B was added and kept at room temperature for 15 minutes. Next, solution B was slowly drained, and distilled water was added and placed at ambient temperature for

3 minutes. At least on each slide, a light microscope (1000× magnification) counted sperm. Finally, the number of sperms with a large or medium halo (normal) and with a small or no halo (broken) was reported as a percentage (24).

**Statistical analysis**

Data were analyzed with SPSS software (version 16, SPSS Inc., USA), their normality was assessed by Kolmogorov-Smirnov test and the results were presented as mean ± SD. Correlation between variables was determined using the Pearson correlation coefficient, and P<0.05 was considered significant.

**Results**

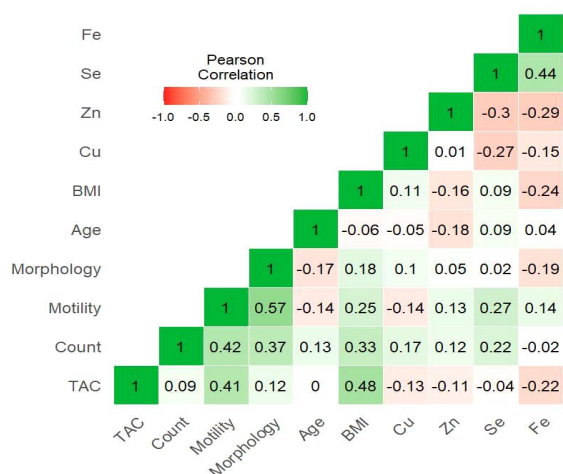
The participants in this study had an average age of 33.63 ± 1.84 (18-48) years and an average height and weight of 178.73 ± 60.1 cm and 79.9 ± 14.5 kg, respectively. The average body mass index (BMI) of these people was 25.01 ± 22.26. Sperm count and motility were 50 ± 30.3×10<sup>6</sup> and 22.83 ± 9.83 µM/seconds, respectively (Table 1). Furthermore, SDF and TAC were 21.33% ± 10.57 and 310.78 ± 215.36 µMol/L. The levels of Cu, Zn, Fe, and Se were shown in Table 1.

**Table 1:** Descriptive indicators of the study values are shown as mean ± SD

Parameter	Mean ± SD	Min-Max
Age (Y)	33.63 ± 1.84	18-48
Height (cm)	178.73 ± 60.1	160-192
Wieght (cm)	79.9 ± 14.55	60-115
BMI (kg/m <sup>2</sup> )	25.01 ± 22.26	18.9-31.8
Sperm morphology (%)	7.3 ± 8.56	2-10
Sperm motility (µM/seconds)	22.83 ± 9.83	20-30
Sperm count (M/ml)	50 ± 30.3	20-100
SDF (%)	21.33 ± 10.57	5-50
TAC (µMol/L)	310.78 ± 215.36	129.6-521.7
Cu (µMol/L)	0.68 ± 0.001	0.1-1.5
Zn (µMol/L)	8552.63 ± 714.97	1951-16425
Se (µMol/L)	76.66 ± 50.62	31.98-141.24
Fe (µMol/L)	21.47 ± 18.42	4.06-72.54

BMI; Body mass index, SDF; Sperm DNA fragmentation, TAC; Total antioxidant capacity, Cu; Copper, Zn; Zinc, Se; Selenium, and Fe; Iron.

The relationship between the studied parameters is shown in Figure 1. There was a weak positive relationship between SDF and BMI, which was not significant (P=0.25, r=0.21) (Table 2). Furthermore, there was a moderate negative correlation between SDF and sperm count (P=0.71, r=-0.71), morphology, and motility (P= 0.75, r-0.61), but these correlations were not statistically significant. The data showed that there was no relationship between SDF and TAC (P=0.92, r=0.01) (Table 2). There is no significant correlation between SDF with the levels of semen Fe, Zn, Cu, and Se (P>0.05, Table 3).



**Fig.1:** Statistical analysis using Pearson correlation test. The relationship between the parameters examined in this study shows the value of the correlation coefficient (r), while those that have reached statistical significance (P<0.05).

**Table 2:** SDF correlation with BMI, TAC, and sperm parameter

Parameter	Pearson correlation with SDF	P value	Number
BMI (kg/m <sup>2</sup> )	0.21	0.25	30
TAC (μMol/L)	0.01	0.92	30
Sperm count (M/ml)	-0.71	0.71	30
Sperm motility (μM/seconds)	-0.61	0.75	30
Sperm morphology (%)	-0.57	0.60	30

Descriptive indicators of the study values are shown as mean ± SD. Data were analyzed by the Pearson correlation coefficient. SDF; Sperm DNA fragmentation, Fe; Iron, Se; Selenium, Zn; Zinc, and Cu; Copper.

**Table 3:** SDF Correlation with Fe, Cu, Zn, and Se

Parameter	Pearson correlation with SDF	P value	Number
Fe (μMol/L)	0.24	0.20	30
Se (μMol/L)	0.02	0.90	30
Zn (μMol/L)	-0.07	0.25	30
Cu (μMol/L)	0.02	0.76	30

Descriptive indicators of the study values are shown as mean ± SD. Data were analyzed by the Pearson correlation coefficient. SDF; Sperm DNA fragmentation, Fe; Iron, Se; Selenium, Zn; Zinc, and Cu; Copper.

## Discussion

The present study investigated the relationship between sperm parameters, metal elements, and the total antioxidant capacity (TAC) of human semen with the state of sperm chromatin. There was a positive correlation between sperm chromatin and BMI, and a negative correlation with sperm count and motility, but there was no correlation with TAC. In addition, there was a weak correlation between this parameter with Fe concentration and no significant correlation with Se, Zn, and Cu concentration. There are conflicting results about the importance of trace elements and oxidative stress in semen. Our understanding in this field is still incomplete and often contradictory and needs to be completed.

In obese people, there is an increase in the permeability

of metabolic endotoxemia in the intestine, which causes systemic inflammation and oxidative stress and damages the male reproductive system, thus leading to an increase in ROS production, oxidative stress, and reduction of sperm DNA integrity (24). A large study has investigated this relationship in 3 years in several centers, including 330 men in infertile couples. The uridine end labeling method showed that the amount of sperm DNA damage is increased in obese men (25). Our data showed that there is a negative correlation between SDF and the number and motility of sperm, this negative correlation was confirmed in previous studies, and the results obtained in the study are in line with previous studies because all samples are normozoospermic (26, 27).

In addition, our data showed that there is no significant relationship between SDF and TAC. Semen plasma TAC is measured by the level of enzymatic and non-enzymatic antioxidants. Low levels of TAC in semen may play an important role in male infertility. Although the amount of ROS is regulated by antioxidants, the excessive production of oxidative stress cannot be neutralized by the antioxidant system in infertile men. A major part of sperm DNA damage is related to oxidative stress. The imbalance between superoxide anion and peroxynitrite with antioxidant capacity of infertile men with abnormal sperm parameters is associated with higher SDF (28). The results of previous studies show a significant correlation between SDF and TAC (29-31), which does not match the results of the present study and is probably due to the difference between the sample of healthy people in our study and infertile people in their study.

A comparison of the SDF relationship with Fe, Cu, Zn, and Se showed that there was a weak correlation between Fe concentration and SDF. In addition, there was no significant relationship between Se, Zn and Cu concentration with SDF. Studies report trace elements' effects on human reproduction are ambiguous. A similar study tested the effect of lead (Pb), cadmium (Cd), Cu, Zn, and Se on SDF and showed that SDF is a dynamic process that increases with rising Pb levels in seminal plasma. The amount of Pb and Cd in the semen plasma of infertile men was higher compared to fertile men. The Zn, Cu, and Se level in semen plasma was higher in men with proven fertility than infertile men and had no significant effect on SDF dynamics. The level of Cd did not have a significant effect on the exacerbation of SDF (32). Due to the contradictory results obtained in this field, these studies should be expanded and continued in the future.

## Conclusion

The results of this study showed that the metal elements measured in the seminal fluid of normozoospermic individuals did not show a noticeable role in sperm chromatin integrity.

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### Authors' Contributions

Sh.R.; Participated in study design and evaluation. N.B.; Data collection. M.R.S.; Contributed extensively to interpreting the data and the conclusion. L.R., N.B.; Wrote the manuscript and editing. M.K.; Conceptualization and validation. All authors read and approved the final version of the manuscript.

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