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Original Article

# Impact of Manganese Levels on Sperm Functional Characteristics Among Men Investigated for Infertility in Benin, Nigeria

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## Abstract

**Background:** Manganese (Mn) is absolutely necessary for several biological functions in the body, but higher concentrations may be extremely harmful to male reproductive health.

**Objectives:** This study aimed to determine Mn, zinc (Zn), and copper (Cu) levels in serum and seminal plasma and correlate their concentrations with sperm quantity and quality among men investigated for infertility.

**Methods:** A total of 70 infertile males in the age range of 25-45 years and 50 control subjects were evaluated in this study. The semen samples were analyzed by the microscopic technique, while Mn, Zn, and Cu were analyzed using an atomic absorption spectrophotometer. Socio-demographic parameters were obtained using a semi-structured questionnaire. Then, unpaired Student's *t* test and chi-square test were used to compare the discrete data between infertile males and controls and categorical data, respectively. Finally, Pearson's correlation coefficient was employed to correlate measured elements with sperm induces.

**Results:** The sperm count, sperm motility, viability, and serum/seminal plasma Zn and Cu levels were significantly lower ($P<0.001$), while the Mn and Cu/Zn ratios were significantly higher ($P<0.001$) among infertile men than control subjects. Serum/seminal plasma Mn levels were inversely correlated with the sperm count ($r=-0.279$, $P=0.02$), motility ($r=-0.279$, $P=0.02$), and morphology ($r=-0.275$, $P=0.04$), while Zn levels were positively correlated with sperm motility ($r=0.238$, $P=0.04$) and morphology ($r=0.258$, $P<0.03$). Eventually, Cu was inversely correlated with motility ($r=-0.237$, $P=0.04$) and morphology ($r=-0.235$, $P=0.04$).

**Conclusion:** Overall, high levels of Mn in the serum and seminal plasma may have an adverse effect on sperm quantity and quality, and thus, there is a need for routine Mn determination as part of the investigation in the evaluation of infertile males irrespective of the occupation of subjects.

**Keywords:** Male, Manganese, Nigeria, Spermatozoa, Infertility

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## Background

Manganese (Mn) is ever present in the environment and is absolutely necessary for humans in small amounts, but may be potentially harmful when present in large amounts. It is readily found in the soil, air, water, and food (1). Mn is an important element that plays a part in several enzymatic reactions. It is involved in alleviating free radical accumulation via its presence in Mn superoxide dismutase (Mn-SOD). Optimum Mn levels in the cells prevent oxidative damage in many living organisms through Nramp transporters (2). Some researchers demonstrated the importance of Mn to structural flexibility for enzyme kinetics since it is needed for RNA to chemically combine to form large molecules (3). Studies indicated that $Mn^{2+}$ has the ability to shorten the oxidation chain length as a result of its reaction with peroxyl radicals in biological systems and stimulates several polymerases at minute levels, but excessive Mn intake may harm the process (4).

Our group has previously reported that essential minerals such as copper (Cu), zinc (Zn), and their Cu/Zn ratio are altered among infertile men, with harmful effects on semen property and infertility. The seminal plasma Zn level is correlated with sperm count and functional indices among infertile men (5). The presence of elevated concentrations of cadmium, lead, and other toxic metals may result in hampering the enzyme systems needed for sperm functional characteristics (6). An experimental study indicated that Mn at a low level of 0.1 mM is essential

for adequate sperm motility in-vitro in an experimental animal at 6 hours than the control without $Mn^{2+}$(7). In addition, the $MnCl_2$ concentration of 0.1 mM was reported to enhance and maintain sperm motility without adverse effects on riddling the mucus and fertilizing ability. Therefore, $Mn^{2+}$is a potent activator of sperm motility by quickening the adenylate cyclase activity (7).

Conversely, there are indications in both animal and human studies that Mn exposure might have adverse effects on spermatogenesis and male fertility. The oral administration of Mn was reported to reduce the sperm count in mice in a dose-dependent manner without histomorphological alterations of the testes (8). In humans, men with the lowest and highest serum Mn levels were observed to have decreased sperm concentrations, suggesting that an optimal Mn level is essential for spermatogenesis (9).

Reproductive biologists are increasingly interested in investigating possible factors that may be responsible for the global downward trend of sperm quality and quantity, a condition that is alarming in the so-called infertility belt of Africa, including Nigeria. This is also imperative because of differences in the geographical location, ethnicity, and available methods of fertility assays, the need to conduct studies that examine the trend in male infertility, and the determination of possible factors affecting male reproductive health cannot be overemphasized. This study was, therefore, designed to determine the levels of Mn, Zn, and Cu levels in both serum and seminal plasma and correlate their concentrations with sperm quantity and quality among men evaluated for infertility.

## Materials and Methods

### *Study Design and Population*

In this cross-sectional study, 70 non-occupationally exposed men aged 25-45 years were investigated for infertility in Central Hospital, Benin, Nigeria in 2017. The subjects were recruited in the study because their partners were unable to conceive after one or more years of unprotected sexual intercourse. Conversely, 50 control male subjects who had no chronic clinical illnesses and had their babies within the last year were enrolled in the study.

### *Inclusion and Exclusion Criteria*

All males aged 25-45 years who presented to the hospital for a fertility evaluation, gave informed consent, and suffered from no physical deformities or chronic diseases were enrolled in the study. Men who had no chronic clinical diseases, had their babies within the last year, and whose seminal fluid analysis gave sperm count ≥ 15 million sperm cells/mL of ejaculate were used as controls. Conversely, men with known sexual/physical deformities or with chronic diseases such as hypertension, diabetes mellitus, sexually transmitted diseases, testicular varicocele, and genital warts were excluded from the study. Those currently on antioxidant supplementation, smokers, alcohol abusers, and with known endocrine diseases were excluded as well.

### *Sample Size Determination*

The sample size (N) was determined using the formula by Lwanga and Lemeshow (10) and considering the 4.0% prevalence of male infertility in Ilorin, Nigeria (11). The microscopic examination of the semen was performed according to the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen (fifth edition), 2010 criteria with interest in the sperm count, percent motility, percent viability, and percent morphology (abnormal sperm head and/or tail defects were recorded) (12).

The calculated minimum sample size was 59, but 70 cases and 50 controls were used based on the purpose of this study.

### *Sample Collection*

The semen specimen was delivered to the laboratory immediately after collection without the use of spermicidal lubricants in a wide-mouth sterile container. Before the collection, the subject was mandated to abstain from sexual intercourse for at least three days. For the purpose of quality control, two specimens were collected and analyzed within 2 months, and the mean value was used accordingly. If the difference between the first and second results was > 10%, a third measurement was made by another analyst and the mean of the closer observation was obtained accordingly.

A blood sample was also collected the same morning the semen was submitted, and 5 mL of the blood was collected in a plain container. The blood was spun after clot retraction at 3000 rpm for 10 minutes, and the serum was separated into another clean test tube. The serum was kept at -80 ˚C until analysis.

### *Semen Analysis*

The semen was kept at 37 ˚C and assessed for volume, appearance, pH, and viscosity. The microscopic examination of the semen was performed according to the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen (fifth edition), 2010 criteria with interest in the sperm count, percent motility, percent viability, and percent morphology (abnormal sperm head and/or tail defects were recorded). The serum/seminal Zn, Cu, and Mn levels were determined by an atomic absorption spectrophotometer as previously described (13).

### *Statistical Analysis*

Data were computed using SPSS, version 23.0 software (SPSS Chicago, IL, USA). The mean values of the infertile males and controls were compared using an unpaired Student's *t* test. The chi-square test was employed to compare the categorical data, while Pearson's correlation coefficient was applied to assess the association between

the metals and sperm induces. Any $P \leq 0.05$ was considered significant. The normal distribution was visually checked by plotting the frequency distribution of the data.

## Results

Table 1 presents the demographics of men investigated for infertility. Of the 70 men, 61.4%, 21.4%, and 17.2% of cases were in the age range of 31-40, 41-45, and 21-30 years, respectively ($P<0.001$). All the respondents had a formal education; in general, 5.7%, 25.7%, and 68.6% had primary, secondary, and tertiary education, respectively ($P<0.001$). More than half (51.4%) of the respondents were civil servants, while 15.7%, 5.7%, and 21.4% were artisans, farmers and industrial workers, and businessmen, respectively ($P<0.001$). The duration of marriage showed that 62.9%, 22.9%, and 14.0% were ≤5, 6-10, and 11-15 years, respectively.

Table 2 provides the comparison of sperm indices among men investigated for infertility and control subjects. Sperm count, sperm motility, sperm morphology, and viability were significantly lower ($P<0.001$) among men evaluated for infertility compared to control subjects. Based on data in Table 3, serum levels of Zn, and Cu were significantly lower ($P<0.001$) while Mn and Cu/Zn ratios were significantly higher ($P<0.001$) among men investigated for infertility than control subjects. Table 4 presents the comparison of the seminal plasma concentrations of Cu, Zn, and Mn between men investigated for infertility and control subjects. Seminal plasma Zn was significantly lower ($P<0.001$), while seminal plasma Mn was significantly higher ($P<0.001$) among infertile men than control subjects. Although the concentration of seminal plasma Cu was higher among infertile men than the control, the difference was insignificant ($P=0.42$). Table 5 indicates the seminal plasma concentrations of Zn, Cu, and Mn among infertile men based on sperm counts. Seminal plasma Mn and Cu increased with decreasing sperm counts, while Zn concentrations increased with increasing sperm count. The differences between the means were statistically significant ($P<0.001$). Table 6 summarizes the occupational distribution of the subjects based on sperm counts. Based on the findings, 11/70 (15.7%), 36/70 (51.4%), and 15/70 (21.4%) were artisans, civil servants, and businessmen, while 4/70 (5.7%) of them were farmers and industrial workers, respectively. There was no significant correlation between sperm count and occupational distribution. The results (Table 7) indicate that Mn levels were negatively correlated with the sperm count (r = -0.279, $P=0.02$) and morphology (r = -0.275, $P=0.04$), while Zn levels were positively correlated with sperm motility (r-0.238, $P=0.04$) and morphology (r = 0.258, $P=0.03$). Moreover, Cu was inversely correlated with motility (r = -0.237, $P=0.05$) and morphology (r = -0.235, $P=0.05$).

**Table 1.** Socio-demographic Characteristics of Infertile Male Subjects

| Variables | Frequency | Percent | Chi-square |
|---|---|---|---|
| Age group (y) | | | |
| 21-30 | 12 | 17.2 | |
| 31-40 | 43 | 61.4 | 0.005 |
| 41-45 | 15 | 21.4 | |
| Education | | | |
| Primary | 04 | 5.7 | |
| Secondary | 18 | 25.7 | 0.001 |
| Tertiary | 48 | 68.6 | |
| Occupation | | | |
| Artisans | 11 | 15.7 | |
| Civil servants | 36 | 51.4 | |
| Farmers | 04 | 5.7 | 0.001 |
| Industrial workers | 04 | 5.7 | |
| Business men | 15 | 21.4 | |
| Age of marriage (y) | | | |
| ≤5 | 44 | 62.9 | |
| 6-10 | 16 | 22.9 | 0.001 |
| 11-15 | 10 | 14.0 | |

**Table 2.** Comparison of Sperm Indices Among Men Investigated for Infertility and Control Subjects

| Variables | Infertile Men (n=70) | Fertile Men (n=50) | *P* Value |
|---|---|---|---|
| Sperm count (×10⁶/mL) | 35.2±2.10 | 68.4±2.01 | 0.001 |
| Motility (%) | 36.8±5.18 | 82.1±7.01 | 0.001 |
| Morphology (%) | 34.7±4.8 | 56.2±6.2 | 0.001 |
| Viability (%) | 32.0±1.2 | 60.2±4.8 | 0.001 |
| pH | 7.8±0.1 | 7.8±0.2 | 1.0 |
| Volume (mL) | 2.9±0.1 | 3.0±0.1 | 0.9 |

## Discussion

Studies indicated decreasing sperm quantity and semen quality among humans over the past decades from all continents of the world. The reason(s) for these decreases is not exactly clear. This worrisome event has motivated reproductive biologists to keep searching for the possible

**Table 3.** Comparison of Measured Essential Elements Among Males Investigated for Infertility and Control Subjects (Mean±SD)

| Parameters Reference Range | Infertile Men (n=70) | Fertile Men (n=50) | *P* Value |
|---|---|---|---|
| Zinc (µg/mL, 0.64-1.24) | 0.81±0.01 | 2.06±0.08 | 0.001 |
| Copper (µg/mL, 0.75-1.53) | 1.41±0.08 | 1.93±0.05 | 0.90 |
| Manganese (µg/L, 0.48-4.3) | 5.61±0.50 | 0.52±0.20 | 0.001 |
| Copper/Zinc ratio (1:1) | 1.73±0.01 | 0.92±0.01 | 0.001 |

*Note*. SD: Standard deviation.

**Table 4.** Comparison of Measured Metal Elements in Seminal Plasma Between Males Investigated for Infertility and Controls (Mean±SD)

| Metals | Infertile Males | Fertile Males | *P* Value |
|---|---|---|---|
| Copper (µg/mL) | 2.01±0.85 | 1.92±0.05 | 0.42 |
| Zinc (µg/mL) | 1.67±0.57 | 2.06±0.08 | 0.001 |
| Manganese (µg/L) | 11.78±2.50 | 7.28±2.15 | 0.001 |

*Note*. SD: Standard deviation.

**Table 5.** Concentration of Metals in Seminal Plasma Among Males Investigated for Infertility Based on Sperm Counts

| Metals | Normozoospermia (n = 35) | Oligozoospermia (n = 25) | Azoospermia (n = 10) | *P* Value |
|---|---|---|---|---|
| Copper (µg/mL) | 1.90 ± 0.86 | 1.92 ± 0.95[a] | 2.68 ± 0.98[b] | 0.001 |
| Zinc (µg/mL) | 2.18 ± 0.91 | 1.30 ± 0.70[b] | 0.83 ± 0.21[b] | 0.001 |
| Manganese (µg/mL) | 6.48 ± 3.21 | 16.21 ± 5.21[b] | 19.22 ± 3.42[b] | 0.001 |

*Note*. a = $P > 0.05$, b = $P < 0.001$.

**Table 6.** Occupational Distribution of the Subjects Based on Sperm Indices

| Occupation | Normozoospermia (>15 Million Sperm Cells/mL) | Oligozoospermia (<15 Million Sperm Cells/mL) | Azoospermia (No Sperm Cells) | Pearson's $\chi^2$ | *P* Value |
|---|---|---|---|---|---|
| Artisans (n = 11) | 03 | 06 | 02 | 46.01 | 0.92 |
| Civil servants (n = 36) | 25 | 08 | 03 | | |
| Farmers (n = 4) | 02 | 02 | 00 | | |
| Industrial workers (n = 4) | 01 | 03 | 00 | | |
| Businessmen (n = 15) | 04 | 06 | 05 | | |
| Total | 35 | 25 | 10 | | |

**Table 7.** Correlation of Essential Elements With Sperm Indices Among Infertile Males

| Variables | Sperm Count | | Motility | | Morphology | |
|---|---|---|---|---|---|---|
| | R | *P* | R | *P* | R | *P* |
| Zinc | 0.225 | 0.06 | 0.238 | 0.04 | 0.258 | 0.03 |
| Copper | -0.226 | 0.06 | -0.237 | 0.04 | -0.235 | 0.05 |
| Manganese | -0.239 | 0.04 | -0.279 | 0.02 | -0.275 | 0.04 |

impacts of environmental factors such as lifestyle, environmental, and occupational exposure to mineral elements and toxic metals, environmental pollutants, and pesticides on semen quality and male reproductive health (14). It was suggested that more studies are needed to investigate the prevailing tendencies of male reproductive disorders and to reconnoiter or seek factors responsible for the declining male reproductive potentials (15,16).

In this study, the concentrations of Mn, Zn, and Cu were determined in both serum and seminal plasma of non-occupationally exposed male partners of infertile couples, and their concentrations were correlated with semen indices. Serum Zn and Cu were significantly lower, while the serum Mn and Cu/Zn ratios were significantly higher among infertile men than control subjects. Similarly, seminal plasma Zn was significantly lower, whereas seminal Mn was higher among infertile men than control subjects. Seminal plasma Mn and Cu increased with decreasing sperm count, while the Zn concentration increased with increasing sperm count. Furthermore, serum Mn was inversely correlated with sperm count, motility, and morphology. Cu was inversely correlated with motility and morphology, while serum Zn was positively correlated with sperm motility and morphology.

Mn is absolutely necessary for human health, even though it is involved in several important functions for normal reproductive health, and higher concentrations than normal may be harmful to reproductive health (7,17,18). The seminiferous epithelium is highly sensitive to the sudden onset of events that can have deleterious effects on sperm production, and these issues should be detected during the male fertility evaluation. Environmental exposures to chemicals for a long time even at low-dose and in nutrients are common, but their identification is difficult. Hence, public health research into the effects of these contaminations from environmental toxicants have increased leading to successive additions of these exposures on human health, including spermatogenesis and male fertility. Additionally, spermatogenesis is a complex process that is modulated by several factors, including genes, the adequate function of the testis, and optimal hormonal quickening from the hypothalamus and pituitary gland. Any aberration at various levels where these regulations occur might impact spermatogenesis (9).

The observed alteration in the concentrations of Zn, Cu, and Mn in both serum and seminal plasma of study participants is consistent with previous studies (7,15). It was demonstrated that the addition of an appropriate concentration (0.1 mM) of $MnCl_2$ had favourable or beneficial effects on sperm motility without harmful effects on mucus penetration and fertilizing potential. The Mn ion is a powerful stimulator of sperm motility via the induction of adenylate cyclase activities (15). In a previous study among 52 Nigerian male partners of infertile couples, it was reported that significantly higher serum Mn levels were observed among normospermic infertile men than oligozoospermic and azoospermic subjects (19). In the present study, the mean Mn concentrations among infertile males, irrespective of the sperm count, were observed to be significantly higher than fertile counterparts. In a study evaluating the soil contents of some metals from five different locations in two states in Nigeria (Cross River and Akwa Ibom), it was found that the Mn concentration was the highest out of the determined trace metals. This is important because most of the trace elements in human diets are derived from plants, water, animal foods, and ultimately the soil (20). In addition, the

serum Mn level was reported to be significantly elevated among infertile men than among fertile control subjects, and a significant inverse association was observed between the Mn concentration and sperm motility and morphology in a general population in China (15). Similarly, severe damaging effects on reproductive health were reported in occupationally exposed men to Mn. For example, prolonged semen liquefaction time and lower percentage of sperm motility among workers exposed to Mn, and lower percentage of motile sperm with longer duration of employment as a miner were reported in another study (21). The activities of some antioxidant enzymes have been found to be lower among workers occupationally exposed to Mn. Specifically, the catalase activity of red blood cells was observed to be lower among workers who spent up to 10 years as Mn miners than in controls, suggesting that high Mn levels might lead to lower activities of antioxidant enzymes, and subsequently cell damage (22). A significantly lower seminal plasma Mn level was reported among men with oligospermia (23). In a study involving 200 men investigated for infertility (24), significantly higher Mn levels were associated with the increased risk of low sperm motility (OR = 5.4, 95% CI: 1.6-17.6) and low sperm concentration (OR = 2.4, 95% CI: 1.2-4.9).

Cheema et al (25) examined the protective role of Mn for cattle bull semen cryopreservation for the purpose of in vitro fertilization and observed that the addition of different concentrations of Mn to egg-yolk-citrate extender + glycerol (EYC-G) resulted in sperm function improvements, decreased lipid peroxidation, and protein leakage. The researchers concluded that the addition of Mn to the EYC-G dilutor may enhance the fertilizing potential of spermatozoa and improve in-vitro fertilization and artificial insemination success rates among cattle.

Conversely, some researchers examined the in vitro effects of Mn on semen quality in healthy men and reported conflicting observations (26-28). A previous study among infertile Nigerian males reported that seminal Mn was significantly lower in both infertile diabetic and non-diabetic subjects than in fertile men (29).

## Conclusion

The findings revealed that the serum Mn concentration was significantly higher among infertile men than among control subjects, and high levels of the essential elements might be harmful to male reproductive health. The need for routine Mn determination as part of the investigation in the evaluation of infertile males is suggested irrespective of the occupation of the subjects. Relatively higher levels of Mn might adversely impact sperm quality given that Mn is necessary for many metabolic processes, including reproduction. The finding related to significantly higher concentrations are critical because the high mean Mn level observed was above the normal range for Mn in the blood, while the low level was within the normal range reported among the control subjects, representing that high levels of Mn are a potential risk factor for poor semen quality.

### Authors' Contribution

**Conceptualization:** Mathias Abiodun Emokpae.
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**Writing – Original Draft:** Winner Oluwarotimi Bamidele.
**Writing – Review & Editing:** Mathias Abiodun Emokpae.

### Competing Interests

The authors declare no potential conflicts of interest relevant to this article.

### Ethical Approval

All individuals that participated in this study gave informed consent to be part of the study. The ethical approval was obtained by the Edo State Ministry of Health, Benin (Reference number: HM.1208.355).

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