CORRELATION OF BLOOD AND SEMINAL PLASMA ZINC LEVELS AND SEMEN PARAMETERS
INFERTILE AND INFERTILE NIGERIAN MEN.

A DISSERTATION SUBMITTED TO THE NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE FELLOWSHIP OF THE COLLEGE IN OBSTETRICS AND GYNAECOLOGY.

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DEDICATION

This book is dedicated to the Almighty God, the giver of life and wisdom, for His guidance and support in all my endeavours. To my dear wife, Ramat and my lovely children, Bryana, Kennice, Daniella and Anora and to the memory of H.R.H Edward Oyahire Ogah.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>4-8</td>
</tr>
<tr>
<td>Introduction</td>
<td>9-12</td>
</tr>
<tr>
<td>Rationale for the study</td>
<td>13-14</td>
</tr>
<tr>
<td>Literature review</td>
<td>15-23</td>
</tr>
<tr>
<td>Objectives and Hypothesis</td>
<td>24</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>25-41</td>
</tr>
<tr>
<td>Results</td>
<td>42-58</td>
</tr>
<tr>
<td>Discussion</td>
<td>59-67</td>
</tr>
<tr>
<td>References</td>
<td>68-83</td>
</tr>
<tr>
<td>Appendix</td>
<td>84-90</td>
</tr>
</tbody>
</table>
CORRELATION OF BLOOD AND SEMINAL PLASMA ZINC LEVELS AND SEMEN PARAMETERS IN FERTILE AND INFERTILE NIGERIAN MEN

ABSTRACT

BACKGROUND: Infertility constitutes a major part of the health problems in our society and male infertility is a serious problem all over the world. Nutritional deficiency of trace elements like zinc play a role in male infertility as zinc plays an important role not only in normal testicular development, but also in spermatogenesis and sperm motility.

OBJECTIVE: To evaluate and compare the concentrations of zinc in blood and seminal plasma with semen parameters among fertile and infertile Nigeria men.

STUDY DESIGN: A prospective case-control study.

SUBJECTS AND METHODS: Eighty five men with primary infertility whose partners had been fully investigated and found essentially normal, aged 20-55 years were enrolled as cases. The control group were made up of eighty five men with normozoospermia whose partners had spontaneous conception. After semen analysis the cases were further grouped as oligozoospermic (n=64), asthenozoospermic (n=13) and azoospermic (n=8). All the semen samples were analyzed according to WHO 2010 criteria. Blood and seminal plasma concentration of zinc were determined by microwave plasma-atomic emission spectrophotometer. The results were coded, fed into the computer and analyzed using
the Statistical Package for Social Sciences (SPSS) IBM version 19. Chi square test was used to compare categorical variables and t-test was used to compare means. Mann Whitney U test and Kruskal Wallis test were used to compare median of two or more than two numerical variables respectively. Spearman rho correlation was used to determine correlation between numerical variables that were not normally distributed. For all statistical test p was considered significant if it was less than 0.05.

RESULTS: Except for semen volume, all the semen parameters were significantly lower among the infertile men than the fertile group. The median blood zinc concentrations were significantly lower in the infertile group compared with those in the fertile group; 1.7 μmol/L (IQR 0.6, 2.7) versus 3.2 μmol/L (IQR 1.6, 5.2), p<0.001. The median seminal plasma zinc concentrations were also significantly lower in the infertile group compared with those in the fertile group; 9.0 μmol/L (IQR 6.1, 11.0) versus 19.4 μmol/L (IQR 14.0, 25.5), p<0.001. There was a significant positive correlation between seminal plasma zinc levels and progressive sperm motility (r=0.251, p=0.045) in the oligozoospermic infertile men. Also, blood zinc levels showed a significant positive correlation with sperm count (r=0.311, p=0.012), progressive motility (r=0.252, p=0.045) and total motility (r=0.285, p=0.022) in the oligozoospermic men. There was no significant correlation between blood zinc levels and seminal plasma zinc levels among the fertile men (r=0.008, p=0.940) and infertile men (r=0.115, p=0.297). However, blood zinc level was significantly positively
correlated with seminal plasma zinc levels in the study population irrespective of their fertility status.

**CONCLUSION:** On the basis of the findings of this study, blood and seminal plasma zinc levels were significantly lower in the infertile group than the fertile group. Blood and seminal plasma zinc levels were significantly positively correlated with semen parameters in oligozoospermic infertile Nigeria men and also blood zinc levels were significantly positively correlated with seminal plasma zinc levels.
CHAPTER ONE

INTRODUCTION

Infertility constitutes a major part of the health problems in our society. It is defined by the World Health Organization as the failure to conceive following 12 months of regular unprotected sexual intercourse. Worldwide, infertility is generally quoted as occurring in 8-12% of couples. However, the incidence varies from one region of the world to the other, being highest in the so-called infertility belt of Africa that includes Nigeria.

In contrast to an average prevalence rate of 10-15% in the developed countries, the prevalence of infertility has been notably highly variable in sub-Saharan Africa ranging from 20-46%. This has been attributed to the high rate of sexually transmitted infections, complications of unsafe abortions, and puerperal pelvic infections. About 30% of infertility is due to female problems, 30% to male problems, and 30% to combined male/female problems, while in 10%, there is no recognizable cause. The four main categories of causes of infertility recognized in clinical practice are: (i) Male Infertility (ii) Female infertility (iii) Infertility in both male and female partners and, (iv) When both partners are individually fertile, yet they are infertile as a couple.

The prevalence of infertility in Nigeria is put between 20-25% among married couples and about 40-50% of all consultations in gynaecology clinics are infertility cases.
related.\textsuperscript{3} Infertility is seen in Nigeria as barrenness and only women are thought to be barren, notwithstanding empirical medical evidence to the contrary.\textsuperscript{6} One of the consequences is that attention and research have been focused on female infertility to the neglect and detriment of male infertility.\textsuperscript{3,6} In Nigeria, the male factor contribution to the incidence of infertility is put between 20-25\%.\textsuperscript{7,8} Male infertility is therefore an area that is still open for more research, in order to improve the quality of healthcare offered to the client. There are some risk factors leading to defective spermatogenesis, and hence male infertility, like varicocele, cryptorchidism, obstructive lesions, cystic fibrosis, trauma, genitourinary infection, environmental agents, and nutritional deficiency of trace elements like, zinc and selenium.\textsuperscript{9,10}

Zinc which is second only to iron as the most abundant element in the body is found in chicken, nuts, meat, fish, milk, and legumes.\textsuperscript{10} Despite this, the World Health Organization estimates that one-third of world population is deficient in zinc.\textsuperscript{11} Zinc is critical to reproductive potential. The zinc content of semen is 87 times that in the blood and has been reported to protect sperm from bacteria and chromosome damage.\textsuperscript{12} Zinc in the body plays an important role in normal testicular development, spermatogenesis, and sperm motility.\textsuperscript{13,14}
Deficiency of zinc is associated with hypogonadism and insufficient development of secondary sex characteristics in human beings and can cause atrophy of the seminiferous tubules in the rat, leading to failure in spermatogenesis and impotence.\textsuperscript{14,15,16} Low seminal zinc levels were correlated with a decrease in fertilizing ability of sperm.\textsuperscript{17} Also, a study with adult males experimentally deprived of zinc showed that leydig cell synthesis of testosterone was decreased.\textsuperscript{18}

Prostate gland contains high concentration of zinc; however, lower concentration of seminal zinc has been reported because of its dilution with vaginal and cervical fluids after ejaculation. It is not clear with certainty, as to how zinc level in seminal plasma affects sperm function. There are still controversies regarding zinc levels in different infertile groups as well as the relationship between seminal plasma zinc and semen parameters. Some authors reported significantly different seminal zinc levels between fertile and infertile groups, indicating low seminal zinc levels in the infertile population.\textsuperscript{19,20} While some others have shown that there is no difference between the two groups.\textsuperscript{21,22,23}

Majority of the research in this area were also carried out in the white population especially Caucasians. There is therefore the need to establish the effect of zinc on semen parameters in our own population, as geographic, racial and even ethnic factors may affect the dynamics of various processes both in the normal and diseased human body.\textsuperscript{24}
There is a paucity of available publications concerning the effect of this trace element on semen parameters in Nigeria. This study is therefore designed to evaluate and compare blood and seminal plasma zinc levels with various semen parameters among fertile and infertile male subjects.
CHAPTER TWO

RATIONALE FOR THE STUDY

Infertility is a problem of public health importance in Nigeria with a high prevalence. It affects 20-25% of married couples in the country and has reached such an alarming level that so much hype is made about it in the Nigerian media.\(^3\)\(^6\) It has been reported that the male partner contributes 40% of the cases of infertility.\(^2\) Male reproductive capacity is often judged from the number of sperms, morphology and their motility. However, secretions from accessory glands also influence overall semen quality. Human semen contains high concentrations of trace elements like zinc, magnesium, copper and selenium in bound and free forms. These trace elements play vital role in affecting various semen parameters.\(^25\)

In the past decade, numerous assays have been performed on both fertile and infertile subjects in an effort to elucidate factors that may contribute to male infertility. The levels of trace elements, cations and toxic metals and their effects on spermatogenesis, sperm characteristics are being investigated. However, much of the research work was done among Caucasians. There is evidence that alteration in seminal plasma levels of zinc affects sperm quality with decreased fertility potential.\(^26\),\(^27\),\(^28\) As earlier stated, there have been conflicting reports on the role of zinc in male infertility. While some authors have
reported significantly different blood and seminal zinc levels between fertile and infertile
groups,\textsuperscript{19,20} others observed no significant change.\textsuperscript{21,22,23} There is therefore need for more
studies on the subject.

Also, because of endemic level of poverty in our society, malnutrition is more common;
hence the deficiency of zinc is likely to be more common in our environment compared to
the developed world. It is pertinent to establish if there is any association between blood
and seminal plasma zinc and semen parameters in our own population. This is because
geographic, racial and even ethnic factors have been known to affect the dynamics of
every process in the human body.

Finally, if zinc deficiency is confirmed in the pathophysiology of male infertility,
intervention may have a more dramatic impact on the management of infertility. This
study is expected to contribute to the body of knowledge on this subject.
CHAPTER THREE

LITERATURE REVIEW

3.1 : General overview of infertility.

Globally 8–12% of couples experience difficulty conceiving a child.\textsuperscript{2,28} Male factors account for the difficulties in 40% of couples. Infertility has been described as a stressor and a life crisis for individuals or couples, which results in a lower quality of life and marital conflicts.\textsuperscript{29,30,31,32} The risk of infertility increases with advanced age of the female partner (>35 years) \textsuperscript{33,34,35,36}. Female infertility may present as anovulation, obstructed fallopian tubes, endometriosis or uterine abnormalities.\textsuperscript{33,35} Male factor infertility is characterized by diminished production of morphologically normal, motile sperm.\textsuperscript{35,37} Causes of male infertility include congenital or acquired urogenital abnormalities, genetic and immunological factors, endocrine disturbances, genital tract infections and erectile dysfunction. Genetic abnormalities, hormonal imbalances and congenital/infectious malformations of the reproductive tract are some of the common causes of male and female infertility.\textsuperscript{33,35,37,38} Lifestyle factors such as obesity,\textsuperscript{39} diet, smoking and alcohol use\textsuperscript{40} along with environmental chemical exposures\textsuperscript{33,37,40} have been increasingly examined as additional modifiers of fertility.
Diagnosis of infertility is varied and may include assessment of sperm quality, hormones and imaging analysis of the uterus/fallopian tubes. Depending on the diagnosis, infertility may be treated by reproductive surgery, administration of hormones and/or assisted reproductive technologies (ART). ART encompass clinical/laboratory procedures wherein male and female gametes are manipulated for the purposes of reproduction and include in vitro fertilization (IVF), intracytoplasmic sperm injection, preimplantation genetic diagnosis, embryo cryopreservation and gestational surrogacy.41

3.1 : Pathophysiology of male infertility.

The hypothalamus controls production of reproductive hormones through the pulsatile secretion of gonadotropin-releasing hormone (GnRH). In turn, GnRH stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH acts on the Leydig cells of the testes to produce testosterone, whereas FSH acts on Sertoli cells to stimulate spermatogenesis. Negative feedback control of reproductive hormone levels is achieved via inhibin (which decreases FSH levels) or via estradiol aromatized from testosterone (which inhibits LH production).42

The classic form of testosterone deficiency is found in individuals with hypogonadotropic hypogonadism. This is the form of testosterone deficiency that is best characterized, and which is often the first consideration of the physician, although this potentially
treatable form of male factor infertility accounts for <1% of cases. The causes of hypogonadotropic hypogonadism can be congenital, acquired, or idiopathic; the congenital etiologies include Prader–Willi syndrome, Lawrence–Moon–Biedl syndrome, and Kallman syndrome, while the acquired causes are radiotherapy to the brain, trauma, or secondary to a pituitary tumor. Kallman syndrome is the most common form of primary hypothalamic deficiency. These patients can be identified by their lack of secondary sexual characteristics and anosmia (which is due to failure of GnRH neurons to migrate from the olfactory placode, along with failure of the olfactory bulb to form). The main mechanism of infertility in patients with Kallman syndrome is a failure to initiate spermatogenesis.

Hyperprolactinemia (defined as a serum prolactin concentration ≥20 ng/ml in men) can also be a cause of infertility in both males and females and is usually caused by a pituitary tumor, hypothyroidism, hepatic disease, or secondary to treatment with psychotropic or antihypertensive drugs. Excess prolactin inhibits the hypothalamic secretion of GnRH and impairs binding of LH to Leydig cells in the testis. In addition to hypogonadotropic hypogonadism, patients with hyperprolactinemia have low ejaculate volumes and can experience visual field defects if a tumor is present.

The use of exogenous androgens can have a profound effect on fertility. Androgen excess caused by the use of steroids impairs spermatogenesis by suppressing GnRH, which results
in decreased LH and FSH levels and a considerable reduction in intratesticular testosterone levels. Exogenous testosterone replacement therapy can result from self-administration in order to increase lean muscle mass (sometimes seen in athletes), or even as an appropriate treatment in a hypogonadal patient in whom fertility was either not a concern initially or not discussed. Though the anabolic to androgenic ratio in testosterone-derived products can vary, they all have pharmacological effects that can lead to male factor infertility.

Infections of the male genitourinary tract account for up to 15% of cases of male infertility. Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process, causing qualitative and quantitative sperm alterations. Recent studies have shown that the simple presence of bacteria in semen samples may compromise the sperm quality. The bacteria responsible for semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse.

The most frequently isolated microorganism in male patients with genital tract infections or semen contamination is *Escherichia coli*. The negative influence of this species on sperm quality is partially due to its effect on motility and to the impaired acrosomal function, as
demonstrated at the ultrastructural level by Diemer et al.\textsuperscript{50,51} Genital ureaplasmas and mycoplasmas may colonize male urethra and contaminate the semen during ejaculation. However, these microorganisms and particularly \textit{Ureaplasma urealyticum} are potentially pathogenic species playing an etiologic role in both genital infections and male infertility.\textsuperscript{52} \textit{Ureaplasma urealyticum}, one of the most frequent causes of the male infertility, due to its ability to reduce semen quality and the fertilizing potential of sperm, negatively influences the sperm motility, density and morphology and reduces the oxidoreductive potential of the ejaculate, which makes sperm more vulnerable to peroxidative damage.\textsuperscript{53}

It is increasingly recognized that reactive oxygen species (ROS) are of significant pathophysiological importance in the etiology of male infertility.\textsuperscript{54} ROS are highly reactive oxidizing agents belonging to the class of free radicals containing one or more unpaired electrons which are continuously being generated through metabolic and pathophysiologic processes.\textsuperscript{55} It has been postulated that oxidants interfere with normal sperm function via membrane lipid peroxidation and fragmentation of nucleic acids, which result in sperm dysfunction.\textsuperscript{56} Due to their abundance of membrane polyunsaturated fatty acids and their capacity to generate ROS, human spermatozoa are highly susceptible to oxidative stress.\textsuperscript{56} The more important marker of lipid peroxidation is malondialdehyde (MDA). This by-product has been used in biochemical assays to monitor the degree of peroxidative
damage sustained by spermatozoa. The results of such assay exhibit an excellent correlation with the degree to which sperm function is impaired.\textsuperscript{55} High levels of seminal MDA represent increased lipid peroxidation rates, which diminishes fertility.\textsuperscript{56,57}

Hence, human spermatozoa are known to possess all of the major antioxidant defensive systems including catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase.\textsuperscript{57} Their effectiveness is impaired by their limited concentrations and distribution. Interestingly, the seminal plasma is well equipped with an array of antioxidant defence mechanisms to protect the spermatozoa against oxidants. Antioxidants that are present in the seminal plasma compensate for the deficiency in cytoplasmic enzymes in the spermatozoa.\textsuperscript{57}

3.1 : Brief overview of zinc and its role in male infertility.

Analysis of trace elements, especially zinc, in different body fluids has been one of the areas of focus because of their correlation to human health. It is known that certain elements play foremost role in a variety of biochemical process that determine the welfare of living organisms. Depending on the concentration of these trace metal ions, they could be either beneficial or injurious.\textsuperscript{58} Seminal plasma is the secretion by the sexual accessory glands at the time of ejaculation to support spermatozoa. It contain proteins, including enzymes like acid phosphatase, alanine transaminase, alkaline phosphatase, aspartate
transaminase in addition to lipids, macroelements like sodium, potassium, calcium, magnesium, phosphate, chloride, and microelements like copper, iron and zinc.\textsuperscript{59} Zinc has been shown to be obligatory to maintain the structure and function of a large number of macromolecules and more than 300 enzymes.\textsuperscript{60} This divalent metal ion has both catalytic and structural role in enzymes.\textsuperscript{61} Zinc is present at high concentrations in human seminal plasma at a mean concentration of 2nanomoles which is 100 times higher than the concentration in serum.\textsuperscript{60,62} The concentration of zinc in human seminal plasma is higher than in any other tissues.\textsuperscript{63}

Trace elements in human semen comprising zinc and selenium have been shown to be essential for testicular development and spermatogenesis.\textsuperscript{64,65,66,67} Zinc is one of the primary elements responsible for deoxyribonucleic acid (DNA) transcription and protein synthesis which are major parts of sperm development.\textsuperscript{66} Its concentrations are very high in the male genital organs, particularly in the prostate gland.\textsuperscript{65} Zinc can oppose the oxidation by binding sulphydryl groups in proteins and by occupying binding sites for copper in lipids and DNA.\textsuperscript{66} Recent studies hypothesized that insufficient intake of zinc impairs antioxidant defences. This may subsequently makes the spermatozoa more susceptible to lipid peroxidation.\textsuperscript{64}
Evaluation of zinc concentration in human seminal plasma was found to be one of the diagnostic measures for human male infertility. Zinc has an imperative responsibility in testis development, sperm as well as semen physiologic functions. Decrease in the level of zinc concentration causes hypogonadism, inadequate development of secondary sexual characteristics, and atrophy of seminiferous tubules that could possibly lead to failure in spermatogenesis.\textsuperscript{68,69} A clinical study demonstrated that adult males experimentally deprived of zinc showed a disturbance of testosterone synthesis in the Leydig cell. The authors concluded that adequate seminal concentration of zinc is required for normal sperm function.\textsuperscript{70}

Total content of zinc in mammalian semen is elevated and has been found to be decisive to Spermatogenesis.\textsuperscript{70,71} Zinc in seminal plasma was proposed to stabilize the cell membrane and nuclear chromatin of sperm.\textsuperscript{71} There is extensive evidence that human seminal zinc has an important role in the physiologic functions of sperm and that reduced levels result in low quality of sperm and reduced chances of fertilization.\textsuperscript{71,72} Chia SE. et al,\textsuperscript{65} in a case control study involving 107 infertile men and 103 fertile men reported that the geometric means of the seminal plasma zinc concentration were significantly lower in the infertile men compared with that in the fertile men; 183.6mg/L versus 274.6mg/L. There was no significant difference in the geometric means of the blood zinc concentration between the two groups. Seminal plasma zinc concentration was found to be significantly
correlated with sperm density, motility and viability. The authors concluded that “zinc may contribute to fertility through its positive effect on spermatogenesis.”

Yosra MA. et al\textsuperscript{13} measured the concentration of zinc in semen samples of 100 infertile male subjects and 50 fertile males using the atomic absorption spectrophotometry. The result showed a significant decrease in the seminal plasma zinc concentration in the infertile males compared to the fertile males; 18.0mg/dl versus 21.1mg/dl. They also concluded that “zinc may contribute to fertility through its effects on semen parameters.”

Akinloye O. et al\textsuperscript{70} reported a significant inverse correlation between serum zinc and sperm count. Seminal zinc was also found to be negatively correlated with spermatozoa viability. Fuse H. et al\textsuperscript{73} found no significant difference in the mean value of seminal plasma zinc levels between infertile and fertile males. Most studies on the effects of blood and seminal plasma zinc levels on semen parameters were carried out in Caucasian and their reports are conflicting.\textsuperscript{17,20,23}
CHAPTER FOUR

OBJECTIVES AND HYPOTHESIS

Main Objective

To evaluate and compare the concentrations of zinc in blood and seminal plasma with semen parameters among fertile and infertile Nigeria men.

Specific Objectives

1. To determine the semen parameters in infertile and fertile Nigeria men.

2. To determine the blood and seminal plasma zinc concentrations in infertile Nigeria men compared to that of fertile men.

3. To determine if blood and seminal plasma zinc concentration has a significant correlation with various semen parameters among fertile and infertile Nigeria men.

Null Hypothesis

There is no significant correlation between the concentration of zinc in blood and seminal plasma with sperm quality.
**Alternative Hypothesis**

There is insignificant correlation between the concentration of zinc in blood and seminal plasma with sperm quality.

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**CHAPTER FIVE**

**MATERIALS AND METHODS**

**5.1: Study Site**

The study was conducted at the department of Obstetrics and Gynaecology of the Lagos State University Teaching Hospital (LASUTH) Ikeja, Lagos in South-western part of Nigeria. The hospital serves as a major referral centre for Lagos, Ogun and Oyo States. Spouses of patients were recruited mainly from referral to the Institute of Fertility Medicine (IFM), LASUTH, the gynaecology clinic and antenatal clinic of the various units in the department, and from the blood donation laboratory. IFM was established in 2011 as a public private partnership between LASUTH and the Bridge Clinic—a foremost assisted conception unit and the first focused In Vitro Fertilization (IVF) centre in Nigeria. It was established with a mandate to provide a low cost IVF without compromising standard; training of gynaecologists, embryologists and fertility nurses from LASUTH; and eventual transfer of technology to LASUTH.
5.2: Study Duration

Patients were recruited consecutively until the desired sample size was attained. The study lasted for eight months.

5.3: Study Design

A prospective case-control study involving infertile men, compared with age-matched fertile subjects, as controls.

5.4: Study Population

The study population consisted of two groups: Cases and controls. Cases were infertile male subjects, age range between 20-55 years who has had adequate unprotected sexual intercourse for at least one year without conception with their partners. Controls were age-matched fertile males whose partners had spontaneous conception within one year and having sperm count of at least 15 million/ml with progressive motility and normal morphology of at least 32% and 4% respectively. The controls were husbands of women attending the antenatal clinic at the Maternal and Child Center (MCC) Ifako-Ijaiye and MCC Isolo which constitute the obstetric units of the Lagos State University Teaching Hospital, Ikeja. A semen sample was collected from each infertile subject on two separate occasions, at least two weeks apart, after 3-5 days of abstinence.
5.5: Sample Size Determination

The prevalence of male infertility in Nigerian is put between 20-25%. Using this figure, and accepting a study power of 80%, confidence interval of 95% and case to control ratio of 1:1. The sample size of each group was determined using the statistical formula for the comparison of proportions.

\[ n = 2 \times \frac{Z_{\text{crit}} \sqrt{2 \bar{p}(1-\bar{p})} + Z_{\text{pwr}} \sqrt{p_1(1-p_1) + p_2(1-p_2)}}{D^2} \]

\[ Z_{\text{crit}} = \text{Significance criterion of 0.05} = 1.960 \]

\[ Z_{\text{pwr}} = \text{Power of 0.80} = 0.842 \]

\[ P_1 = \text{Proportion of Cases (Infertility)} = 20\% (0.20) \]

\[ P_2 = \text{Proportion of Fertility (control). This is usually set relative to } P_1 \text{ and with proposed effect size of 30\%. } P_2 = 0.20 + 0.30 = 0.50 (50\%) \]

\[ D = \text{Minimum expected difference (} |p_1 - p_2| \text{)} \]

\[ \bar{p} = \frac{(p_1 + p_2)}{2} = \frac{(0.2 + 0.5)}{2} = 0.35 \]
\[ n = 2 \times \frac{[1.96 \sqrt{2(0.35)(1-0.35)} + 0.842 \sqrt{0.2 (1-0.2) + 0.5(1-0.5)}]^2}{0.09} = 76.98216 \]

Approximately 77 patients were used for infertile men (cases) and 77 patients; fertile men (control). A minimum sample size of 154 was required for the study.

In order to accommodate possible attrition or unforeseen errors in completing the study questionnaire or blood and semen sample processing, an additional 10% (16 subjects) of the calculated figure were recruited to bring the figure to a minimum of 170 subjects (85 infertile men and 85 fertile men).

5.6: Sampling Technique

Stratified sampling (a probability sampling technique) was used to recruit subjects, following the inclusion and exclusion criteria. Two strata were observed: infertile (case) and fertile subjects (control). Equal number of fertile and infertile subjects was assigned to each stratum. One out of every two patients was selected within the strata until the desired sample size was reached. A minimum of 85 patients were recruited in the infertile group and a minimum of 85 patients were recruited in the control group.

5.7: Sampling Procedure
Eighty five infertile male subjects, aged 20-55 years who has had regular unprotected sexual intercourse for at least one year without conception were recruited from IFM and the gynaecology clinic into this study. Thereafter, the purpose of the study was explained to the subjects and informed consent was obtained. Patient information was collected by a structured questionnaire (Appendix 1). Semen specimens were collected in sterile polystyrene containers through masturbation after 3-5 days of abstinence. Semen samples were incubated for 30 minutes at 37 degree centigrade for liquefaction and they were subsequently analyzed for descriptive semen parameters within one hour of collection.

Analysis were performed according to the WHO guidelines of 2010 to measure volume, sperm concentration, progressive motility, total motility and morphology. Infertile male patients were then divided into the following three groups according to their sperm count, motility and morphology. Group I: Azoospermic (sperm count= zero), Group II: Oligozoospermic (sperm count < 15x10^6/ml), Group III: Asthenozoospermic (sperm count ≥ 15x10^6/ml, progressive motility < 32%, irrespective of morphology). Eighty five fertile males whose partners had spontaneous conception within one year and having sperm count ≥ 15x10^6/ml with progressive motility and normal morphology of at least 32% and 4% respectively were selected via the antenatal clinic and taken as normozoospermic control group.
It is customary for husband of patients attending antenatal clinic of our hospital to donate blood for their wives. They were approached at the point of blood donation in the laboratory and the purpose of the study was explained to them. Also females attending the antenatal clinic of the hospital were approached by female obstetric nurse. The purpose of the study was explained to the pregnant women by the nurse. Those that agreed to the study were encouraged to seek their husband’s consent to participate. Only couples who had never attended an assisted reproductive program were recruited for the study. All who agreed to participate in the study were given a consent form to sign. These fertile men were similarly interviewed with the same questionnaire that was used for infertile men.

After liquefaction, a portion of the sample was centrifuged at 2000g for 15-20 minutes and the supernatant (the seminal plasma) was transferred into fresh tubes and store frozen at -20 degree centigrade until analyzed for the zinc. Also, about 5mls of blood samples was taken from each subject in the morning. The samples were centrifuged at 2000g for 15-20 minutes and the supernatant (the serum) were transferred into fresh tubes and store frozen at -20 degree centigrade until analyzed for the zinc. The samples were labeled with patient’s name and identification number. The concentrations of zinc in blood and seminal plasma of each infertile patient and fertile control were determined by Microwave Plasma-Atomic Emission Spectrophotometer (MP-AES).
Sample processing analysis was done at the Department of Chemical Pathology of Lagos State University Teaching Hospital.

5.8: Seminal Fluid Analysis Procedure

The seminal fluid analysis was done with the aid of the following instrument:

- Olympus light microscope, manufactured in Philipines. Instrument model CX41RF
- Manenfeed neubauer counting chamber, manufactured in Germany. Neubauer improved model. REF 0610030. WT20104.
- Olympus counter AC-15. Instrument model CX41. Ph1,2,3 & DF.

Sperm count: Total sperm count was done using the Neubauer counting chamber which 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 x 3mm pattern of gridlines etched on the glass surface. It was use with a special thick coverslip. Each counting area is divided into nine 1 x 1mm grids. Each grid holds 100nl with a depth of 100µm. Four of these grids (nos 1,3,7 and 9) contain 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 20nl per row. The semen was immobilize 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.

**Liquefaction:** Semen samples were examined under room temperature after the sample has been allowed to liquefy, that is the semen becoming thinner. Semen should completely liquefy within 30 minutes at room temperature, although it may rarely take up to 60 minutes sometimes at room temperature.

**Semen volume:** The semen volume were measured by a modified graduated glass measuring cylinder with a wide mouth, into which the semen was collected. The volume was read directly from the graduation on the measuring cylinder.

**Sperm motility:** Sperm motility was assessed after liquefaction of the semen sample. An aliquot of semen was removed after mixing the semen sample. Subsequently, a wet preparation was made on a slide and examine microscopically. Percentage of different motile categories according to WHO guideline was graded as progressive motility, non progressive motility and immotile.
**Sperm morphology:** Sperm morphology was determined by preparing a smear of semen on a slide, air drying, fixing and staining of 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.

**5.9: Zinc Measurement in Seminal Plasma and Serum**

Before measurement of zinc level in seminal plasma, glass wares and plastic materials for the procedure were soaked in 1:3 nitric acid solutions to remove organic materials, wash in detergent solutions, rinse with tap water then rinse with deionized distilled water. This is because small amount of metal ions contained in the glass wares and plastic materials can affect the result of analysis. In order to ensure proper sample handling and identification, laboratory procedures were strictly adhere to. Samples were clearly labelled with adhesive sample label to reflect the following: Log in number, location, acid added and sample matrix. The samples were carefully digested with ASTM D 4698 – 92B (1996) in fume cupboard. The method is described below:

**Sample Preparation**

- Seminal plasma sample was first allowed to thaw in room temperature.
• Using an analytical weighing balance AG 204 METTLER TOLEDO, 0.5g seminal plasma was measured, and aspirated with a pipette into a conical flask.

• Concentrated nitric acid (2mls) and 2mls of perchloric acid was added to the 0.5g of seminal plasma for the process of deproteinization (digestion), this release the seminal plasma zinc from the protein matrix. Subsequently, the suspension of seminal plasma in acid in the conical flask was moved to the digestion chamber to continue the digestion process. The sample was heated by hotplate until complete dissolution, this occurred when the suspension that was appearing as golden yellow becomes a transparent solution. A blank sample was also prepared for control, this was to correct for any background error.

• The pellet obtained was diluted 100 times with deionized distilled water for measurement of zinc concentration.

• The concentration of zinc in seminal plasma was determined by Microwave Plasma-Atomic Emission Spectrophotometer (MP-AES). Instrument model MY14280004. Maker: Agilent technologies.

• The measurement was conducted (zinc wavelength) at 481.053 nm wavelength. The coefficient of variation for the instrument was 0.997.

• Zinc concentration was determined by direct aspiration of the acidic sample into the Microwave Plasma-AES.
The above procedure was also repeated to determine the level of zinc in the serum.

**Principles of Microwave Plasma-AES for metal analysis**

This relatively new and simple instrumental technique is a fast sequential multi-element analytical technique that has microwave-induced nitrogen plasma as an excitation source and optical dispersion and detection components similar to inductively coupled plasma atomic emission spectrometry (ICPAES). This instrument uses a microwave excitation assembly to create a concentrated axial magnetic field around a conventional torch. This focuses the microwave energy where it is needed to produce a toroidal plasma with a cooler central channel that is suitable for stable introduction of liquid samples using a conventional sample introduction system. The principle of this technique is similar to any other emission technique such as a flame emission technique or well-known ICP-AES. The intensity of each emitted line will be directly proportional to the concentration of a particular element.

Microwave Plasma-AES is used for simultaneous multi-analyte determination of major and minor elements. It employs microwave energy to produce a plasma discharge using
nitrogen supplied from a gas cylinder or extracted from ambient air, which eliminates the need for sourcing gases in remote locations. Samples are typically nebulized prior to interaction with the plasma in Microwave Plasma-AES measurements. The atomized sample passes through the plasma and electrons are promoted to the excited state. The light emitted electrons return to the ground state light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured with a working range of low part per million (ppm) to weight percent. Microwave Plasma-AES is a technique comparable to traditional atomic absorption but with several potential advantages including lower cost of operation and elimination of the requirement for flammable gasses.

**Calculation of Zinc Concentration in μmol/L**

1ppm=1mg/dl=100μg/dl

To convert to μmol/L, zinc concentration in mg/dl is multiplied by 100 to convert to µg/dl, and then multiplied by a factor of 0.153.

= Zn in mg/dl x 100 x 0.153

= Zinc concentration in μmol/L
5.10: W.H.O Semen Analysis Manual and Definition of Terms

W.H.O, in 2010 published a new manual for semen analysis and clinicians were encouraged to use it for evaluation of male fertility. The semen parameters in this study was based on the new manual for semen analysis.

Definitions relating to semen quality based on W.H.O manual

<table>
<thead>
<tr>
<th>Terms</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normozoospermia</td>
<td>Sperm concentration ≥ 15 million/ml</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>Sperm concentration &lt; 15 million/ml</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>Progressive motility&lt; 32%</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>&lt; 4% normal morphology</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>Disturbance of all three variables</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>No spermatozoa in the ejaculate</td>
</tr>
</tbody>
</table>

5.11: Inclusion criteria
a. A minimum of 85 fertile males whose partners had conceived within one year without any previous history of treatment were taken as controls.

b. A minimum of 85 infertile males who has had regular unprotected sexual intercourse for at least one year without conception with their partners formed the study group. Wives of the infertile subjects that were included had no obvious cause of infertility like tubal blockage, endometriosis, pelvic inflammatory diseases or ovulatory disorders. These women were identified through clerking, examination and investigations which include endocervical swab microscopy, culture and sensitivity, hysterosalpingography, transvaginal scan and hormone profile.

5.12: Exclusion criteria

a. Refusal of consent

b. History of pelvic surgery or hernia repair

c. Diabetes mellitus

d. Thyroid disease

e. Patients on antipsychotic or antihypertensive drugs.

f. Urinary tract infections
g. Sexually transmitted infections

h. Testicular injury

i. Small testes (volume < 10ml)

j. Varicoceles

k. Alcohol consumption

l. History of smoking or drug addiction

m. Patients taking vitamins and mineral supplementation.

5.13: Data processing and statistical analysis

The data obtained was entered and analyzed using the Statistical Package for Social SciencesIBM version 19. Histogram plot of outcome variables were plotted to determine if they were normally distributed and hence the appropriate statistical tests to use. Percentages, mean, median, standard deviation and inter quartile range of numerical variables were determined. Chi square test was used to compare categorical variables and t-test was used to compare means. Mann Whitney U test and Kruskal wallis test were used to compare median of two or more than two numerical variables respectively. Spearman rho correlation was used to determine correlation between numerical variables that were
not normally distributed. Scatter diagram was plotted to show the pattern of relationship between numerical variables. For all statistical test p was considered significant if it was less than 0.05.

5.14: Study limitations

Assay of zinc levels in blood and seminal plasma was carried out once in each sample in this study. A better assay method that limits intra assay coefficients of variation of assay would have been to repeat the measurement of zinc levels in each sample thrice and obtain a mean value for each sample. This would have enormously increased the cost of conducting the study.

The exact dietary intake of the study participants was unknown. Some participants may have been on diet rich or deficient in zinc, which could have affected the levels of zinc in the blood and seminal plasma.

5.14: Ethical Consideration
Approval for the study was obtained from the Ethics Committee of Lagos State University Teaching Hospital, Ikeja, Lagos State. The subjects for the study were fully briefed on the research protocol in language they understand.

They were told that information gathered would contribute to the knowledge on future management of infertility. They were encouraged to ask questions on any aspect of the study. They were also allowed the right of refusal to take part in the study or withdraw at any point during the study, which would not affect the standard of their care in anyway. They were assured that all information given would be treated as confidential. They were also informed that the researcher would bear the cost of the seminal fluid analysis and other investigations. Informed written consent was then obtained (Appendix 2).
CHAPTER SIX

RESULTS

Table 1 shows the socio-demographic characteristics of fertile and infertile men. These were similar in the two groups. The mean age of the fertile men was 38.6±6.3 years, while that of the infertile men was 38.5±5.9 years. With regards to educational level, most of the respondents had tertiary education, 64(75.2%) in the fertile group and 56(65.9%) in the infertile group. The major tribes in the country were equally represented in the two groups. However, Yoruba tribe accounted for most 51(60%) of the fertile group and 52(61.2%) of the infertile group. Most of the respondents in both groups were civil servants, business men or professionals. In the fertile group 25(29.4%) were civil servant, 24(28.2%) were business men and 21(24.7%) were professionals, while in the infertile
group 20(23.5%) were civil servant, 29(34.1%) were business men and 27(31.8%) were professionals. With regards to duration of marriage, most of the respondents were married for less than 5 years, 59(69.4%) in the fertile group and 61(71.8%) in the infertile group.

Table 2 shows semen parameters of fertile and infertile men. Except for semen volume, other semen parameters studied were reduced in the infertile group compared to the fertile group. Except for semen volume, significant difference was observed between fertile and infertile group in relation to sperm count, progressive motility, total motility and morphology (p <0.001). There was no significant difference in the semen volume between the two group (p =0.433).

Fig 1 and Fig 2 are histograms showing the distribution of blood and seminal plasma zinc levels among respondents. Blood and seminal plasma zinc levels were not normally distributed and hence the use of median and inter quartile range instead of mean and standard deviation as a measure of average and dispersion respectively in this study.

Table 3 shows the median blood and seminal plasma zinc levels and the median semen parameters among fertile and infertile men. The median blood and seminal plasma zinc levels were significantly lower in the infertile men than the fertile men. Except for semen volume, the median of other semen parameters studied were significantly lower in the
infertile men than the fertile men. The median blood zinc level was 3.2 μmol/L (IQR 1.6, 5.2) in the fertile men, while it was 1.7 μmol/L (IQR 0.6, 2.7) in the infertile men. The median seminal plasma zinc levels in the fertile men was 19.4 μmol/L (IQR 14.0, 25.5) while it was 9.0 μmol/L (IQR 6.1, 11.0) in the infertile. The difference in blood zinc levels between the fertile and infertile men was statistically significant (p<0.001). The difference in seminal plasma zinc levels between the fertile and infertile men was also statistically significant (p<0.001).

Table 4 shows blood and seminal plasma zinc levels in the normozoospermic, fertile group and the oligozoospermic, asthenozoospermic and azoospermic infertile groups. The median blood zinc level was 3.2 μmol/L (IQR 1.6, 5.2) in the normozoospermic, 1.7 μmol/L (IQR 0.6, 2.7) in the oligozoospermic, 1.7 μmol/L (IQR 0.9, 3.8) in the asthenozoospermic and 0.2 μmol/L (IQR 0.1, 1.6) in the azoospermic group. The median seminal plasma zinc level was 19.4 μmol/L (IQR 14.0, 25.5) in the normozoospermic, 8.9 μmol/L (IQR 5.8, 10.6) in the oligozoospermic, 10.3 μmol/L (IQR 6.7, 14.6) in the asthenozoospermic and 9.4 μmol/L (IQR 6.5, 16.4) in the azoospermic group. Blood and seminal plasma zinc levels were significantly lower in the azoospermic, oligozoospermic and asthenozoospermic infertile groups than in the normozoospermic fertile group (p<0.0001, p<0.001).
Table 5 shows the correlations of blood and seminal plasma zinc levels with semen parameters among the fertile and infertile men. Blood zinc levels showed a weak negative correlation with semen volume ($r=-0.186 \ p=0.088$), sperm count($r=-0.008 \ p=0.940$) and morphology ($r=-0.014 \ p=0.896$); and a weak positive correlation with progressive motility ($r=0.189 \ p=0.083$) and total motility ($r=0.080 \ p=0.469$) among the fertile men. These correlations were not statistically significant. Meanwhile among the infertile men, blood zinc levels showed a weak positive correlation with semen volume ($r=0.057 \ p=0.604$), sperm count ($r=0.008 \ p=0.941$), progressive motility ($r=0.016 \ p=0.883$), total motility ($r=0.065 \ p=0.553$) and a weak negative correlation with morphology ($r=-0.029 \ p=0.794$) These correlations were also not statistically significant. Seminal plasma zinc levels showed a weak negative correlation with sperm count($r=-0.054 \ p=0.626$), progressive motility($r=-0.193\ p=0.077$), total motility ($r=-0.056 \ p=0.613$) and a weak positive correlation with semen volume ($r=0.065 \ p=0.557$) and morphology ($r=0.088 \ p=0.423$) among the fertile men. These correlations were not statistically significant. Also among the infertile men, seminal plasma zinc levels showed a weak positive correlation with all the semen parameters studied; semen volume ($r=0.031 \ p=0.775$), sperm count ($r=0.014 \ p=0.898$), progressive motility ($r=0.136 \ p=0.215$), total motility ($r=0.118 \ p=0.282$) and morphology ($r=-0.155 \ p=0.157$). These correlations were not statistically significant.
Fig 3 and figure 4 are scatter plots of correlation between blood zinc level and seminal plasma zinc level among the fertile men and infertile men respectively. Blood zinc levels showed a weak positive correlation with seminal plasma zinc level in both fertile men ($r=0.008\ p=0.940$) and infertile men ($r=0.115\ p=0.297$). These correlations were not statistically significant.

Correlation coefficient of blood and seminal plasma zinc levels with semen parameters in normozoospermic fertile men, azoospermic, oligozoospermic and asthenozoospermic infertile men are depicted on Table 6. Blood zinc showed a significant positive correlation with sperm count ($r=0.311\ p=0.012$), progressive motility ($r=0.252\ p=0.045$) and total motility ($r=0.285\ p=0.022$) and a non-significant negative correlation with semen volume ($r=-0.045\ p=0.722$) and morphology ($r=-0.109\ p=0.389$) in the oligozoospermic men. Among the asthenozoospermic group, blood zinc levels showed a non-significant weak positive correlation with semen volume ($r=0.298\ p=0.322$), sperm count ($r=0.014\ p=0.964$), progressive motility ($r=0.063\ p=0.838$), total motility ($r=0.367\ p=0.217$); and a weak non-significant negative correlation with morphology ($r=-0.322\ p=0.283$). There were no significant correlations between blood zinc levels and semen parameters in the normozoospermic and azoospermic group.
Among the oligozoospermic group, seminal plasma zinc level was significantly positively correlated with progressive motility ($r=0.251 p=0.045$). It was also positively correlated with semen volume($r=0.064 \ p=0.613$), sperm count($r=0.086 \ p=0.497$), total motility($r=0.218 \ p=0.083$) and morphology($r=0.165 \ p=0.192$), but not statistically significant. Among the asthenozoospermic group, seminal plasma zinc levels showed a non-significant weak positive correlation with sperm count ($r=0.438 \ p=0.134$), progressive motility($r=0.172 p=0.574$), total motility($r=0.391 p=0.187$), morphology($r=0.096 p=0.756$), and a non-significant weak negative correlation with semen volume($r=-0.307 p=0.308$). There were no significant correlations between seminal plasma zinc levels and semen parameters in the normozoospermic and azoospermic group.

Table 7 shows blood zinc levels in subjects with normal and different types of abnormal semen parameters irrespective of their fertility status. Except for semen volume, blood zinc levels were lowered in subjects with abnormal semen parameters compared to those with normal semen parameters. The difference was statistically significant for sperm count ($p<0.001$), progressive motility ($p=0.002$), total motility($p=0.003$) and morphology ($p<0.001$). However, blood zinc levels were lower in subjects with normal semen volume compared to those with abnormal semen volume. The difference was not statistically significant($p=0.276$).
Table 8 shows seminal plasma zinc levels in subjects with normal and different types of abnormal semen parameters irrespective of their fertility status. Except for semen volume, seminal plasma zinc levels were lower in subjects with abnormal semen parameters compared to those with normal semen parameters. The difference was statistically significant for sperm count \( (p<0.001) \), progressive motility \( (p<0.001) \), total motility \( (p<0.001) \) and morphology \( (p<0.001) \). However, seminal plasma zinc levels were slightly lower in subjects with normal semen volume compared to those with abnormal semen volume. The difference was not statistically significant \( (p=0.400) \).

Table 9 shows correlation of blood and seminal plasma zinc levels with semen parameters among subjects irrespective of their fertility status. Except for semen volume, blood zinc levels were significantly positively correlated with sperm count \( (r=0.272 \ p<0.001) \), progressive motility \( (r=0.286 \ p<0.001) \), total motility \( (r=0.229 \ p=0.003) \) and morphology \( (r=0.309 \ p<0.001) \). However, blood zinc levels showed a non-significant negative correlation with semen volume \( (r=-0.099 \ p=0.197) \). Except for semen volume, seminal plasma zinc levels were significantly positively correlated with sperm count \( (r=0.555 \ p<0.001) \), progressive motility \( (r=0.467 \ p<0.001) \), total motility \( (r=0.503 \ p<0.001) \) and morphology \( (r=0.712 \ p<0.001) \), however seminal plasma zinc levels showed a non-significant negative correlation with semen volume \( (r=-0.046 \ p=0.584) \).
Fig 5 shows scatter plots of correlation between blood zinc levels and seminal plasma zinc levels among respondents irrespective of their fertility status. Blood zinc levels showed a significant positive correlation with seminal plasma zinc levels ($r=0.299 \ p<0.001$).

<p>| Table 1: Socio demographic characteristics of fertile and infertile respondents |
|---------------------------------|-----------------|-----------------|----------|--------|
| Variables                       | Fertile n = 85 (%) | Infertile n = 85 (%) | Test     | p      |
| Age group                       |                  |                  |          |        |
| &gt;30                             | 5 (5.90)         | 4 (4.7)          | 0.050**  | 0.960  |
| 30 – 39                         | 45 (52.0)        | 48 (56.5)        |          |        |
| 40 – 49                         | 27 (31.8)        | 29 (34.1)        |          |        |
| ≥50                             | 8 (9.4)          | 4 (4.7)          |          |        |
| Mean±SD                         | 38.6±6.3         | 38.5±5.9         |          |        |
| Education level                 |                  |                  |          |        |
| Primary                         | 2 (2.4)          | 1 (1.2)          | 2.59*    | 0.274  |
| Secondary                       | 19 (22.4)        | 28 (32.9)        |          |        |
| Tertiary                        | 64 (75.2)        | 56 (65.9)        |          |        |
| Tribe                           |                  |                  |          |        |
| Yoruba                          | 51 (60.0)        | 52 (61.2)        | 1.107*   | 0.775  |
| Hausa                           | 4 (4.7)          | 6 (7.1)          |          |        |</p>
<table>
<thead>
<tr>
<th>Variables</th>
<th>Fertile n = 85 (%)</th>
<th>Infertile n = 85 (%)</th>
<th>x2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semen volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (&lt;1.5 mls)</td>
<td>18 (21.2)</td>
<td>14 (16.5)</td>
<td>0.616</td>
<td>0.433</td>
</tr>
<tr>
<td>Normal (≥1.5 mls)</td>
<td>67 (78.8)</td>
<td>71 (83.5)</td>
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<td></td>
</tr>
<tr>
<td><strong>Sperm count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt;15 x 10^6/ml)</td>
<td>85 (100.0)</td>
<td>13 (15.3)</td>
<td>124.898</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low (&lt;15 x 10^6/ml)</td>
<td>0 (0.0)</td>
<td>72 (84.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Progressive motility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≥32%)</td>
<td>85 (100.0)</td>
<td>18 (21.2)</td>
<td>110.583</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low (&lt;32%)</td>
<td>0 (0.0)</td>
<td>67 (78.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total motility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≥40%)</td>
<td>85 (100.0)</td>
<td>37 (43.5)</td>
<td>59.953</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NB: * = Chi square test
** = T-test
<table>
<thead>
<tr>
<th>Morphology</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;4%)</td>
<td>0 (0.0)</td>
<td>48 (56.5)</td>
</tr>
<tr>
<td>Normal (≥4%)</td>
<td>85 (100.0)</td>
<td>6 (7.1)</td>
</tr>
<tr>
<td>Low (&lt;4%)</td>
<td>0 (0.0)</td>
<td>79 (92.9)</td>
</tr>
</tbody>
</table>
Fig 1. Histogram showing the distribution of blood zinc levels among respondents

Fig 2. Histogram showing the distribution of seminal plasma zinc levels among respondents
Table 3: Median blood and seminal plasma zinc levels and median semen parameters in fertile and infertile men

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fertile Median (IQR)</th>
<th>Infertile Median (IQR)</th>
<th>U</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood zinc</td>
<td>3.2 (1.6, 5.2)</td>
<td>1.7 (0.6, 2.7)</td>
<td>2095.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Seminal plasma zinc</td>
<td>19.4 (14.0, 25.5)</td>
<td>9.0 (6.1, 11.0)</td>
<td>488.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volume</td>
<td>2.0 (1.5, 3.1)</td>
<td>2.5 (1.8, 3.2)</td>
<td>3210.0</td>
<td>0.207</td>
</tr>
<tr>
<td>Sperm count</td>
<td>28 (23.0, 40.0)</td>
<td>6.0 (2.5, 11.0)</td>
<td>636.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>40.0 (35.0, 45.0)</td>
<td>20.0 (10.0, 30.0)</td>
<td>767.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total motility</td>
<td>56 (50.0, 64.0)</td>
<td>35.0 (26.0, 45.5)</td>
<td>778.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology</td>
<td>5 (5.0, 5.0)</td>
<td>1.0 (0.0, 2.0)</td>
<td>198.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NB: U = Mann Whitney U test
IQR = Interquartile range

Table 4: Blood and seminal plasma zinc levels among different groups of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Median (IQR)</th>
<th>K</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood zinc levels (μmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normozoospermic</td>
<td>85</td>
<td>3.2 (1.6, 5.2)</td>
<td>27.913</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oligozoospermic</td>
<td>64</td>
<td>1.7 (0.6, 2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthenozoospermic</td>
<td>13</td>
<td>1.7 (0.9, 3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azoospermic</td>
<td>8</td>
<td>0.2 (0.1, 1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seminal plasma zinc levels (μmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normozoospermic</td>
<td>85</td>
<td>19.4 (14.0, 25.5)</td>
<td>97.198</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oligozoospermic</td>
<td>64</td>
<td>8.9 (5.8, 10.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthenozoospermic</td>
<td>13</td>
<td>10.3 (6.7, 14.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azoospermic</td>
<td>8</td>
<td>9.4 (6.5, 16.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NB: K = Kruskal Wallis test
IQR = Interquartile range
Table 5: Correlations of blood and seminal plasma zinc level with semen parameters among fertile and infertile men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fertile spearman’s rho correlation</th>
<th>p</th>
<th>Infertile spearman’s rho correlation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Zinc level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>-0.186</td>
<td>0.088</td>
<td>0.057</td>
<td>0.604</td>
</tr>
<tr>
<td>Sperm count</td>
<td>-0.008</td>
<td>0.940</td>
<td>0.008</td>
<td>0.941</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>0.189</td>
<td>0.083</td>
<td>0.016</td>
<td>0.883</td>
</tr>
<tr>
<td>Total motility</td>
<td>0.080</td>
<td>0.469</td>
<td>0.065</td>
<td>0.553</td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.014</td>
<td>0.896</td>
<td>-0.029</td>
<td>0.794</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminal plasma zinc level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>0.065</td>
<td>0.557</td>
<td>0.031</td>
<td>0.775</td>
</tr>
<tr>
<td>Sperm count</td>
<td>-0.054</td>
<td>0.626</td>
<td>0.014</td>
<td>0.898</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>-0.193</td>
<td>0.077</td>
<td>0.136</td>
<td>0.215</td>
</tr>
<tr>
<td>Total motility</td>
<td>-0.056</td>
<td>0.613</td>
<td>0.118</td>
<td>0.282</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.088</td>
<td>0.423</td>
<td>0.155</td>
<td>0.157</td>
</tr>
</tbody>
</table>
Fig 3. Scatter plots showing correlation between blood zinc and semen zinc levels among fertile men. Spearman rho’s correlation coefficient = 0.008,  \( p = 0.940 \)

Fig 4. Scatter plots showing correlation between blood zinc and semen zinc levels among infertile men. Spearman rho’s correlation coefficient = 0.115,  \( p = 0.297 \)
Table 6: Correlations of blood and seminal plasma zinc level with semen parameters among different groups of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normozoospermic spearman’s rho correlation (p)</th>
<th>p</th>
<th>Azoospermic spearman’s rho correlation (p)</th>
<th>p</th>
<th>Oligozoospermic spearman’s rho correlation (p)</th>
<th>p</th>
<th>Asthenozoospermic spearman’s rho correlation (p)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Zinc level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>-0.186</td>
<td>0.088</td>
<td>0.282</td>
<td>0.498</td>
<td>-0.045</td>
<td>0.722</td>
<td>0.298</td>
<td>0.322</td>
</tr>
<tr>
<td>Sperm count</td>
<td>-0.008</td>
<td>0.968</td>
<td>-</td>
<td>0.311</td>
<td>0.012</td>
<td>0.014</td>
<td>0.964</td>
<td>0.838</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>0.189</td>
<td>0.083</td>
<td>-</td>
<td>0.252</td>
<td>0.045</td>
<td>0.063</td>
<td>0.217</td>
<td>0.283</td>
</tr>
<tr>
<td>Total motility</td>
<td>0.079</td>
<td>0.472</td>
<td>-</td>
<td>0.285</td>
<td>0.022</td>
<td>0.367</td>
<td>0.217</td>
<td>0.283</td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.014</td>
<td>0.896</td>
<td>-</td>
<td>-0.109</td>
<td>0.389</td>
<td>-0.322</td>
<td>0.283</td>
<td>0.283</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>0.065</td>
<td>0.557</td>
<td>-0.233</td>
<td>0.578</td>
<td>0.064</td>
<td>0.613</td>
<td>-0.307</td>
<td>0.308</td>
</tr>
<tr>
<td>Sperm count</td>
<td>-0.054</td>
<td>0.626</td>
<td>-</td>
<td>0.086</td>
<td>0.497</td>
<td>0.438</td>
<td>0.134</td>
<td>0.574</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>-0.193</td>
<td>0.077</td>
<td>-</td>
<td>0.251</td>
<td>0.045</td>
<td>0.172</td>
<td>0.187</td>
<td>0.574</td>
</tr>
<tr>
<td>Total motility</td>
<td>-0.056</td>
<td>0.613</td>
<td>-</td>
<td>0.218</td>
<td>0.083</td>
<td>0.391</td>
<td>0.187</td>
<td>0.187</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.088</td>
<td>0.423</td>
<td>-</td>
<td>0.165</td>
<td>0.192</td>
<td>0.096</td>
<td>0.756</td>
<td>0.756</td>
</tr>
</tbody>
</table>
Table 7: Blood zinc levels in subjects with normal and abnormal semen parameters irrespective of their fertility status

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Median (IQR) blood zinc</th>
<th>U</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>138</td>
<td>1.8 (IQR 1.0, 4.0)</td>
<td>1935.0</td>
<td>0.276</td>
</tr>
<tr>
<td>Low</td>
<td>32</td>
<td>2.4 (IQR 1.6, 5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sperm count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>98</td>
<td>3.1 (IQR 1.5, 5.1)</td>
<td>2163.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low</td>
<td>72</td>
<td>1.6 (IQR 0.5, 2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Progressive motility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>103</td>
<td>2.2 (IQR 1.5, 5.0)</td>
<td>2477.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Low</td>
<td>67</td>
<td>1.7 (IQR 0.8, 2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total motility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>122</td>
<td>2.0 (IQR 1.3, 4.6)</td>
<td>2540.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Low</td>
<td>48</td>
<td>1.7 (IQR 1.0, 3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>91</td>
<td>3.1 (IQR 1.5, 5.2)</td>
<td>2269.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low</td>
<td>79</td>
<td>1.7 (IQR 0.7, 2.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NB: U = Mann Whitney U test
IQR = Interquartile range
Table 8: Seminal plasmazinc levels in subjects with normal and abnormal semen parameters irrespective of their fertility status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Median (IQR) seminal plasma zinc level</th>
<th>U</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>138</td>
<td>11.9 (IQR 8.5, 18.4)</td>
<td>1997.0</td>
<td>0.400</td>
</tr>
<tr>
<td>Low</td>
<td>32</td>
<td>12.5 (IQR 8.9, 19.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sperm count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>98</td>
<td>18.4 (IQR 12.5, 25.2)</td>
<td>687.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low</td>
<td>72</td>
<td>8.9 (IQR 5.9, 10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Progressive motility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>103</td>
<td>17.9 (IQR 12.2, 24.3)</td>
<td>1006.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low</td>
<td>67</td>
<td>8.9 (IQR 6.1, 11.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total motility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>122</td>
<td>15.1 (IQR 11.1, 23.3)</td>
<td>1083.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low</td>
<td>48</td>
<td>8.7 (IQR 6.1, 10.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>91</td>
<td>18.5 (IQR 14.1, 25.5)</td>
<td>407.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low</td>
<td>79</td>
<td>8.7 (IQR 6.0, 10.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NB: U = Mann Whitney U test  
IQR = Interquartile range
Table 9: Correlation of blood and seminal plasma zinc levels with semen parameters among subjects irrespective of their fertility

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spearman rho correlation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood zinc level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>-0.099</td>
<td>0.197</td>
</tr>
<tr>
<td>Sperm count</td>
<td>0.272</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>0.286</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total motility</td>
<td>0.229</td>
<td>0.003</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.309</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Seminal plasma zinc level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>-0.046</td>
<td>0.584</td>
</tr>
<tr>
<td>Sperm count</td>
<td>0.555</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>0.467</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total motility</td>
<td>0.503</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.712</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig 5. Scatter plots showing correlation between blood zinc and semen zinc levels among respondents irrespective of their fertility.  Spearman rho’s correlation coefficient = 0.299 p = <0.001
CHAPTER SEVEN

DISCUSSION

The aim of this study was to evaluate and compare the concentrations of zinc in blood and seminal plasma with semen parameters among fertile and infertile Nigerian men. The sociodemographic characteristics of the two groups were similar hence good basis for comparison. The results of this study showed that there were significantly lower levels of blood and seminal plasma zinc in infertile males compared to fertile male controls. This finding was in agreement with what was reported in Pakistan by Hasan et al.\textsuperscript{19} and in Singapore by Chia et al\textsuperscript{65} who observed significant decreased in seminal plasma zinc in infertile males. Hasan et al.\textsuperscript{19} reported that zinc concentration in seminal plasma should be considered as one of the factors responsible for decreased testicular function in infertile male subjects.

In contrast, Fuse et al.\textsuperscript{73} and Umeyama et al.\textsuperscript{75} found no significant difference in the mean value of seminal plasma zinc of infertile men compared to fertile men. This could be due to the small sample size for the fertile control group for the two studies. It must be noted that the number of men in the fertile control group in these two studies were 8 and 22 respectively. The result of blood and seminal
plasma zinc of our study was not in accordance with what was reported by Akinloye O et al.\textsuperscript{70}, who observed higher levels of blood and seminal plasma zinc in infertile males compared to fertile male control.

Seminal plasma zinc levels in the oligozoospermic and azoospermic infertile men were significantly lower than in normozoospermic fertile men. These findings were similar to results obtained by Hasan et al.\textsuperscript{19} They studied the relationship of zinc concentrations in blood and seminal plasma with various semen parameters in infertile subjects. The average blood and seminal plasma zinc levels in oligozoospermic infertile subjects were 1.7 μmol/L and 8.9 μmol/L respectively in our study; while it was 1.07 μmol/L and 8.28 μmol/L respectively in the work done by Hasan et al\textsuperscript{19}. The concentration of blood and seminal plasma zinc in normozoospermic fertile men who were the control in our study were 3.2 μmol/L and 19.4 μmol/L respectively; while the control group for the study by Hasan et al\textsuperscript{19} who were also normozoospermic had blood and seminal plasma concentration of 1.26 μmol/L and 9.79 μmol/L respectively. The control group for the study by Hasan et al were men with normal sperm count selected from the general population after semen analysis; while the control for our study were men with normal sperm count and proven fertility. This could account for the wide
variations in the seminal plasma zinc concentrations in the 2 control groups of both studies.

This study was able to establish correlations between blood and seminal plasma zinc levels and semen parameters, which is consistent with other studies and inconsistent with some previous studies. In our study seminal plasma zinc levels were positively correlated with sperm count in oligozoospermic and asthenozoospermic men, though the relationship was not statistically significant. This was in accordance with what was reported by some studies and contrary to the findings of other studies. Sperm counts are largely variable and are influenced by various factors such as past illnesses, smoking status, use of medication, and time of abstinence. In this study, time of abstinence was kept constant and special care was taken to ensure identical conditions during sampling, storage, and analysis. The other factors were also eliminated since none of the subjects had any urogenital infection and none was using any medication.

This study revealed a significant positive correlation between seminal plasma zinc levels and progressive sperm motility in the oligozoospermic infertile group. This was in accordance with several results which have been reported elsewhere.
but differs from the result of some studies.\textsuperscript{80,94} Total motility was also positively correlated in this group and in the asthenozoospermic subjects, but not statistically significant.

This study revealed a weak positive correlation between seminal plasma zinc levels and sperm morphology in the normozoospermic, oligozoospermic and the asthenozoospermic subjects. This was not in accordance with what was reported in Japan by Fuse et al.\textsuperscript{73} who observed no correlation between seminal plasma zinc level and sperm morphology; but was in agreement with the studies done in Singapore by Chia et al.\textsuperscript{65} and in India by Doshi et al.\textsuperscript{20}. Our study revealed semen volume to be positively correlated with the seminal plasma zinc in infertile subjects, though not statistically significant. This result was in accordance with the findings of Omu et al.\textsuperscript{79} and contrary to some other studies.\textsuperscript{92,93}

Some authors have reported high concentration of seminal plasma zinc to be associated with enhanced sperm parameters including sperm count\textsuperscript{65,73,81}, motility\textsuperscript{73,82} and normal morphology\textsuperscript{65}, whereas another study\textsuperscript{83} reported a high concentration of seminal plasma zinc to be associated with poor motility of sperm. Other studies could not find a significant association between zinc concentration in seminal plasma and semen parameters.\textsuperscript{25,84,85}
This study revealed a significant positive correlation between blood zinc and sperm count and between blood zinc and sperm motility (progressive and total) in oligozoospermic infertile subjects. These findings were not in accordance with what was reported by Hasan et al\textsuperscript{19} and Madding et al\textsuperscript{95} who reported no significant correlation between blood zinc and semen parameters. This difference may be due to the small sample size for the fertile control group in the two studies. The number of men in the fertile control group in these two studies were 25 and 11 respectively. A weak positive correlation was observed between blood zinc levels and seminal plasma zinc levels among the fertile men and infertile men in this study (fig 3 and fig 4). However, this was not statistically significant. This was in accordance with some other studies.\textsuperscript{19,95}

This study observed significant difference between fertile and infertile group in relation to sperm count, progressive motility, total motility and morphology. It was clear that the infertile group had poorer semen parameters compared to the fertile group. Using the WHO criteria (2010), the men in the infertile group had median semen parameter values that were below the WHO-designated normal values, except for semen volume (Table III). This finding could explain why the couples had been unable to conceive for at least a year.
Further comparisons were made in our study between subjects with normal and different types of abnormal semen parameters irrespective of whether or not they were fertile. Except for those with abnormal/normal semen volume, decreased blood and seminal plasma zinc levels were observed in those with abnormal semen parameters compared to those with normal semen parameters. These differences were statistically significant for all the semen parameters studied except for semen volume. We also observed a statistically significant positive correlation between blood zinc levels and seminal plasma zinc levels among all the respondents, irrespective of their fertility status (Fig 5).

Zinc is present in high concentrations in the seminal fluid and there is evidence that it may act in vivo as a scavenger of excessive oxygen production by defective spermatozoa and/or leukocytes in semen after ejaculation and may also play a multifaceted role in sperm functional properties. A clinical study demonstrated that adult males experimentally deprived of zinc showed a disturbance of testosterone synthesis in the Leydig cells. The authors concluded that adequate seminal concentration of zinc is required for normal sperm function. Studies have demonstrated that zinc therapy results in significant improvement in sperm quality with increase in sperm density, progressive motility, and improved conception and pregnancy outcome.
Our study clearly indicated that there is a relationship between zinc and semen parameters. However, further studies are needed in our population to observe whether zinc supplementation will improve semen quality in males with sub normal semen parameters. Such studies will be useful in deciding the necessity of zinc supplementation in cases of male infertility.
CONCLUSION

On the basis of the findings of this study the following conclusion may be drawn from this study.

- There was significant difference between the semen parameters (sperm count, progressive motility, total motility and morphology) of fertile and infertile men.
- The blood and seminal plasma zinc levels were significantly lower in the infertile group than the fertile group.
- Blood and seminal plasma zinc levels were significantly positively correlated with semen parameters in oligozoospermic infertile Nigeria men.
- Blood zinc level was significantly positively correlated with Seminal plasma zinc level.
RECOMMENDATIONS

This study has demonstrated that zinc may have a role in male fertility even in our environment, there is therefore;

- An urgent need for more research in this area in Nigeria as this will further shed more light on the role of zinc in male infertility and also enable a standard reference value to be set for the general population to enhance objective comparison. It will also enable us to know if zinc supplementation should be adopted for use in the treatment of male infertility.

- A need to harmonise the various methods of determination of zinc to enable proper comparison of results.

- A need to include blood and semen zinc assessment in the routine investigation of infertile male as the investigation of this group of people may not be complete without them.
REFERENCES


APPENDIX 1

PROFORMA FOR DATA COLLECTION

Serial no...........  Patient code.............  Sample code........

1. Hospital No.:  

2. Age in years:  

3. Marital Status: single ( ), married ( ), divorced( ), widowed( ), separated( ).  

4. Educational Status : primary( ), secondary( ), tertiary( ), none( )  

5. Occupation:  

6. Religion:  

7. Tribe:  

8. Height (m) ............., Weight (kg) ................... , BMI ...............................  

9. How long have you been married ......................................  

10. Have you had children in the past: Yes( ), No ( )  

11. If yes to Q10, Are the children from your present wife: Yes( ), No ( )  

12. Are you exposed to any of the following during the course of your work:  
   (i) metals ( ), (ii) pesticides ( ), (iii) solvents ( ), (iv) heat( ). If yes how long have you been exposed .................................  

13. Are you diabetics: Yes ( ), No ( ). If yes how long ago were you diagnosed ................  

14. Are you hypertensive : Yes ( ), No ( )  

15. Any history of chronic illness: Yes ( ), No ( ). If yes specify .................................
16. Any history of heat or cold intolerance: Yes ( ), No ( )

17. Are you on any routine medication: Yes ( ), No ( ). If yes, what is the medication for.........................

18. Are you taking vitamins or mineral supplementation: Yes ( ), No ( )

19. Have you had any pelvic surgery: Yes ( ), No ( ). If yes, what type ............

20. Have you had any injury to your testes: Yes ( ), No ( )

21. Any history of sexually transmitted infections: Yes ( ), No ( )

22. Do you have frequent or painful micturition: Yes ( ), No ( )

23. Do you use any contraceptive: Yes( ), No ( )

24. If yes to Q23, which contraceptive do you use minipills( ), COCP( ), Injectables( ), IUCD( ), Condom( ), Tubal ligation( ), Vasectomy( )

25. Do you smoke cigarette: Yes ( ), No ( )

26. If yes to Q25 :
   a. How many sticks do you smoke per day ....................
   b. How long have you been smoking ....................

27. Do you take any hard drugs: Yes ( ), No ( ). If yes, specify the name ............

28. Do you drink alcohol: Yes ( ), No ( )

29. If yes to Q28
   a. How frequent
   b. How much do you consume per day (bear bottles)....................
LABORATORY REPORT

30. Semen parameters

Method of collection: Masturbation ( ), Coitus interruptus

Abstinence.......... days. Time collected..............................

Time received.......... Time examined..............................

Volume............ Total motility.........%

Progressive motility.........% Normal forms.......% Abnormal forms.....%

Total count (concentration)........................................10^6cells/ml

Vitality (Live).........% Dead............%

WBC.........................10^6

31. Concentration of zinc in seminal plasma: ..............................................

32. Concentration of zinc in the blood: .....................................................
APPENDIX 2

PARTICIPANT CONSENT FORM

Title of the Research: Correlation of Blood and Seminal plasma Zinc levels and Semen parameters In Fertile and Infertile Nigerian men

Name and Affiliation of Researcher
This study is being conducted by Dr. J.K Ogah of the Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital (LASUTH), Ikeja, Lagos state-Nigeria.

Sponsors of Research
This study is self- sponsored.

Purpose of Research
The purpose of this research is to evaluate and compare the concentration of zinc in blood and seminal plasma with various semen parameters among fertile and infertile Nigeria men.

Procedure of the Research
A proforma for Data collection will be administered to obtain information including patient’s demographic data. Semen and blood sample will be collected from each subject and sent to the laboratory scientist for analysis. About 170men will participate in this study.

Expected Duration of Research
The duration of the study is expected to be about 4-6 months.

Risk
The participants in this research are not at any risks. All precaution will be taken to ensure that the process of sample collection does not cause you any harm.
Cost to the Participants, if any, of joining the Research
Your participation in this research will not cost you anything. You will not be required to stay longer in the Hospital because of your participation in this study. The researcher will bear the cost of the investigations.

Benefits
The goal of this study is to determine if blood and seminal plasma zinc levels have a role in the pathophysiology of male infertility in order to improve the management of infertility. It will also afford participants an opportunity to know their blood and seminal plasma zinc level at no costs.

Confidentiality
All information collected in this study will be given code numbers and no name will be recorded. This cannot be linked to you in anyway and your name or any identifier will not be used in any publication or reports from this study. As part of my responsibility to conduct this research properly, officials of NAFDAC, NHREC and ethics and food and drugs regulators from the United States may have access to these records.

Voluntariness
Your participation in this research is entirely voluntary.

Alternatives to Participation
If you choose not to participate, this will not affect your treatment in this hospital in any way.

Due Inducement
You will not be paid any fees for participating in this study.

Consequences of Participant’s decision to withdraw from research and procedures for orderly termination of Participation
You can also choose to withdraw from the research at anytime. Please note that some of the information that has been obtained about you before you chose to withdraw may
have been modified or used in reports and publications. These cannot be removed anymore. However, the researcher promises to make good faith effort to comply with your wishes as much as is practicable.

**Modality of Providing treatments and Action to be taken in case of injury or adverse events**
If you suffer any injury as a result of your participation in this research, you will be treated at the Lagos State University Teaching Hospital, Ikeja and the research will bear the cost of this treatment.

**What happens to Research Participants and communities when the Research is over**
The Researcher will inform you of the outcome of the research through a news bulletin. During the course of this research, you will be informed about any information that may affect your participation or your health.

**Statement about Sharing the Benefits among Researchers and Whether this includes or excludes Research Participants**;
If this research leads to commercial products, the Lagos State University Teaching Hospital shall own it. There is no plan to contact any participant now or in future about such commercial benefits.

**Any Apparent or Potential Conflict of Interest**
The researcher does not have any interest or information that may prevent her from doing her work without fear or favour.

**Statement of Person Obtaining Informed Consent**:
I have fully explained this research to .............................. and have given sufficient information, including about risks and benefits, to make an informed decision.

Date: ..........................
Signature: ..........................
Name: ..........................
Statement of Person giving Consent

I have read the description of the research or have had it translated into language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

Date: ......................................................
Signature:...................................................
Name:........................................................
Witness’ Signature (if applicable): .....................
Witness name (If applicable): ...........................

This research will be approved by the Health Research Ethics Committee of the Lagos State University Teaching Hospital and the Chairman of this Committee can be contacted at the Health and Ethics Committee Office, Lagos State University Teaching Hospital, 1-5 Oba Akinjobi Road, Ikeja-Lagos. Tel: 01-4710670, www.lasuth.org.

In addition, if you have any question about your participation in this research, you can contact the principal investigator, Dr. James Kolawole Ogah at the Department of Obstetrics and Gynaecology, LASUTH, Ikeja
Phone No: 08033884387, email: kolaogah@yahoo.com

PLEASE KEEP A COPY OF THE SIGNED INFORMED CONSENT