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Original Article

Serum and Seminal Fluid Zinc Concentrations among Infertile Men with Oligozoospermia at University of Ilorin Teaching Hospital

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ABSTRACT

Introduction: Infertility is a major reproductive health challenge and usually a source of concern for the affected couples. The Sub-Saharan African community is known for its patriarchal nature, where male factor infertility is often under-reported. Seminal fluid analysis (SFA) abnormality is a central factor in male infertility, and deficiency of some micronutrients, like zinc, has been implicated because of their roles in sperm production and functions.
Aim: This study aimed to determine the serum and seminal fluid zinc levels among men with oligozoospermia at the University of Ilorin Teaching Hospital.
Methods: This is a cross-sectional study of 76 men with oligozoospermia at the Assisted Reproductive Technology (ART) Unit of University of Ilorin Teaching Hospital (UITH) Nigeria. The recruited participants had SFA on two occasions, 4 to 6 weeks apart. Their serum and seminal fluid zinc concentrations were determined using spectrophotometry. Data was analyzed using SPSS version 26.0, and the results were presented in tables and figures.
Results: The mean age of the participants was 38.01 ± 6.38 years, the majority (72.4%) were employed, and a large proportion (89.5%) had a tertiary education. About 78.9% had primary infertility. Mean serum zinc levels were low among participants, but seminal fluid zinc concentration was unaffected.
Conclusion: The study reported reduced zinc levels in men with infertility, further supporting the use of this micronutrient for routine supplementation in our fertility clinics.

Keywords: Infertility; Micronutrient; Oligozoospermia; Seminal Fluid Analysis; Zinc

INTRODUCTION

He Infertility is a multifaceted issue, influenced by biological, lifestyle, and sociocultural factors, and it affects individuals uniquely based on gender, personal choices, and cultural context. It is defined as the inability to achieve pregnancy after 12 months of regular, unprotected sexual intercourse. Primary infertility is when a woman has never been pregnant or conceived, while secondary infertility is when a woman has conceived previously, irrespective of the outcome of pregnancy. Male infertility contributes to about 50% of infertility, either as a sole factor or in conjunction with the female factor (Ali *et al*., 2007; Devranoğlu *et al*., 2024). In Nigeria, male factor accounts for about 42.4% of infertility, with oligozoospermia and asthenozoospermia being the most common aetiological factors (Ikechebelu *et al*., 2003).

The diagnosis of male infertility is made by assessing semen parameters, including volume, count, concentration, motility, and morphology (Akang *et al*., 2023). Infertile men usually show abnormalities in the pattern of semen parameters, ranging from oligozoospermia (abnormality in sperm count (<39 million per ejaculate) or sperm concentration (<15 million/ml)), asthenozoospermia (abnormality in total motile sperm or progressive motility), and teratozoospermia (abnormal morphology), or a combination of varying degrees of these abnormalities (National Institute for Health and Care Excellence [NICE], 2014; Owolabi *et al*., 2013).

On the other hand, zinc is an essential micronutrient and trace element that plays a key role in human reproduction. It plays a significant role in testicular development and spermatogenesis.

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It plays a critical role in stabilizing the membrane and protecting sperm from oxidative stress, due to the high zinc concentrations in prostatic fluid and seminal plasma (Adewunmi *et al.*, 2018). It has been shown that the concentration of zinc in the seminal plasma and semen of infertile men is usually significantly lower, thereby giving the correlation that zinc plays a vital role in reproduction. The normal reference value for seminal plasma zinc level is ≥2.4 µmol (Centre base ≥1.5 µmol/ L) (WHO, 2010). However, studies have shown higher values of up to 100-200mg/L (1768 µmol/L) among the Japanese population (Zečević *et al.*, 2025), which is higher than the serum zinc level because of the high concentration of zinc in prostatic secretion; however, the normal range serum zinc level in adults is 11.1 to 19.5 µmol/L (center base/lab base range). It is noteworthy that some infertile males exhibit dwindling serum zinc concentrations simultaneously with the depletion of seminal fluid zinc levels, suggesting a potential relationship in zinc distribution and utilization in the reproductive system (Adewunmi *et al.*, 2018). Zinc is highly beneficial in human physiology and plays a vital role in males especially in testosterone balance, several enzymatic reactions, prostatic sexual health antibacterial and antioxidant roles but its role in the evaluation of male infertility is still limited and unclear (Hibi *et al.*, 2020). In sub-Saharan African countries where male infertilities are vastly under-reported, serum zinc analysis may serve as an adjunct to seminal fluid analysis in the diagnosis of male infertility. This study aims to determine the pattern of semen parameters and seminal zinc level among oligozoospermic men at UITH.

MATERIALS AND METHODS

Study design/ Study setting

This is a cross-sectional study conducted in an Assisted Reproductive Technology (ART) Unit of UITH. The ART Unit is a section of the Obstetrics and Gynaecology Department and is staffed by 3 consultants. There is an attached laboratory with a chemical pathologist, 2 andrologists and 3 laboratory scientists. The study involved 76 infertile men with oligozoospermia who were recruited and had semen analysis on two occasions, 4-6 weeks apart. Informed consent was obtained from participants who met the inclusion criteria after proper counselling sessions in simple language that they understood.

Inclusion criteria

Infertile men aged 20-59 years with a sperm concentration less than 15 million/ml on two occasions, 4-6 weeks apart, were included in the study.

Exclusion criteria

Men with azoospermia, aspermia, varicocele, erectile dysfunction, a history of smoking, medical conditions such as diabetes mellitus and hypertension, and participants currently receiving treatment for sexually transmitted infections were excluded.

Sample size determination

This was done using the formula (Araoye, 2003):

$$n = \frac{z^2pq}{d^2}$$

n: Minimum sample size

z: Standard deviation set at 1.96, which corresponds to the 95% confidence interval.

P: 4.8% (0.048) prevalence of male infertility in sub-Saharan Africa from a previous study (Agarwal *et al.*, 2015)

Q: 1-p = (1 - 0.048) = 0.952

d: degree of accuracy desired = 0.05

n = 70

Adjusting for attrition using the formula:

N= n/ (100-r %)

Where r% is the anticipated attrition rate, which is 10%.

r = anticipated attrition rate in percentage

N = 78

A total of 78 participants were recruited for this study; however, 76 men were included in the final analysis, as 2 subjects had incomplete data.

A convenience sampling technique was used, and Information on sociodemographic and fertility history was obtained from the clients.

Procedures

Semen analysis

The semen samples were obtained from the participant after masturbation in a sterile wide-mouthed container at a designated area within the ART unit, which was next to the laboratory, following abstinence from sexual intercourse for a minimum of 3-5 days. The samples were immediately handed over to the laboratory scientist after collection to preserve spermatozoa viability. Samples were allowed to liquefy at room temperature (25°C–30°C), as monitored by a thermometer mounted on the laboratory wall, for at least 45 min. After liquefaction, samples were analyzed for volume, sperm count, motility, and morphology. The basic laboratory procedures for SFA were in accordance with the World Health Organization (WHO) guidelines (WHO, 2010)

The procedure of Seminal Fluid Analysis

Sperm Count: Total sperm count was carried out using the Neubauer counting chamber.

The Improved Neubauer Haemocytometer Counting Chamber

It is made of glass material with a chamber depth of 0.1mm. It has two separate counting chambers, each of which has a microscopic 3 mm × 3 mm pattern of gridlines etched on the glass surface. It was used with a special thick coverslip (thickness number 4, 0.44 mm), which lies over the grids and is 33 supported by glass pillars 0.1 mm above the chamber floor. Each counting area is divided into nine 1 mm ×1 mm grids. With a depth of 100µm, each grid holds 100nl. Four of these grids (nos 1, 3, 7, and 9) contained four rows of four squares, each holding 6.25 nl; two grids (nos 2 and 8) contain four rows of five squares, each of 5 nl; two grids (nos 4 and 6) contain five rows of four squares, each of 5 nl; and the central grid (number 5) contains five rows of five squares, each of 4 nl. Each of the 25 squares of the central grid (number 5) is subdivided into 16 smaller squares. Thus, grids 1, 2, 3, 7, 8, and 9 each have four rows holding 25 nl per row, while grids 4, 5, and 6 each have five rows holding 20 nl per row. The semen was immobilized and diluted using WHO diluent; dilutions 1:10, 1:20, 1:50, 1:100 depending on sperm density; count sperm number in all 25 squares of the central grid; multiply total sperm number by 0.1, 0.2, 0.5 or 1.0 (depending on dilution) to obtain sperm concentration in millions/ml (WHO, 2010).

Macroscopic Examination

Semen samples undergo a macroscopic examination at room temperature and as the sample liquefied, the semen became thinner. Complete liquefaction of semen was completed in 30-60 minutes at room temperature (WHO, 2010). The semen volume was measured by using a modified graduated measuring cylinder with a wide mouth, into which the semen was collected. The volume was read directly from the graduation on the measuring cylinder (WHO, 2010). Sperm motility was assessed as soon as the liquefaction of the semen sample is completed. The semen sample was mixed well; an aliquot of semen was removed immediately. The semen sample was remixed and a replicate aliquot was removed again. A wet preparation was made on a slide and examined microscopically (MshOt microscope, ×40 lens). The percentage of different motile categories according to World Health Organization (WHO, 2010) guidelines was graded as, progressive motility, non-progressive motility, and immotile. Finally, Sperm morphology was determined by preparing a smear of semen on a slide, air drying, fixing and staining the slide, mounting the slide with a cover slip, examination of the slide under the microscope, and assessing the percentage of normal and abnormal forms and calculations of sperm morphology. The semen smear for morphological analysis was air dried, fixed, and stained with eosin–nigrosin stain (Papanicolaou stain) (WHO, 2010).

Seminal fluid zinc analysis

The remaining semen sample following the semen analysis was centrifuged at 3000 revolutions per minute for 10 minutes, and the supernatant was stored at -20 °C for assaying the seminal zinc level. The samples were pooled together, and semen zinc levels were measured.

Procedure for seminal fluid zinc analysis

Following semen analysis, the remaining semen sample was centrifuged at 1000g (3000rpm) for 10 minutes. The remaining semen sample was decanted and the sperm- free seminal plasma was stored at -20 ^{0}C until when the sample was analyzed. Sperm-free seminal plasma was pooled with other samples to provide a standard for internal quality control in future assays. The sperm-free seminal plasma was thawed and mixed well with a vortex mixer. At the same time, an aliquot of pooled seminal plasma was mixed for internal quality control. Dilutions of each sample of seminal plasma were prepared to replicate this by adding 5 µl of seminal plasma using a positive displacement pipette to 300 µl of purified water in each of the two 1.5-ml tubes and mixed by vortexing for 5 seconds. 40 µl of the replicated aliquots of the diluted seminal plasma sample from step 3 was added to a 96-well plate. Replicate of blanks was included (40 µl) of purified water) and 40 µl replicates of each of the standards. 200 microliters of the colored reagent were added to each well and mixed for five minutes on a 96-well plate shaker. The plate was read at 560nm wavelength; the blank water was used to set zero (WHO, 2010).

Serum zinc analysis

A venous blood sample was collected by the researcher using a labelled plain Vacutainer bottle, following sterile procedure and observing basic universal precautions. A tourniquet was applied around the arm. The cubital fossa was swabbed with spirit, which was then allowed to dry, and 5 ml of blood was drawn from median cubital vein. The sample was also centrifuged at 3000 rpm for 10 minutes, and the supernatant was stored at -20 °C for analysis of the serum zinc concentration using a spectrophotometer.

Procedure serum zinc level

Following overnight fast blood sample was taken from the vein, the red cells were allowed to settle, and the serum was centrifuged at 3000 rpm for 10 minutes. The serum sample was decanted, and the serum free sample was stored at -20^{0}C until when the sample was analyzed. The serum free sample was thawed and mixed well. A reagent blank was prepared by adding 1 ml of reagent R into a test tube. The standard was prepared by adding 1 ml of reagent R and 50 µl of standard solution into another test tube. For each test sample, the appropriate volume of serum was added to 1 mL of reagent R in a separate test tube. The blank, standard, and samples were mixed thoroughly and incubated for 10 minutes at 25°C or 5 minutes at 37°C. They were subsequently transferred into a cuvette and the cuvette was placed in the Rx Monza flow cell holder for absorbance reading. The absorbance of the sample was recorded as sample absorbance, while the absorbance of the standard was recorded

as standard absorbance, both measured against the reagent blank (WHO, 2010)

Data Analysis

Data was analyzed using IBM Statistical Package for the Social Sciences (SPSS) version 26.0 (Armonk, NY). Descriptive statistics were computed, and the results were presented in tables and charts. Continuous variables were expressed as mean ± standard deviation (SD),

Ethical Consideration

Ethical review was obtained from the University of Ilorin Teaching Hospital (UITH) Ethics Review Committee, with ethical approval number ERC PAN/2021/05/0152. Participants' data and all personal information were kept confidential

RESULTS

The mean age of the participants was 38 ± 6.38 years. The majority of participants were married (100%), employed (72.4%), and had tertiary-level education (89.5%). Overall, 70 (92.1%) of the participants were Yorubas, as shown in Table 1.

The majority (71.1%) of participants had infertility for less than 5 years, and 93.4% were in a monogamous relationship, while the mean number of wives was 1.08 ± 0.32, as shown in Table 2. Figure 1 shows the type of infertility among the study participants, with more than two-thirds of the participants having primary infertility (78.9%), and 21.1% had secondary infertility.

Table 3 shows reduced sperm count, concentration, and motility, but normal morphology, in comparison with the NICE guideline 2014. Also, the serum zinc level was slightly lower than the normal values expected for an adult, whereas a normal seminal fluid zinc level is reported.

More than two-thirds of the participants had primary infertility 78.9%, with 21.1% having secondary infertility.

Table 1: Socio-demographic variables of the study participants (N=76)

Variable	Frequency	Percentage (%)
Age group (Years)		
20 – 29	2	2.6
30 – 39	41	53.9
40 – 49	26	34.2
50 – 59	7	9.2
Mean ± SD (38 ± 6.38)		
Range (23 – 58 years)		
Marital status		
Married	76	100.0
Unmarried	0	0.0
Employment status		
Employed	55	72.4
Unemployed	20	26.3
Student	1	1.3
Educational status		
Secondary	8	10.5
Tertiary	68	89.5
Ethnicity		
Yoruba	70	92.1
Igbo	1	2.6
Others	4	5.3

Table 2: Duration of infertility and family settings of participants

Variable	Frequency	Percentage
Duration of Infertility		
≤ 5 years	54	71.1
6 – 10 years	17	22.4
> 10 years	5	6.6
Mean ± SD (5.00 ± 3.79 years)		
Range (2 – 26 years)		
Family setting		
Monogamous	71	93.4
Polygamous	5	6.6
Mean no of wives ± SD (1.08 ± 0.32)		
Range (1 – 3)		

Table 3: Serum Zinc concentrations, Semen parameters and seminal fluid zinc levels among the participants.

Variables	Mean ± SD	Reference limits
Seminal zinc levels (µmol/l)	16.81±10.31	≥2.4
Serum zinc level (µmol/l)	19.24±8.79	≥19.5
Sperm count (million/ ejaculate)	10.96±10.03	≥39
Sperm concentration (million/ml)	4.48±4.24	≥15.0
Progressive Motility (%)	11.57±14.81	≥32
Normal Morphology (%)	10.48±3.69	≥4%

DISCUSSION

This study reported a mean age of 38.01 ± 6.38 among infertile men with oligozoospermia, which is similar to a survey of semen parameters among oligozoospermic men in India (Firdous *et al.*, 2023). This corresponds to the age when the pressure of infertility and the urgency to seek medical intervention by most couples in our local environment. The majority of research participants were married, mostly educated to a tertiary level, and Yoruba. This is likely because the study site is in the state capital, where most of the working class reside, and because literate individuals tend to seek medical care more than less educated individuals. The study was conducted in an ART unit of UITH. Assisted conception remains very expensive and is beyond the reach of low-income earners and the unemployed. This finding is similar to that of a study in South-Western Nigeria by Ottun *et al*. (2023), which highlighted that ART is still not within the reach of most Nigerian couples. Despite the heterogeneity and tribal distribution of North-Central Nigeria, we found a predominantly Yoruba group of participants, which may be due to the city's Yoruba population and its commercial activities drifting more toward the South-Western part of the country.

Generally, all semen parameters were affected except morphology, and participants had slightly reduced blood zinc concentrations, while their seminal fluid zinc levels remained normal. The role of zinc in male fertility, particularly regarding sperm quality, has been extensively studied. Zinc's essential role in spermatogenesis, sperm membrane stability, and overall fertility has been consistently emphasized in both fertile and infertile men (Allouche-Fitoussi & Breitbart, 2020). Our findings show normal seminal plasma zinc concentrations in infertile men (oligozoospermia). This is in contrast with reports from Zečević *et al*. (2025), Chia *et al*. (2000), Kothari & Chaudhari (2016), Ajose *et al*. (2024), Firdous *et al*. (2023) and Igibah *et al*. (2023) who observed lower zinc levels in the seminal plasma of infertile men compared to fertile controls, highlighting zinc's key role in sperm motility, viability, density, and chromosomal regulation. Although most studies report a correlation between reduced serum zinc and seminal fluid zinc, our study found only lower blood zinc levels; this difference may suggest that a decrease in plasma zinc takes time before manifesting in other bodily fluids. The discrepancy might also reflect population differences and dietary zinc intake. The data further suggest that serum zinc levels could serve as a potential biomarker for male infertility. Notably, the relationship between blood and seminal plasma zinc concentrations remains unclear. However, this study indicates that blood zinc levels may provide a quick overall indicator of zinc status, while seminal plasma zinc levels might offer a more long-term assessment of male fertility.

Also, Hibi *et al*. (2020) evaluated 2010 infertile males and found that 45.9% had serum zinc deficiency, although the study was conducted among Japanese whose diets varied widely from those in Africa. Furthermore, as highlighted in the review by Devranoğlu *et al*. (2024) male infertility is a multifactorial condition, with genetic, hormonal, and environmental factors contributing to its development. Zinc deficiency, in particular, may exacerbate existing infertility conditions and may also be influenced by lifestyle factors such as smoking, excessive alcohol consumption, and poor diet. It is essential, therefore, to consider these additional factors when assessing the role of zinc in male infertility. A broader approach, including lifestyle modification, zinc supplementation, and targeted medical interventions, may offer more effective solutions for treating male infertility.

Conclusion: Our study supports the growing body of evidence indicating that zinc may play a critical role in male infertility, particularly in relation to sperm quality. Maintaining optimal zinc levels in seminal fluid and plasma can improve sperm counts and overall sperm function, providing a potential biomarker for infertility diagnosis and an avenue for targeted treatments. Further research, particularly randomized controlled trials evaluating the effects of zinc supplementation on sperm quality, is warranted to confirm these findings and explore the therapeutic potential of zinc in male infertility.

Strength and Limitations: The limitations of this study include the relatively small sample size and the observational nature of the analysis, which restricts the ability to draw causal inferences. Also, while seminal zinc concentrations were measured, the impact of zinc supplementation on sperm quality was not evaluated, which could have provided valuable insights into the therapeutic potential of zinc in male infertility. Additionally, serum zinc concentration is influenced by factors such as time of day, hunger, and stress, which were not accounted for in this study. Another limitation of this study might be the fact that we do not have a control group to compare with. However, the study's strength lies in the fact that the seminal fluid analysis was repeated on two occasions, at least 4-6 hours apart, before the diagnosis.

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