CORRELATION OF SEMINAL PLASMA ZINC LEVELS AND SEMEN PARAMETERS IN NORMOZOOSPERMIC AND OLIGOZOOSPERMIC MEN ATTENDING AN INFERTILITY CLINIC

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I hereby declare that this work is original unless otherwise acknowledged. This has neither been presented to any college, faculty or school for the award of a degree, diploma or fellowship nor has it been submitted elsewhere for publication.

----------------------------------------
Dr. Omu Patrick
DEDICATION

To my dear wife, Ejiro and sweet children Orezioghene and Oghenekarho, for their sacrifice and endurance while the programme lasted.
I worship the Almighty God for his divine assistance in making this dissertation a reality.

My gratitude goes to all the great professors and consultants in the department for the rare privilege to learn under them.

I am particularly grateful to my supervisors Professor. A..A.E Orhue and Dr. M.E Aziken for painstakingly going through the dissertation and tirelessly guiding me through the study.

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Finally my sincere gratitude goes to my wife, children, parents and siblings for their support, encouragement and understanding. Glory be to God for His multiple blessings and success in our lives through Jesus Christ our Lord. Amen.
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ABSTRACT

**Background:** Seminal plasma zinc level is significantly correlated with sperm concentration, motility and morphology. Abnormal level of this essential trace element has been associated with abnormal semen parameters. Routinely, this element is not assessed during investigation of male factor infertility. With its important role in normal testicular development, spermatogenesis and sperm motility, assessment of zinc levels during evaluation of male partners of infertile couples and possibly replacement therapy may improve abnormal sperm parameters.

**Objective:** To determine the levels of zinc in seminal plasma and its relationship with sperm quality in normozoospermic and oligozoospermic males.

**Study Design:** A comparative analytical case control study of male partners of infertile couples presenting for infertility assessment.

**Methods:** Semen parameters of 116 healthy consented male partners of infertile couples oligozoospermia (n=59), normozoospermia (n=57) undergoing evaluation for infertility were assessed. Seminal plasma zinc levels were determined using Flame Atomic Absorption Spectrophotometer. Zinc concentration was expressed as μmol/L. Primary outcome measure was zinc levels in seminal plasma in oligozoospermic men. Data entry and analysis was performed using SPSS software for windows version 16.0. An independent t test was used to compare the scores of each of the measures and mean of parameters between the two groups. The Pearson’s correlation of coefficient test and Linear Regression were used to analysis and examine relationship between
the semen parameters and zinc concentration scores. Test of significant was by chi square and Fisher exact test when appropriate and a probability level of ≤ 0.05 was considered statistically significant.

**Results:** The mean concentration of zinc in seminal plasma was significantly higher in oligozoospermic males when compared to normozoospermic subjects, 31.86 ± 3.92 μmol/L vs 16.90 ± 4.20 μmol/L, respectively (P< 0.000). Negative significant correlations were found between seminal plasma zinc concentrations and semen parameters among the study population: morphology (r = -0.770, P< 0.000), motility (r = -0.7444, P< 0.000), count (r = -0.712, P< 0.000), viability (r = -0.750, P< 0.001) but semen volume did not reach significant correlation (r = -0.91, P= 0.332). In oligozoospermic men, a significant (P< 0.001) correlation was observed between seminal plasma zinc levels and sperm counts, while in normozoospermic men only weak correlations was reached between seminal plasma zinc levels and semen parameters.

**Conclusion:** Sperm counts, motility, morphology and viability are affected by variations of seminal plasma zinc concentrations. Assessment of zinc levels in male infertility with severe semen abnormality is a useful tool in evaluation of male factor infertility.
INTRODUCTION

Infertility is a major public health concern, with economic and psychosocial consequence Worldwide including Nigeria. Infertility could be defined as the inability to achieve a pregnancy after one year of regular intercourse without contraception,¹ and may affect about 15% of couples in the reproductive age group.¹ Male factor infertility describes a situation where the inability to conceive is associated with an anomaly in the male partner, in a normal female. It is found in about 20% of infertility and is often a contributory factor in another 30% to 40%.² In a male dominated societies like Nigeria, a couple’s infertility is almost always attributed to the female partner often to the neglect of male related causes of infertility which is often poorly or never evaluated in our environment.

Semen fluid analysis (SFA) is the initial and most essential step of infertility evaluation³ as a screening and when there are persisting anomalies in 2 separate samples, further screening includes; a physical examination, hormonal evaluation, sperm function testing, and where applicable genetic analysis. SFA is performed according to the WHO criteria⁴ and the results are used to categorise the severity of male factor infertility (MFI).³

Human spermatozoa display marked heterogeneity, and therefore a variety of sperm abnormalities may be found in the semen sample; even in those from fertile men. Male infertility generally involves a status in which the inability to conceive is due to anomalies present in the male partner with a normal female. This anomaly may be associated with low sperm production (oligozoospermia), poor sperm motility (asthenozoospermia) or abnormal sperm morphology (teratozoospermia); and a combination of these Oligoasthenoteratozoospermia
(OAT syndrome), is considered to be the most common and severe cause of male infertility.\textsuperscript{5} A normal seminal fluid analysis does not necessarily indicate satisfactory fertility potential. Owing to these inherent limitations in the methods of assessment, an accurate diagnosis of male factor infertility (MFI) can be made in only 40% of affected males seeking assistance.\textsuperscript{6}

There is evidence that is suggesting that human semen quality has been decreasing in the last few decades. For example, sperm density is said to have fallen by 40% over the past 50 years and there had been a decline in sperm density, motility and the percentage of morphologically normal sperm in fertile men.\textsuperscript{7-9} These findings led to much speculation about the cause and the mechanism of the decline of semen quality. Although many causes have been identified as causing male factor infertility, most of the report remains inconclusive, because of differences in definitions of infertility and by different methods in semen analysis used in these studies.

Aetiological factors for male infertility and subfertility are diverse. These include abnormalities of sperm production and functions arising from genetic, endocrine and congenital disorders, the presence of varicocele, and genitourinary infections. Autoimmune factors with the presence of antisperm antibodies; which affects transport of sperm through the cervical mucus. Erectile dysfunction and premature ejaculation also impairs adequate sperm deposition. Age also impacts on the fertility potentials of a man because spermatogenesis increases during puberty, reaches a plateau phase at the age of 55 years, and subsequently decreases.\textsuperscript{10} Lifestyle factors that may affect semen quality are smoking,\textsuperscript{11,12} alcohol use,\textsuperscript{13} stress,\textsuperscript{14} and high temperature.\textsuperscript{15} Many physical, chemical, and pharmacological agents may also affect semen quality.\textsuperscript{15,16}
The relationship between good nutrition and reproduction is well established.\(^1\) However, the impact of nutrition on male factor infertility and subfertility has not been sufficiently studied. Nutritional deficiencies may be an important cause of reproductive impairment in males. In animals, vitamin A deficiency causes germinal cell degeneration.\(^2\) Human spermatozoa are particularly susceptible to peroxidative damage because they contain high concentrations of polyunsaturated fatty acids and also have a significant ability to generate reactive oxygen species, mainly superoxide anion and hydrogen peroxide. Antioxidants such as vitamin E (\(\alpha\)-tocopherol), vitamin C (ascorbic acid), and carotenoids can restore the proper peroxidant-antioxidant balance (oxidative stress) and maintain the genetic integrity of sperm cells by preventing oxidative damage to sperm DNA.\(^3\) Selenium and vitamin E supplementations seem to improve sperm motility and morphology.\(^4,5\) Several animal studies have shown the importance of dietary vitamins C and D on semen quality and reproductive functions.\(^6,7\)

Much attention has been given to the impact of the nutritional trace element zinc on reproductive functions in man.\(^8\) Zinc plays an important role in normal testicular development, spermatogenesis, and sperm motility. Zinc has been reported to be an essential cofactor for 80 metalloenzymes and plays an important role in the polymeric organization of macromolecules such as RNA and DNA, protein synthesis, cell division, and stability of biomembranes. Zinc also has numerous important functions and is essential for conception, implantation, and a favourable pregnancy outcome.\(^9,10\) Zinc is present in high concentrations in the seminal fluid and may play a multifaceted role for sperm functional properties. It influences the fluidity of lipids, and thus the stability of biological
membranes. It affects the stability of sperm chromatin. It is involved in the formation of free oxygen radicals and may play a regulatory role in the process of capacitation and the acrosome reaction.

The concentration of zinc in the male genital organs and human semen is extremely high compared with that of other body fluids and tissues. Zinc is secreted predominantly by the prostate. Therefore, it is likely that zinc levels in seminal plasma mostly reflect the prostatic secretory function. The main sources of Zinc are meat and seafood. Zinc deficiency causes hypogonadism and Zinc is thought to be important in the stabilization of sperm chromatin. Consensus exists that the Zinc concentration in seminal plasma is of importance for spermatogenesis, although the results of several studies are still elucidating the other multiple very essential role of this trace element.

Seminal plasma zinc levels are related to multiple determinants of semen quality such as progressive motility, the total motile sperm count, the formation of local sperm antibodies, and other prostatic or epididymal markers. Sperm count, motility, and morphology are parameters used to evaluate potential male fertility. Zinc is believed to be important for normal spermatogenesis and sperm motility. This study investigated the relationship of this nutritional trace element to male factor infertility and semen parameters by quantifying the concentrations of zinc in seminal plasma of normozoospermic and oligozoospermic males.
LITERATURE REVIEW

BACKGROUND

Male infertility implies a lack of sufficient numbers of competent sperm, resulting in failure to fertilize the normal ovum. Male factor is the sole cause of infertility in 20% of infertile couples, but it may be a contributing factor in as many as 30% to 40% of cases. However, the combined effects of our increased understanding of genetic causes in male factor infertility and the efficacy of intracytoplasmic sperm injection (ICSI) in assisted reproductive technique (ART) have revolutionized modern treatment of male factor infertility. Semen analysis is inexpensive and non-invasive, and remains fundamental to the infertility evaluation. The value and interpretation of the semen analysis and other tests for male infertility should be considered within the context of male reproductive physiology. With the considerable progress in our understanding of male infertility, it is often possible to treat the specific disorder in the man medically or surgically and natural conception may be possible with appropriate therapy.

Male infertility in Nigeria is reported to be an important but neglected reproductive health issue. Published studies indicated that the male factor accounts for 20% – 50% of the causes of infertility in different parts of the country. However, very little has been done to identify the original causes of male infertility in the country, and several reports on the major part of male infertility is unexplained. A study conducted among infertile men in Ibadan and screened with the alpha-glycosidase test, found cases where occlusion of the vas deferens may be responsible for infertility. Similar studies revealed high
rates of hyperprolactinemia\textsuperscript{40,41} anti-sperm antibodies\textsuperscript{42} and genital infections\textsuperscript{43} in Nigerian men presenting with infertility. However, these studies had limitations of not exploring the background causes of these abnormalities, and also the absence of a control group also made it difficult to interpret the findings. Studies from several populations around the world,\textsuperscript{44-46} indicated that smoking; types of occupation, alcohol and coffee intake and deficient nutritional factors are risk factors of male infertility. Human seminal plasma contains several nutritional trace elements that play an important role in the normal function of sperm and zinc is one such element with highest concentration in seminal plasma than any other part of the body. This study set out to measure seminal plasma zinc levels in both normozoospermic and oligozoospermic men, and determine if any, correlation with semen parameters.

**SEMINAL FLUID ANALYSIS**

Semen Analysis remains the most feasible test for male fertility even though it is not a conclusive indicator of fertility or Infertility. Semen guidelines such as those of the World Health Organization (WHO)\textsuperscript{4} have been established to determine semen quality limits below which the chance of achieving pregnancy becomes increasingly low. Abnormal semen quality is a threat to male reproductive health with serious psychological and mental consequences. The aetiology of abnormal semen and its diagnosis and evaluation in many developing countries are imprecise for many reasons. The method of semen collection, analysis and interpretation of results are not uniform. Thus it becomes
difficult to appreciate the degree of normality in a particular sample of semen because of various definitions of normal and abnormal semen.

WHO published manuals in 1980, 1987, 1992, and 1999 with the objective of standardizing semen analysis procedures in andrology laboratories Worldwide. Most human semen analysis laboratories have adopted the WHO-recommended reference values for assessing semen characteristics. However, there is a prevailing concern among clinicians that the current WHO reference values are too stringent. The 1999 manual widely in use was formulated based on the clinical experiences of many investigators who have studied populations of healthy fertile men. Since these values are not the minimum values needed for conception, they are referred to as ‘reference’ rather than ‘normal’ values. Before adopting the values provided in these manuals, it is important to implement the techniques specified by the WHO and establish reliable quality control procedures, both internal (within the establishment) and external (in comparison to other regional and national units). Interpreting a semen analysis report, even with the provision of the reference values, will require some information on quality control data, including both, the inter- and intra-observer coefficients of variance or at least some understanding of their role.

At least two semen analysis tests should be carried out to minimise the effect of the confounding variables on the semen analysis results.\(^4\) This eliminates the seasonal variation effect as well as other technical artefacts. The Royal College of Obstetrics and Gynaecology (RCOG) guidelines\(^{47}\) also made the same recommendation. It also advised that General Practitioners who make
the first contact with the couple should send semen for analysis to the same laboratory use by the fertility expert to whom the couple will be referred to, in a bid to reduce technique error. Laboratory under-taking the analysis should adopt the WHO methodology, practise quality control and belong to an external quality control scheme.

SAMPLE COLLECTION

The WHO manual\textsuperscript{4} stated that sample should be collected in a private room near the laboratory, in order to limit the exposure of the semen to fluctuation in temperature and to control time between collection and analysis. That a sample may be collected at home in exceptional circumstances such as demonstrated inability to produce sample by masturbation in a clinic or the lack of adequate facility near the laboratory, and that clear and special instruction concerning collection and transport of the semen sample be provided to the man and the sample should get to laboratory within 1 hour of collection. Also, sample may be collected in a condom during sexual intercourse only in exceptional circumstances such as a demonstrated inability to produce a sample by masturbation. Only special non-toxic condom design for semen collection should be used. The WHO manual\textsuperscript{4} also stated that coitus interruptus is not a reliable means of semen collection, because the first portion of the ejaculate, which contains the highest number of spermatozoa, may be lost. Moreover, there may be cellular and bacteriological contamination of the sample and the low pH of the vaginal fluid could adversely affect the semen analysis. And that the postcoital test can be done if the man cannot provide semen sample. If a man cannot
provide a semen sample, the postcoital test may provide some information about his spermatozoa. Sample should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence.

Elzanaty et al. compared semen parameters in sample collected by masturbation from 379 consecutive non-azoospermic men undergoing infertility assessment at the fertility centre. Of the 379 samples, 273 were collected at the clinical laboratory, and 106, at home. Sperm concentration and total sperm count were significantly higher in semen samples collected at home than in those provided at the clinic (P=0.01 and P=0.02, respectively). Rapid progressive motility was observed in a significantly larger proportion of the home-collected samples compared with those obtained at the clinic (P=0.02). The same trend was found regarding total count of progressive motility (P=0.046). However, the samples in the home collection and the clinic-collection group did not differ significantly (P>0.05) with respect to semen volume, proportions showing slow progressive; progressive; or local motility; immotility, or normal morphology. This deterioration may be at least partly a result of the psychological stress experienced in the clinical environment.

**SEMEN PARAMETERS**

Complete semen analysis includes ejaculate volume, sperm count, concentration, motility, and morphology. Reference ranges determined by WHO are listed in Table 1.0.
Table I: Reference ranges for semen analysis

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<tr>
<td>Ejaculate volume</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>20 million/ml</td>
</tr>
<tr>
<td>Total sperm count</td>
<td>40 million/ejaculate</td>
</tr>
<tr>
<td>Motility</td>
<td>50% (grade a &amp; b) or 25% (grade a) within 60 minutes of ejaculation</td>
</tr>
<tr>
<td>Normal morphology (strict criteria)</td>
<td>14%</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
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</table>


TOTAL SPERM COUNT AND CONCENTRATION

Sperm count, or sperm concentration, measures the concentration of sperm in a man’s ejaculate, as distinct from total sperm count, which is the sperm count multiplied by volume. Over 20 million per milliter is considered normal. The average sperm count today is around 60 million per milliliter in the Western world, having decreased by 1 - 2% per year from a substantially higher number decades ago. Azoospermia, defined as the complete absence of sperm on standard microscopic semen analysis, is confirmed by repeated centrifugation of the semen specimen. Azoospermia should be further classified as obstructive (normal spermatogenesis) or non-obstructive (diminished or absent spermatogenesis). Obstructive azoospermia can occur anywhere in the ductal system from the efferent ducts to the ejaculatory ducts. Non-obstructive azoospermia may result from primary or secondary testicular failure. Low levels
of spermatogenesis may be present in nonobstructive azoospermia, but not in sufficient quantity for epididymal transit and ejaculation.\textsuperscript{50}

Oligozoospermia is defined as sperm concentration less than 20 million/ml and severe oligozoospermia is sperm concentration less than 5 million/ml. Varicocele, the most common aetiology for male infertility, often presents as oligozoospermia and abnormalities of sperm motility and forward progression\textsuperscript{51}. Severe oligozoospermia is often idiopathic, but known aetiologies include varicocele, endocrine deficiencies, and genetic disorders such as microdeletion of the Y chromosome.\textsuperscript{52}

**MOTILITY**

Motility is the percentage of sperm with any degree of tail motility. Forward progression is expressed as the percentage of motile sperm progressing in one general direction. Deficient sperm movement (asthenozoospermia) can result from various pathologic processes. The presence of antisperm antibodies (ASA) in the semen may induce sperm aggregation and impaired motility. ASA testing detects antibodies in both serum and semen. Genital tract infections are another cause of impaired sperm motility by increasing the number of leukocytes in the semen white blood cells.

A more specified measure is motility grade, where the motility of sperm are divided into four different grades:\textsuperscript{4}

- **Grade 4:** Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility a.
• **Grade 3**: (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility b.

• **Grade 2**: These have non-progressive motility because they do not move forward despite the fact that they move their tails.

• **Grade 1**: These are immotile and fail to move at all.

**MORPHOLOGY**

Sperm morphology predicts the adequacy of spermatogenesis, and also the likelihood of achieving conception. It is the parameter that has undergone the most significant revision among editions of the WHO laboratory manual.  

WHO normal morphology percentages have decreased sequentially from 50% to 30% to 14% in the most recent guidelines, which endorse the Kruger “strict” criteria. Strict criteria are based on the finding that IVF success rates were significantly higher in a population with greater than 14% normal morphology compared to a population with less than 14% normal morphology. This study established morphology standards for IVF, the relationship between the strict criteria and natural conception is not well established. Menkveld et al. proposed a reduction of strict criteria cut-off for normal spermatozoa to 3%.  

The detection of sperm morphologic abnormalities is a highly subjective evaluation and prone to significant variability. Defects in sperm morphology (teratozoospermia) are categorized by location: head, neck (midpiece) or tail. All three segments must be normal for a sperm to be designated as such. Varicocele and testicular failure are two causes of teratozoospermia. Directed treatment of the specific disorder can improve morphology.
EJACULATE VOLUME

Decreased ejaculate volume may be seen in ejaculatory duct obstruction, primary testicular dysfunction, congenital bilateral absence of the vas deferens (CBAVD), and retrograde ejaculation. In ejaculatory duct obstruction and in most men with CBAVD, the alkaline seminal vesicle contribution is absent from the ejaculate. The acidic prostatic fluid component predominates resulting in an acidic shift (<7.2) of the semen pH. Seminal vesicles contribute fructose to the ejaculate; diminished levels of fructose in the semen correlates with the degree of ejaculatory duct obstruction. In the classic case of complete, bilateral ejaculatory duct obstruction, semen analysis should demonstrate low volume azoospermia with acidic pH and absence of fructose. Seminal vesicle abnormalities (absence, cystic dilation) may accompany CBAVD. Bilateral testicular atrophy caused by primary or secondary testicular failure resulting in hypogonadism can also result in low ejaculate volume. Androgens control seminal vesicle and prostate secretions; as a result, ejaculate volume is reduced in the presence of low circulating testosterone.

THE WHO MANUAL FOR SEMEN ANALYSIS 5TH EDITION 2010

WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction was first published in 1980, because of the need for the standardization of procedures for the examination of human semen.

It has since been updated three times, and used extensively by research and clinical laboratories all over the world; helping to provide global standards in investigating male factor infertility. Even with its acclaimed success, it became
apparent that some recommendations from previous editions of the manual needed to be revised in light of new evidence, and that some concepts needed more explanation and supporting evidence.

In arriving at reference ranges and reference limits used in the 5th edition, data characterizing the semen quality of fertile men, whose partners had a time to pregnancy of 12 months or less, provided the reference ranges for this manual. Raw data from between about 400 and 1900 semen samples, from recent fathers in eight countries on three continents, were used to generate the reference ranges. Conventional statistical tradition is to take the 2.5th centile from a two-sided reference interval as the threshold below which values may be considered to come from a different population. However, a one-sided reference interval was considered to be more appropriate for semen, since high values of any parameter are unlikely to be detrimental to fertility. The 5th centile is given as the lower reference limit. These groups of men had significantly better semen quality than semen samples tested in the general population. It is important to note that falling below these levels does not necessarily mean that a male factor is causing a couple's infertility. However, with lower semen parameters, it is a possible that there is a male factor in the couple's failing to conceive. It is recommended that any clinic performing a semen analysis for a couple use these WHO guidelines\textsuperscript{57} to evaluate and interpret the sample.
Table II  Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics\textsuperscript{57}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower reference limit</th>
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<tr>
<td>Semen volume (ml)</td>
<td>1.5</td>
</tr>
<tr>
<td>Total sperm number (10\textsuperscript{6} per ejaculate)</td>
<td>39</td>
</tr>
<tr>
<td>Sperm concentration (10\textsuperscript{6} per ml)</td>
<td>15</td>
</tr>
<tr>
<td>Total motility (PR + NP, %)</td>
<td>40</td>
</tr>
<tr>
<td>Progressive motility (PR, %)</td>
<td>32</td>
</tr>
<tr>
<td>Vitality (live spermatozoa, %)</td>
<td>58</td>
</tr>
<tr>
<td>Sperm morphology (normal forms, %)</td>
<td>4</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**ZINC A NUTRITIONAL TRACE ELEMENT**

Zinc is an essential trace element for human nutrition that is an integral part of many enzyme systems, including DNA polymerase complex. Zinc deficiency has been associated with stunting of growth and sexual immaturity. As essential nutrient, the importance of zinc was first recognized for the growth of plant life in the 1860s. In humans, a rare genetic disorder called acrodermatitis enteropathica\textsuperscript{58} resulted in skin parakeratosis and diarrhoea in infants fed cows’ milk but not human milk. In 1961, Prasad et al\textsuperscript{59} described a syndrome of hypogonadism, anaemia, and stunting in human who were geophagic. Supplementation with zinc restored growth and sexual maturation, demonstrating the essentiality for dietary zinc in humans.

Zinc is a widely distributed element in foodstuffs (shellfish, liver, milk, and wheat bran). In the human body, zinc is widely distributed in many tissues, blood
cells, bone, and teeth. However, zinc at these sites is firmly bound to protein, and during deficiency and refeeding the concentrations of zinc in tissues (with the exception of blood, milk, hair, and liver) do not change significantly. Endogenous stores of zinc are mobilized in the fasting state, but do not meet metabolic needs during anabolism, because the net movement of zinc is into tissues and circulating zinc is reduced.

Although 86% is in skeletal muscle, there are certain areas where zinc concentration is especially high and may represent functional importance. They are the prostate, hippocampus, pancreas, and kidney cortex. Zinc has been identified as a part of about 120 enzymes. Among them are carbonic anhydrase, carboxypeptidase, alkaline phosphatase, oxidoreductases, transferases, ligases, hydrolases, lyases, and isomerases. Although the syndrome of zinc deficiency cannot be identified with the dearth of any one enzyme, zinc deficiency does have a pronounced effect on nucleic acid metabolism, thus influencing protein and amino acid metabolism. Zinc is an integral constituent of DNA polymerase, reverse transcriptase, RNA polymerase, tRNA synthetase, and the protein chain elongation factor.

Zinc deficiency can alter protein synthesis at a number of different points, and it is not surprising that in the absence of zinc, growth arrest occurs. Furthermore, zinc deficiency is teratogenic as determined by animal studies and observations in patients with untreated acrodermatitis enteropathica. This finding suggests that zinc deficiency may affect gene expression. In experimental studies in unicellular organisms, it has been shown that zinc deficiency changes
the nature of RNA polymerase and the base composition of mRNA. The translated peptides contain a preponderance of arginine-rich peptides that can bind to anions such as phosphate groups in nucleic acids and alter their action. Such an alteration could affect the synthesis of histones, proteins that are known to reduce the activity of DNA as a template.60

Plasma zinc levels are normally regulated to between 80 and 120 g/l or 12–18 mol/l62. Although circulating zinc levels fall in the deficient state, there are other causes of low circulating zinc levels that make this measurement unreliable.62 Hair zinc levels are low when there is low-grade chronic deficiency, but in acute deficiency hair does not grow, and with profound deficiency hair loss occurs and the remaining hair may have normal zinc concentrations.65 It has recently been shown that leukocyte zinc levels are a reliable indicator of zinc deficiency, but this is not an easy measurement to perform.66 Currently, the best way of assessing zinc status and requirements is through multiple clinical parameters.

ZINC AND REPRODUCTIVE PHYSIOLOGY

Zinc plays a key role in reproductive physiology.24,67 Children with habit of eating clay or earth and those who subsist on cereal proteins alone become zinc deficient and exhibit both growth retardation and hypogonadism, which are reversed by zinc administration.68,69 Furthermore, zinc deficiency has been associated with low serum testosterone concentrations in men with uremia, sickle cell anaemia, and infertility.70-72 Mild or marginal zinc deficiency is probably common through-out the world. Much attention has been given to the impact of
the nutritional trace element zinc on reproductive functions in man. Zinc plays an important role in normal testicular development, spermatogenesis, and sperm motility. Zinc has been reported to be an essential cofactor for metalloenzymes and plays an important role in the polymeric organization of macromolecules such as RNA and DNA, protein synthesis, cell division, and stability of biomembranes.

Zinc deficiency in male can occur in cases of decreased zinc intake like vegetarianism, men on slimming diet and starvation. Malabsorption conditions, like inflammatory disease of the bowel in Crohen’s disease and colitis ulcerosa. Men with protein losing entropathies, parasitic diseases have low serum zinc levels. Zinc deficiency also results from condition of increased zinc requirement like in men with chronic diseases like exfoliative dermatitis, chronic infectious disease and cancer. Acrodermatitis enteropathica which is a genetic defect also leads to increased zinc need and subsequent deficiency. Men with Liver, and renal disease have zinc deficiency. The clinical symptoms of zinc deficiency are hair loss, skin lesions, delayed wound healing, dysfunction of the immune system, growth retardation, and gonadal hypofunction.

The concentration of zinc in the male genital organs and human semen is extremely high compared with that of other body fluids and tissues. The lower reference limit for zinc in seminal plasma is 2.4µmol per ejaculate. Zinc is secreted predominantly by the prostate. Therefore, it is likely that zinc levels in seminal plasma mostly reflect the prostatic secretory function. Zinc is also found in the maturing spermatozoa, and there is evidence that zinc in seminal plasma
influences the oxygen consumption of the spermatozoa,\textsuperscript{74,75} nuclear chromatin decondensation,\textsuperscript{76} and acrosin activity.\textsuperscript{77} Zinc deficiency causes hypogonadism,\textsuperscript{78} and zinc is thought to be important in the stabilization of sperm chromatin.\textsuperscript{33} Clinical studies with adult males experimentally deprived of zinc show that Leydig cell synthesis of testosterone depends on adequate dietary zinc.\textsuperscript{79-81} Moreover, zinc plays an important role in the 5a-reductase enzyme that is necessary for the conversion of testosterone into the biologically active form, 5a-dihydrotestosterone (DHT). Netter \textit{et al}.\textsuperscript{82} found a significant increase in testosterone, DHT, and zinc plasma levels after 40–50 days of zinc administration in 37 patients with idiopathic male factor subfertility of 5 years.

**ZINC DEFICIENCY AND SEMEN PARAMETERS**

Zinc deficiency leads to oligozoospermia, impotence, and hypogonadism in rats\textsuperscript{78} and men.\textsuperscript{83} In man, dietary zinc deficiency was first recognized in 1961.\textsuperscript{25} The correlation between the zinc concentration in seminal plasma and semen quality is still evolving. Kvist \textit{et al}.\textsuperscript{33} reported that seminal zinc concentrations were lower in patients with idiopathic subfertility than in normal controls. Saaranen \textit{et al}.\textsuperscript{84} found slightly higher seminal fluid zinc concentration in men with a sperm density of \(>20 \times 10^6/mL\) than in azoospermic and oligozoospermic men. Stankovic and Mikac-Devic\textsuperscript{85} also reported increased motility in such patients. This is in contrast to the results of other studies that indicated that high semen zinc concentrations are related to decreased sperm motility in infertile men\textsuperscript{86}. Carpino \textit{et al}.\textsuperscript{86} found a decreased seminal zinc fraction bound to high molecular weight proteins (HMW-Zn%) and increased unbound seminal zinc in 90 asthenozoospermic patients compared with a
normozoospermic group. The intake of zinc was restricted in men in an experiment performed by Abbasi et al\textsuperscript{81}. Oligozoospermia (total sperm count of \( <40 \times 10^6 \) /ejaculate) was observed in four of five male volunteers after zinc restriction (2.7–5.0 mg/d) for 24–40 weeks. The oligozoospermia recovered to normozoospermia after zinc supplementation.\textsuperscript{81} Clinical studies with adult males experimentally deprived of zinc show that Leydig cell synthesis of testosterone is decreased due to a decreased activity of the Zn-dependent metalloenzyme 5\( \alpha \)-reductase, which is responsible for the conversion of testosterone (T) to the biologically active form, 5a-dihydrotestosterone (DHT).\textsuperscript{79–81} Zinc also has a fundamental role in the antimicrobial activity of the seminal plasma,\textsuperscript{87} sperm nuclear chromatin condensation in men,\textsuperscript{76,77} and acrosin activity.

**ORAL ZINC SUPPLEMENTATION AND SEMEN QUALITY**

A search of the literature revealed that some studies have shown that oral zinc supplementation improves sperm count,\textsuperscript{85,88} motility,\textsuperscript{87} and morphology in infertile men with idiopathic asthenozoospermia and/or oligozoospermia.\textsuperscript{88} However, all these studies included a small number of volunteers, and thus the impact of their conclusions is limited. In a nonrandomized intervention study performed by Kynaston et al\textsuperscript{87} 33 patients with idiopathic asthenozoospermia and/or oligozoospermia attending a male infertility clinic were prescribed oral zinc sulfate (220 mg two times a day) for a period of 3 months. A significant increase was reported in the mean percentage progressive (60%) and total sperm motility (55%). No significant changes in the percentage of dead or abnormal sperm forms were noted. Mean (\( \pm \)SD) seminal fluid zinc concentrations increased significantly from 1.7 \( \pm \) 1.02 to 2.0 \( \pm \) 1.1 mmol/L (\( P<0.3 \))
PREVIOUS STUDIES ON ZINC LEVELS IN SEMINAL PLASMA AND MALE INFERTILITY

Zinc levels in seminal plasma and sperm parameters have previously been studied. Evidence from studies demonstrated that zinc improves the quality of sperm.

Colagar et al. conducted a study in Iran, investigating the association between zinc levels in seminal plasma with sperm quality in fertile and infertile men. Seventy-two semen samples were collected from fertile non-smokers (n = 19), fertile smokers (n = 17), infertile non-smokers (n = 21), and infertile smokers (n = 15). After adjusting for other confounders like alcohol use; use or abuse of other substances and drugs; and a history of orchitis, testicular trauma, sexually transmitted disease, varicocele surgery for inguinal hernia, and cryptorchism. Sperm count and percentage motile sperm were evaluated according to standards set by the World Health Organization. Sperm morphology was evaluated according to the criteria by Kruger.

The authors reported that fertile groups (smokers or non-smokers) demonstrated significantly higher Zn levels in their seminal plasma than any infertile groups (P<0.001). and that a trend was also observed for a lower mean Zn levels in seminal plasma of smokers compared with non-smokers. Fertile non-smokers had significantly high levels of Zn in their seminal plasma than infertile non-smokers (P<0.001); moreover, fertile smokers had significantly high levels of Zn in their seminal plasma compared with infertile smokers (P<0.001). They suggest that poor Zn nutrition may be an important risk factor for low quality of
sperm and idiopathic male infertility. Routine determination of Zn levels during infertility investigation was therefore recommended.

In another study conducted in Netherlands, Wong et al. investigating the impact of calcium, magnesium, zinc and copper in blood and seminal plasma on semen parameters. Blood and semen samples were collected from one hundred and seven fertile and one hundred and three infertile male. Exclusion criteria for both groups included chromosomal disorders related to a fertility disorder, cryptorchidism, vasectomy, and the use of folic acid and/or Zn supplements or medications within three months before recruitment. Subsequently, a semen analysis was performed according to the World Health Organization guidelines to obtain volume, pH, sperm concentration, motility, and morphology. The sperm concentration, motility, and normal morphology were significantly (P < 0.05) lower in subfertile males than in fertile males. The concentrations of Ca, Mg, Zn, and Cu in both compartments were not significantly different between the subfertile and fertile group. Calcium, Mg, and Zn were significantly higher in semen than in blood plasma, with a semen-to-plasma ratio of 69.7 for Zn, 3.7 for Mg, and 2.7 for Ca. The Cu concentration was significantly higher in blood plasma, with a semen-to-plasma ratio of 0.4. No significant correlation was found between blood and seminal plasma concentrations of these elements except for Cu concentrations (P < 0.0001).

The investigators in this study did not establish difference between fertile and infertile males in the concentration of the elements Ca, Mg, Zn, and Cu in the blood and seminal plasma. They reported that that zinc concentrations in seminal
plasma were significantly higher than in blood plasma. The study also revealed a significant correlation with motility, and morphology, and between seminal plasma zinc concentrations and sperm concentration. The authors concluded that although calcium, magnesium, zinc and copper play an essential role in spermatogenesis and fertility, the determination of these elements in the blood and seminal plasma did not discriminate on the basis of fertility in this group of men.

LOCAL STUDIES ON ZINC LEVELS IN SEMINAL PLASMA AND MALE INFERTILITY

A local study on zinc levels in seminal plasma and male infertility was identified from the literature search. We are aware that in our society the incidence of male subfertility, idiopathic infertility and infertility is on the increase, so there is a need to thoroughly evaluate the male that is presenting with this clinical condition.

Akinloye et al\(^3\) studied serum and seminal plasma hormone profile levels of infertile Nigerian male. This study was mainly on hormonal disturbance in male infertility, whereby male reproductive hormone levels in human serum and seminal plasma were evaluated in infertile Nigerian males. Akinloye et al\(^3\) investigated Serum and seminal plasma male reproductive hormones (Leutinizing hormones, Follicular stimulating hormone, Prolactin and Testosterone) in sixty (60) infertile adult male Nigerians (Oligospermic; \(n = 40\) and azoospermic; \(n = 20\)) and forty controls of proven fertility (Normospermic subjects; \(n = 40\)). The results show that the serum concentrations of
gonadotropins (LH and FSH) were significantly higher (P<0.05) in infertile subjects than controls. Patterns of serum prolactin levels were similar. The values of gonadotropins in serum were significantly higher (P<0.05) than those of seminal plasma. Seminal plasma testosterone in infertile subjects was significantly higher (P<0.005) than that of controls but the serum levels of testosterone were significantly higher (P<0.05) in azoospermic than oligospermic subjects and controls. There was no significant correlation between serum hormonal level and seminal plasma hormonal level in all the groups (P<0.05). The study concluded that, male infertility in Nigerians is characterized by hyperprolactinaemia, raised serum gonadotropins (LH, FSH), and raised seminal plasma testosterone. That hormonal profile in serum and seminal plasma were not significantly correlated, and hence cannot be used as exclusive alternative in male infertility investigations.

This study on zinc levels in seminal plasma of normozoospermic and oligozoospermic men and its correlation with semen parameters, will define the effect of the variation in levels of this essential nutritional trace element on sperm count, motility and morphology.
JUSTIFICATION OF STUDY

The relationship between nutritional trace element zinc and semen parameters is presently receiving much attention because of the relationship of zinc with essential bodily functions. Zinc is so ubiquitous in cellular metabolism that even minor impairment of an adequate supply is likely to have multiple biological and clinical effects. Zinc is essential for a wide range of important functions and required by important enzyme systems including the synthesis of DNA and required for spermatogenesis. Correlation of zinc levels in seminal plasma and semen parameters remains an area of controversy, with some investigators ascribing improved semen parameter with increasing levels of zinc while other authors stated contrary findings.

In human semen zinc plays an important role in spermatozoa physiology and the current knowledge on the relationship between seminal plasma zinc levels and different parameters of human semen remain inconsistent. This study is to determine the zinc levels in normozoospermic and oligozoospermic men attending an infertility clinic and also determine, if any relationship between the various semen parameters and zinc levels in these group of men.
AIMS AND OBJECTIVES

OBJECTIVES

MAIN OBJECTIVE

To determine the zinc content in seminal plasma and analyse its relationship and significance with semen parameters in male infertility.

SECONDARY OBJECTIVES

1. To determine the levels of zinc in seminal plasma in normozoospermic and oligozoospermic men.
2. To assess the relationship between zinc levels in seminal plasma with sperm quality in normozoospermic and oligozoospermic men.
3. To evaluate the possibility for routine assessment of zinc level in seminal plasma in the evaluation of male factor infertility.

STUDY HYPOTHESIS

Null Hypothesis ($H_0$): There is no relationship between seminal plasma zinc levels and semen parameters in male infertility.

Alternative Hypothesis ($H_A$): There is a significant relationship between seminal plasma zinc levels and semen parameters in male infertility.
MATERIALS AND METHODS

STUDY LOCATION

This study was conducted at the department of Obstetrics and Gynaecology, of the University of Benin Teaching Hospital, (UBTH) Benin City. The hospital serves as a major referral centre for Edo, Delta and Ondo States. Patients were referred from General hospitals, Government owned health centres, private hospitals and from other departments in the hospital. Patients were recruited from the Gynaecology Clinic of the various units in the department, Urology Clinic and referral to the Human Reproduction Research Programme (HRRP), which is one of the four academic units of the department. This is dedicated infertility research unit in the department of Obstetrics and Gynaecology, UBTH, responsible for the management of infertile couples including the use of Assisted Reproduction Technology (ART). The HRRP was established as an academic unit with the support of WHO since 1st June 1989 and relied on the management of the infertile couple using the standard WHO guideline with its conventional infertility treatment methods. However since 2007, the unit acquired competence in Assisted Conception Technologies including in-vitro fertilization (IVF), Intrauterine Insemination (IUI), Intracytoplasmic Sperm Insemination (ICSI), and cryopreservation for the treatment of infertility.

STUDY DESIGN

A comparative analytical case control study of male partners of infertile couples presenting for infertility assessment.
STUDY POPULATION

The study population were male partners of infertile couples presenting for infertility management. Initially, couple were seen together with detailed history obtained from them. Thereafter a detailed physical examination was done for both of them and seminal fluid obtained from the man for analysis. The subjects were subdivided into two groups based on their sperm count. Study group consisted partners of infertile couples with sperm count less than 20 million/ml oligozoospermia (n=59); and control group consisted of male partners of infertile couples with sperm count greater than or equal to 20 million/ml normozoospermia (n=57). Both groups were attending the same infertility clinic.

A semen sample was collected from each subject on two separate occasions, at least two weeks apart, after about 3 to 5 days abstinence.

INCLUSION CRITERIA

Men who presented for infertility evaluation and within the reproductive age of 20 – 59 years, in stable marital relationship, living together and having regular unprotected intercourse for a year or more. There must be normal descended testes. They gave informed consent before been recruited.

EXCLUSION CRITERIA

Men with the following conditions were excluded: Aspermia, azoospermia, cryptorchidism, testicular varicocele, genital infections like urethritis, prostatitis, and ongoing sexually transmitted diseases, chronic illness and serious systemic diseases like diabetes, endocrine and metabolic disorders, heavy smoking and chronic alcohol intake and previous groin or scrotal surgery. Male using contraceptives like condoms and spermicides. Men taking medications for long-
term medical conditions like hypertension and arthritis. Also medications that suppress spermatogenesis like steroids and immunosuppressants and known human immunodeficiency virus (HIV) positive patients were excluded from the study.

SAMPLE SIZE CALCULATION

Adeniji et al\(^4\) working in University College Hospital, Ibadan studied 824 semen samples from male partners of infertile couples. Inferring from this study there were 225 semen samples that showed some abnormalities which constituted 27.3% of all samples studied. One hundred and fifty three (18.9%) of these semen samples revealed oligozoospermia. Therefore the incidence of oligozoospermia was 18.9%. Using the result obtained from the above study, and accepting a study power of 80%, confidence interval of 95%, study to control ratio of 1:1 and an acceptable dropout rate of 10% (attrition rate). The sample size of each group is determined using the statistical formula for the comparison of proportions.\(^5\)

\[
n = \frac{1}{1 - f} \left[ \frac{2 \times (Z_\alpha + Z_\beta)^2 \times P \times (1 - P)}{(P_0 - P_1)^2} \right]
\]

\[n\] = Minimum sample size

\[P_0\] = percentage of subjects with oligozoospermia 18.9% or 0.189.

\[P_1\] = the proportion of subjects with abnormal sperm parameters that are expected to have oligozoospermia. This is usually set relative to \(P_0\) and with proposed effect size of 30%. \(P_1 = 0.189 + 0.30\) (effect size) = 0.489
Z\(_\alpha\) is determined from statistical table based on the value of the level of significance 0.05 for this study. Therefore, Z\(_\alpha\) = 1.96.

Z\(_\beta\) is determined from statistical table based on the acceptable power of comparison i.e. 80% between the two groups. Therefore, Z\(_\beta\) = 0.84.

\(f\) = Proportion of study participants who are expected to be loss to follow up (attrition rate). For this study, \(f = 10\% \ (0.1)\)

\[
P = \frac{P_0 + P_1}{2} = \frac{0.189 + 0.489}{2} = 0.38
\]

Therefore, the minimum sample size required for each study group for it to be statistically significant was:

\[
n = \frac{1}{1 - 0.1} \left[ \frac{2 \times (1.96 + 0.84)^2 \times 0.399 \times (1 - 0.339)}{(0.189 - 0.489)^2} \right]
\]

\(n \approx 43\) participants per group approximately. Therefore a total of 86 subjects would be required to make the results statistically significant.

One hundred and twenty-two (122) participants were recruited to increase the power of study.

**SUBJECTS SELECTION**

The study participants were male partners of infertile couples presenting for infertility management of the general infertility clinic of the various units of the department and the infertility clinic of the Human Reproduction Research Programme unit of the department of Obstetrics and Gynaecology, University of Benin Teaching Hospital. Also, male partners of infertile couples referred to the gynaecology clinic by the Urologist that met the inclusion criteria were recruited.
for the study. There is no formal joint infertility clinic by the Gynaecologist and 
Urologist, but there is clear and effective liaison between both units for patient’s 
referral, optimal assessment and management, especially those with male factor 
infertility.

Upon recruitment, the purpose of the study was explained to the 
participants and informed consent was obtained. All consecutive subjects that 
met the inclusion criteria were recruited until one hundred and twenty-two 
participants were recruited. Data was obtained using pretested data retrieval 
form after clients were assured of confidentiality. Data extracted included: socio-
demographic characteristics (age, level of education, occupation, ethnic group 
social class) type of infertility (primary or secondary), abstinence period.

Thereafter each male produced semen for analysis. Semen was produced 
in the hospital. A private comfortable room at the HRRP, clinic is usually provided 
for couple. A sterile sample container was provided for the collection of the 
semen sample. The semen produced was sent to the laboratory where the 
researcher under the guidance of the medical microbiologist staff performed 
complete semen analysis. The semen sample remaining after semen analysis 
was centrifuged at 2000rpm for 10 minutes, and the supernatant stored at –20°C 
for assaying the zinc levels. These samples were pooled together and taken to 
the Chemical pathology department of the hospital, for the analysis of seminal 
plasma zinc levels.


SPECIMEN COLLECTION, STORAGE AND ANALYTICAL PROCEDURES

Semen samples were obtained in the hospital either by masturbation or coitus interruptus into a prewarmed clean wide-mouthed sterile container made of glass or plastic after a recommended 3 - 5 days period of sexual abstinence. After collection, specimens was allowed to liquefy at room temperature for 30 minutes and used for analysis. The specimen was analysed in the medical microbiology laboratory attached to the Human Reproduction Research Programme unit of the University of Benin Teaching Hospital by the researcher under the supervision of Medical Microbiology staff. On microscopic examination, sperm count, percentage of motile sperm and sperm with normal morphology was objectively evaluated. Sperm count and percentage of motile sperm were evaluated according to the recommendation World Health Organization².

Seminal plasma zinc levels were determined with the use of Flame Atomic Absorption Spectrophotometer.

SEMINAL FLUID ANALYSIS PROCEDURE

Sperm count: Total sperm count was done 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
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 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.

**SFA Procedure**

**(1) Liquefaction**

Semen samples were examined under room temperature and as the sample liquefied, the semen became thinner. Complete liquefaction of semen was completed in 15 minutes at room temperature, although it rarely took up to 60 minutes at room temperature.

**(2) Semen Volume**

The semen volume was measured by a modified graduated glass measuring cylinder with a wide mouth, into which the semen was collected. Volume was read directly from the graduation on the measuring cylinder.
(3) **Sperm Motility**

Sperm motility was assessed as soon as liquefaction of the semen sample was completed. The semen sample was mixed well, removing an aliquot of semen immediately, after making sure no time was allowed for spermatozoa to settle out of suspension.

The semen sample was now remixed and a replicate aliquot removed again. Subsequently a wet preparation was made on a slide and examined microscopically. Percentage of different motile categories according to WHO guideline was graded, as, progressive motility, non-progressive motility and immotile.

(4) **Sperm Vitality:** Vitality was assessed as soon as liquefaction of semen sample was completed with 30 – 60 minutes. Vitality test was carried out using 1% eosin reagent.

The semen sample was mixed well removing an aliquot of semen and mixed well with an equal volume of 1% eosin suspension. For each suspension, a smear was made on a glass slide; allowed to dry in air and examined under the microscope. The total number of stained (dead) and unstained (vital) cell were counted with the Neubauer counter; reporting the average percentage of the vital spermatozoa.

(5) **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.

ZINC MEASUREMENT IN SEMINAL PLASMA

Before measurement of zinc level in seminal plasma, glass wares to be used were soaked in 1:3 nitric acid solutions, to remove organic materials, washed in detergent solutions, rinsed with tap water and then rinsed with deionized distilled water. All the plastic bottles and other plastic materials were equally soaked in 1:3 nitric acid solutions, washed in detergent solutions, rinsed with tap water and deionized distilled water. This is because small amount of metal ions contained in the glass and plastic materials could have affected the result of analysis. In order to ensure proper sample handling and identification, laboratory procedures were strictly adhered 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. Method is described below.

Sample Preparation

— Seminal plasma sample was first allowed to thaw in room temperature.
— Using a pipette, 0.5g of seminal plasma was aspirated into a conical flask; pipette and conical flask already made sterile by method described above. The 0.5g seminal plasma was measured using an analytical weighing balance AG 204 METTLER TOLEDO.
— Concentrated nitric acid (2mls) and 2ml 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 occurs when the suspension that was appearing as golden yellow becomes a transparent solution. Method of digestion was wet digestion ASTM D 4698 – 92B (1996). A blank sample was also prepared for control, this is to correct for any background error.

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

— Zinc concentration in seminal plasma analysis performed was by Atomic Absorption Spectrophotometer. Flame pg instruments AA500. Instrument model 500F. Serial Number 20 – 0930 – 21 – 0025.

— Analysis Parameters for the instrument are: Analytical line 213.9nm, Bandwidth 0.4nm. Filterfactor 1.0 Lamp Current 3.0ma. Integration time 3.0sec Backgroup D2/SR. Flame type Air/Natural Gas (acetylene gas was used). Flame Setting 400ml/min. Sensitivity 0.01mg/dl, detection limits 0.004mg/L, working range 0.02 – 3.

— The measurement was conducted (zinc wavelength) at 213.86nm wavelength and with 0.4nm bandwidth.

— Coefficient of variation for the instrument 0.99.

— Zinc concentration was determined by direct aspiration of the acidic sample into the flame absorption spectrophotometer (AAS).
Principles of Absorption Spectrophotometer

The concept of atomic absorption spectrometry (AAS) was proposed by two groups in 1955. A Walsh of Australia and another CTJ Alkamade and JMW Malatz from the Netherlands. AAS is a quantitative method of analysis that is applicable to many metals and a few non-metals. In atomic absorption spectrophotometry the atomisation is performed by aspirating the sample solution into a flame where the analyte element is converted into gaseous phase atoms. As the temperature of atomisation is low; most of the atoms remain in the ground state which can absorb characteristic radiation from the radiation source made from the analyte element. The atomic vapours containing free atoms of an element in the ground state are illuminated by a radiation source emitting the characteristic radiation of the analyte. The radiation is absorbed by the analyte vapours and its intensity decreases. The degree of absorption is a quantitative measure of the concentration of ground state atoms in the vapours. The analysis is done by comparing the observed absorption with the one obtained by suitable standard samples of the analyte under similar experimental conditions; so a calibration curve method is generally employed.

The flame atomization system offers the following several advantages, relatively free from interference, low capital cost, low running cost and rapid and simple operation.

Instrument Calibration and Sample Analysis: The instruments were set up and optimized for zinc metal as recommended by the manufacturer. Working standards prepared from dilution of 1000ppm stock standards, and in
concentration range of the same order of magnitude as in the concentrated samples, were used to standardize the instrument. In cases where the sample absorbance were very close to the lower end of the linear response range for the element, the instrument was operated in the absorbance mode, it could also be operated in the direct concentration mode.

The concentrated samples were aspirated into the flame and absorbance values or concentrations were read as appropriate. Absorbance values were converted to concentration using the calibration graph for the zinc element. The reference solution (blank) was double deionised distilled water and acids used.

**Determination of Sensitivity of Spectrophotometer**

**Sensitivity:** This is defined as the concentration of an aqueous solution of the element which absorbs 1% of incidence resonance radiation or the concentration which gives an absorbance of 0.0044. Sensitivity is determined by the slope of calibration line. Sensitivity in this instrument is indirectly expressed as characteristics concentration (i.e. such concentration (µg/ml) of the elements that gives the absorbance 0.0044). Sensitivity determination also includes appropriate detection limit which has to be 2 – 5 lower than the characteristics concentration; quoted detection limit for the spectrophotometer used was 0.0044mg/L. Appropriate working range, which is 2 – 3 orders of magnitude, quoted working range for this instrument 0.002 – 3. Repeatability of the instrument under optimum conditions RDS 0.5–1.5% (Intraassay and interassay coefficients of variation).

Measures to assure sensitivity and quality of analytic results in AAS include appropriate calibration, optimization of fuel – oxidant gas composition,
optimization of burner height and addition of suitable reagents (releasing or
deionizing) to the sample in necessary cases.

**Calibration of AAS Used for the Study**

**Zinc in sperm samples**

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.247</td>
</tr>
<tr>
<td>1</td>
<td>0.466</td>
</tr>
<tr>
<td>1.5</td>
<td>0.667</td>
</tr>
<tr>
<td>2</td>
<td>0.875</td>
</tr>
</tbody>
</table>

Slope rec. = 2.2172949

Conc in. μg/ml = Abs x Slope rec. x Dil fac

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

Concentration of zinc μmol/ml \(\rightarrow\) divided by a factor of 6.54

\[
= \frac{Zn - C \ \mu g/ml}{6.54}
\]

= Zinc concentration in μmol/L
W.H.O SEMEN ANALYSIS MANUAL AND DEFINITION OF TERMS

W.H.O, in 2010 published a new manual for semen analysis and clinician were encourage to use this new manual for evaluation of male fertility. The semen parameters in this study were based on the 1999 W.H.O manual for semen analysis. Our laboratory standards for semen parameters are still based on the 1999 manual. Also most of the studies quoted, and the sample size calculation in this study use semen parameters based on the 1999 manual.

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 greater than or equal to 20million/ml</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>Sperm concentration less than 20million/ml</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>Less than 50% for progressive forward motility</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>Less than 30% have normal morphology. OR less than 14% (using the strict criteria for normal morphology)</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>Signifies disturbance of all three variables (combinations of only two prefixes may also be used)</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>No spermatozoa in the ejaculate</td>
</tr>
<tr>
<td>Aspermia</td>
<td>No ejaculate</td>
</tr>
<tr>
<td>Cryptozoospermia</td>
<td>Few spermatozoa recovered after centrifugation</td>
</tr>
</tbody>
</table>
OUTCOME MEASURES

Primary outcome measure was zinc levels in seminal plasma in oligozoospermic men. Secondary outcome measure was sperm count, percentage of motile sperm and sperm with normal morphology.

DATA ANALYSIS

All data are reported as means ± SD. An independent t test was used to compare the scores of each of the measures and mean of the parameters data between the 2 groups. The Pearson correlation test and Linear regression was used to analyse and examine the relationship between the sperm parameters and zinc concentrations scores. A probability of less than 0.05 was considered as significant. All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago IL, USA).

ETHICAL CONSIDERATIONS

Approval for the study was obtained from the Ethical and Research Committee of the University of Benin Teaching Hospital in September, 2011. Also, the study was carefully explained to the patients and informed consent obtained before being recruited into the study. The three principles of ethics in biomedical research were ensured as follows. Confidentiality: All information obtained was known only to the investigator. Data were coded and put in a data bank. There was no identification of the patient by name while the data was being analysed. Participants were informed that the results of the study may be published in scientific journal without identifying them by name. Benefits: The subject may not benefit directly from the study. However, they received standard
clinical care and did not facing any additional risks on account of this study.

Justice: All consenting participants to this study were handled with the same levels of fairness and equity in their selection for the study and ascribing into the two groups of participants. The principle of distributive justice was applied in conducting this research with equitable distribution of potential burdens and benefits among the study participants.

Participation in this research was entirely voluntary. If there were unwilling at any point to participate, they were completely at liberty to refuse to, and this was not held against them in any way, now or in future, in their management in the University of Benin Teaching hospital or any of its affiliated institutions. Appendices C and D contains participant information sheet and the certification from the Ethics and Research Committee respectively.
RESULTS AND TABLES

One hundred and twenty-two male partners of infertile couples were recruited for this study from April to July 2012. Out of these 116 (95%) participants had complete clinical information and investigation results and these formed the basis of analysis for this study; 6 (5%) of the participants who did not perform the second seminal fluid analysis were therefore excluded from the study. The two groups were made up of 57 participants with normozoospermia and 59 participants with oligozoospermia. The principle of per protocol analysis was used for analysis of data obtained in this study, since only participants who completed the study with complete data were included in the analysis. The principle of intention to treat was not applied.

The socio-demographic characteristics of the study participants are summarized in Table I. The mean age of the participants was 41.69 ± 5.83 years range (29 – 57 years). The mean age of normozoospermic men was 41.98 ± 5.85 years and that for oligozoospermic was 41.41 ± 5.84 years. This difference was not statically significant (t = 0.530) (P Value = 0.597). Men age group 41 – 45 years constituted the largest number {22(38.6%)} of men with normozoospermia, while men 36 – 40 years made up the largest number {30(33.9%)} of men with oligozoospermia. The other admission characteristics of educational attainment and social class among the 2 study population were similar with no statistically significant difference.
Table I: Socio-demographic characteristics of the study participants in each group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normozoospermia (n = 57)</th>
<th>Oligozoospermia (n = 59)</th>
<th>Test</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (years (Mean ± SD)</td>
<td>41.98 ± 5.85</td>
<td>41.41 ± 5.84</td>
<td>0.530&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.597</td>
</tr>
<tr>
<td>Patient age group (years) (N (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=30</td>
<td>1 (1.8)</td>
<td>0 (0.0)</td>
<td>5.627&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.195</td>
</tr>
<tr>
<td>31 - 35</td>
<td>5 (8.8)</td>
<td>10 (16.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 - 40</td>
<td>17 (29.8)</td>
<td>20 (33.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41 - 45</td>
<td>22 (38.6)</td>
<td>13 (22.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;46</td>
<td>12 (21.1)</td>
<td>16 (27.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSCE</td>
<td>10 (17.5)</td>
<td>12 (20.3)</td>
<td>1.208&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.904</td>
</tr>
<tr>
<td>OND</td>
<td>3 (5.3)</td>
<td>4 (6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HND</td>
<td>9 (15.8)</td>
<td>12 (20.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.Sc.</td>
<td>32 (56.1)</td>
<td>29 (49.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masters</td>
<td>3 (5.3)</td>
<td>2 (3.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social class</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16 (28.1)</td>
<td>18 (30.5)</td>
<td>1.897&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.642</td>
</tr>
<tr>
<td>2</td>
<td>20 (35.1)</td>
<td>19 (32.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20 (35.1)</td>
<td>18 (30.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 (1.8)</td>
<td>4 (6.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD = Standard Deviation  
N = Number  
% = Percentage  
<sup>a</sup> t-test  
<sup>b</sup> χ² text  
<sup>c</sup>Fisher’s Exact Test
Table II showed seminal plasma zinc concentration and semen parameters among the 2 groups of men. Seminal plasma zinc levels was 16.90 ± 4.20 μmol/L in normozoospermic subjects and 31.86 ± 3.92 μmol/L in oligozoospermic subjects and this difference was statistically significant (t = -19.837) (P < 0.0001). The semen parameters of the oligozoospermic subjects were found to be significantly lower than those of the normozoospermic group, except for sperm volume where values were similar in both groups. The normozoospermic group has a mean semen volume of 3.59 ± 1.71ml, sperm count of 36.39 ± 16.42 million, motility 50.96 ± 12.65%, normal morphology 42.19 ± 9.07% and normal viability 55.65 ± 11.09%. The oligozoospermic subjects have a mean semen volume of 2.96 ± 1.89ml, sperm count 8.10 ± 4.95 million, motility 15.39 ± 10.74%, normal morphology 6.37 ± 7.94% and normal viability 16.39 ± 12.97%.
Table II: Seminal plasma zinc concentration and semen biophysical characteristics in the 2 groups of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normozoospermia (Mean ±SD) (n = 57)</th>
<th>Oligozoospermia (Mean ±SD) (n = 59)</th>
<th>Test</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc Level (µmol/L)</td>
<td>16.90 ± 4.20</td>
<td>31.86 ± 3.92</td>
<td>-19.837a</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Semen Volume (ml)</td>
<td>3.59 ± 1.71</td>
<td>2.96 ± 1.89</td>
<td>1.877a</td>
<td>0.063</td>
</tr>
<tr>
<td>Sperm Count (×10^6/ml)</td>
<td>36.39 ± 16.42</td>
<td>8.10 ± 4.95</td>
<td>12.649a</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>50.96 ± 12.65</td>
<td>15.39 ± 10.74</td>
<td>16.348a</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>42.19 ± 9.07</td>
<td>6.37 ± 7.94</td>
<td>22.660a</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Normal viability (%)</td>
<td>55.65 ± 11.09</td>
<td>16.39 ± 12.97</td>
<td>17.492a</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

SD = Standard Deviation  
* Statistically significant  
a t-test

Table III shows the correlation matrix of seminal plasma zinc levels and semen parameters in the study participants. A negative statistically significant correlation was identified between seminal plasma zinc levels and all the semen parameters investigated in this study except for semen volume. Morphology was highly significantly correlated with seminal plasma zinc levels (normal morphology: r = -0.770, P< 0.0001). Seminal plasma zinc levels was also significantly correlated with sperm motility (total motility r = -0.744, P<0.0001), sperm count (total count r = 0.712, P< 0.0001) and viability (live sperm r = -0.750, P< 0.001). Semen volume also showed inverse correlation (r = -0.091, P= 0.332), which did not reach significant level.
Table III: Correlation matrix of seminal plasma zinc and semen parameters in the study participants

<table>
<thead>
<tr>
<th></th>
<th>Zinc Level</th>
<th>Volume</th>
<th>Count</th>
<th>Total Motility</th>
<th>Morphology</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc Level</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>-0.0908802</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. value</td>
<td>0.332</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>-0.71218102*</td>
<td>+0.0942426</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. value</td>
<td>0.000</td>
<td>0.314</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Motility</td>
<td>-0.74054395*</td>
<td>+0.04037166</td>
<td>+0.78444</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. value</td>
<td>0.000</td>
<td>0.667</td>
<td>0.000</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.7701791*</td>
<td>+0.1812055</td>
<td>+0.693602*</td>
<td>+0.761488592*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. value</td>
<td>0.000</td>
<td>0.052</td>
<td>0.000</td>
<td>0.000</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.74966879*</td>
<td>+0.12581951</td>
<td>+0.81094*</td>
<td>+0.867635423*</td>
<td>+0.852530352*</td>
<td></td>
</tr>
<tr>
<td>Viability</td>
<td>0.000</td>
<td>0.178</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1</td>
</tr>
</tbody>
</table>

+ Positive correlation; – negative correlation; *Significant correlation p<0.05
Fig. 1: Scatter plots showing correlation between seminal plasma zinc levels and semen parameters in the study participants
Table IV shows the correlation matrix of seminal plasma zinc levels and semen parameters in the two groups of participants. Semen volume ($r = +0.107$, $P = 0.418$) and morphology (normal morphology $r = +0.016$, $P = 0.902$) showed positive correlation which was not statistically significant in the oligozoospermic group; while sperm count (total count $r = -0.35$, $P = 0.006$), motility (total motility $r = -0.037$, $P = 0.783$) and viability($r = -0.47$, $P = 0.724$), negatively correlated with seminal plasma zinc levels. Only, sperm count was statistically significantly correlated. Among the normozoospermic subjects, no statistical significant correlation was observed between seminal plasma zinc concentration and semen parameters. Semen volume ($r = +0.159$, $P = 0.237$), morphology (normal morphology ($r = +0.227$, $P = 0.089$) and viability($r = +0.070$, $P = 0.605$) showed positive correlations, while sperm count ($r = -0.83$, $P = 0.539$) and motility (total motility $r = -0.021$, $P = 0.878$) showed negative correlation.
Table IV: Correlation matrix of seminal plasma zinc and semen parameters among the two groups of subjects

<table>
<thead>
<tr>
<th>Semen Parameters</th>
<th>Zinc Level</th>
<th>Volume</th>
<th>Count</th>
<th>Total Motility</th>
<th>Morphology</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normozoospermic (n=57)</strong></td>
<td>Zinc Level</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volume</td>
<td>+0.15909954</td>
<td>0.237</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>0.08312277</td>
<td>0.539</td>
<td>0.641</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Motility</td>
<td>-0.0236663</td>
<td>0.163</td>
<td>0.124</td>
<td>0.000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-0.0205949</td>
<td>0.474805</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morphology</td>
<td>+0.22734982</td>
<td>0.089</td>
<td>0.202</td>
<td>0.551</td>
<td>0.161</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-0.096265</td>
<td>-0.002372</td>
<td>0.588181455</td>
<td>+0.001039987</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Viability</td>
<td>+0.06993569</td>
<td>0.605</td>
<td>0.476</td>
<td>0.000</td>
<td>0.994</td>
</tr>
<tr>
<td><strong>Oligozoospermic (n=59)</strong></td>
<td>Zinc Level</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volume</td>
<td>+0.1074032</td>
<td>0.418</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-0.3508554*</td>
<td>0.006</td>
<td>0.496</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Count</td>
<td>-0.0365418</td>
<td>0.783</td>
<td>0.336</td>
<td>0.000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-0.002749</td>
<td>0.4670108*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Motility</td>
<td>+0.01644842</td>
<td>0.902</td>
<td>0.685</td>
<td>0.000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-0.00285</td>
<td>+0.3383555*</td>
<td>0.650729709*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morphology</td>
<td>+0.0469872</td>
<td>0.724</td>
<td>0.983</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>+0.4527918</td>
<td>+0.626823668*</td>
<td>0.710186438*</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

+ Positive correlation; – negative correlation; * Significant correlation p<0.05
Fig. 2: Scatter plots showing correlation between seminal plasma zinc levels and semen parameters in the two groups of subjects.
DISCUSSION

This study showed that oligozoospermia is associated with increasing seminal plasma zinc levels. Also, seminal plasma zinc levels were higher among subjects with asthenzoospermia and teratozoospermia. Normozoospermic subjects had significantly reduced levels of seminal plasma zinc when compared with oligozoospermic men and these subjects with normal sperm count showed weak correlation between their seminal plasma zinc levels and all the semen parameters studied. Morphology, viability and volume were positively correlated while count and motility were negatively correlated in normozoospermic men.

The mean value of zinc in seminal plasma measured in this study was 24.5 ± 8.53 μmol/L, which is comparable with some similar studies; Umeyama et al., (26.2μmol/l),97 and Chia et al., (28.1μmol/L).98 However, the reported zinc levels in some other studies are considerably lower than that of this study; Dissanayake et al., (18.5μmol/L),99 Lewis Jones et al.,(18.0μmol/L),100 Wong et al.,(14.8μmol/L),101 and Kruse et al.,(18.6μmol/L).102 Even, further lower levels were reported by Wong et al.,(14.0μmol/L)92 and Tikkiwal et al.,12.1μmol/L.103 Observations, from above quoted studies showed the varied value of seminal plasma zinc concentrations in human semen; some investigators have suggested measuring the levels of zinc in both serum and seminal plasma to determine its role in male infertility. Abou-skakra et al.104 postulated that the role of trace elements in infertility was more directly related to their sperm and serum levels than to the seminal plasma level.
This study was able to establish correlations between seminal plasma zinc levels and semen parameters, this is consistent with results from other studies\cite{34,72,105,106} and inconsistent with some previous findings.\cite{96,102,104,107}. Seminal plasma zinc levels in oligozoospermic men were significantly higher than in normozoospermic men. These findings were similar to results obtained by Akinloye \textit{et al.}\cite{105} working in Ibadan. They studied the impact of blood and seminal zinc and copper concentrations on spermogram and hormonal changes in infertile Nigerian men. The seminal plasma zinc level in oligozoospermic subjects was 31.86$\mu$mol/L in this study and 32.77$\mu$mol/L in the work of Akinloye \textit{et al.}\cite{105}. The concentration of seminal plasma zinc level in normozoospermic men who were control in this study was 16.90 $\pm$ 4.20$\mu$mol/L, while the control group for the study by Akinloye \textit{et al}\cite{105} who were also normozoospermic was 29.85 $\pm$ 1.67$\mu$mol/L. The control group in our study were also subfertile men with normal sperm count whose partners were being investigated for infertility; while the control for the work by Akinloye \textit{et al}\cite{105} were men with normal sperm count and proven fertility, this may account for the wide variations in the seminal plasma zinc concentrations in the 2 control groups of both studies. Some studies indicated that there are no significant difference between zinc content in normozoospermic (fertile) and oligozoospermic (infertile) men\cite{86,92,108,109} but others found a significant difference between them\cite{98,110,111} which was in agreement with our results. Our study demonstrated that oligozoospermic subjects had significantly higher levels zinc in their seminal plasma than normozoospermic group.
This study observed significant different levels between normozoospermic and oligozoospermic groups in relation to sperm count, motility, morphology and viability. In patients with oligozoospermia, a significant negative correlation was found between the seminal plasma zinc level and sperm count. Sperm motility and viability were also negatively correlated but not statistically significant. Semen volume and normal morphology were positively correlated but did not reach significant levels. In the normozoospermic group seminal plasma zinc levels did not show any significant correlation with any of semen parameters. A positive correlation was observed between zinc levels and semen volume, normal forms and viability. Sperm count and motility were negatively correlated. Some authors have reported high concentration of zinc to be associated with enhanced sperm parameters including sperm count,98,109,110 motility 85,109, and normal morphology,98 whereas another study112 reported a high concentration of zinc to be associated with poor motility of sperm. Others studies could not find a significant association between total zinc concentration in seminal plasma and semen parameters.92,99,108,109 Deng et al.,113 reported that biologic zinc treatment has a positive effect on sperm motility, and supplementation of biologic zinc was an effective method for the treatment of infertile males with chronic prostatitis.

Evidently from this study and results from other studies cited, very high levels seminal plasma zinc is associated with oligozoospermia and reduced levels is associated with some abnormal sperm parameters, while normal levels of seminal plasma zinc level is associated with normal sperm functions. Normal levels of zinc in seminal plasma stabilize the cell membrane and nuclear
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{114,115} Total content of zinc in mammalian semen is high and has been found to be critical to spermatogenesis, but there have been conflicting reports on the precise effect of seminal zinc on sperm quality. Zinc plays an essential role in many enzymes and proteins and thus in cellular homeostasis. As a consequence, these elements are strictly regulated, a process in which the blood-seminal plasma barrier plays a pivotal role. However, the precise mechanism by which these elements are transferred from the circulating blood into the seminal plasma is unclear.

The very high seminal plasma zinc level in oligozoospermic men could be toxic to sperm cells especially during the process of spermatogenesis. During spermatogenesis a functional locomotor apparatus is formed in the spermatozoa\textsuperscript{116} and considerable amount of zinc are incorporated into the spermatid. Spermatozoa in the rete testis and caput epididymis with their very high zinc content show only sluggish and non-progressive movement.\textsuperscript{117} It is only during epididymal transit that spermatozoa obtain their progressive motility.\textsuperscript{118} During their course at the epididymal sperm maturation, when all other semen parameters becomes well developed, zinc content is reduced by approximately 60\%\textsuperscript{119} leading to increased stabilization of outer dense fibres (ODFs) proteins which surround the axoneme of the sperm cells and required for normal motility and maturation by the process oxidation of sulphydryl group to
disulfide bridges. If there is failure of excreting this high amount of zinc incorporated during testicular spermatozoa, content of zinc in the seminal plasma becomes very high with abnormal motility and other semen parameters.

In this study, seminal plasma zinc concentration showed significant negative correlation with all the semen parameters except sperm volume. Sperm volume showed weak negative correlation. This could be due to the very high values obtained for seminal plasma zinc concentration in oligozoospermic subjects, which was approximately twice the value for normozoospermic subjects, tilting the balance of the study subjects to the oligozoospermic group. Weak positive correlation was found between seminal plasma zinc levels and sperm volume, normal morphology and viability in normozoospermic men, while weak negative correlation was found with sperm motility and in the same group. Among the oligozoospermic subjects, weak positive correlation was noted between the seminal plasma zinc levels, volume and normal morphology. While count, total motility and viability had negative correlation and count was significantly correlated.

Our study clearly indicated that there is a relationship between zinc and semen quality. However, further studies are needed in our population to observe whether supplementation improves the semen quality in males with subnormal zinc levels or subnormal semen parameters. Such studies will be useful in deciding the necessity of assessing seminal plasma zinc levels in the evaluation of male subfertility.
CONCLUSION AND RECOMMENDATIONS

There are many semen parameters to reflect semen quality. It is possible that one trace element has differing impacts on different semen parameters. Therefore, the influences of trace elements on semen quality are complicated. High levels of seminal plasma zinc levels as seen in oligozoospermic subjects and possibly zinc toxicity appears to contribute to poor semen quality seen in this group of infertile men. Even though data on correlation of zinc with semen parameters remains inconclusive, we have been able to establish correlation between seminal plasma zinc level and semen parameters.

Based on the critical mass of knowledge on zinc in the basic sciences, numerous opportunities exit for translational research in inter and multidisciplinary settings; bringing together expert on reproductive infertility, nutritionist and toxicologist, to properly assess the role of zinc and other trace nutritional elements in male infertility. With regards to readily available zinc supplements advocated for use by some health care provider in male infertility and planned interventional trials in humans there is need for additional experimental research concerning this trace element before its adoption for above purposes. We are also suggesting the adoption of a standardized reference value for normal levels of zinc in seminal plasma possibly by a consensus statement by experts in this field and the evaluation of seminal plasma zinc levels in male infertility with severe semen abnormality.
LIMITATIONS OF STUDY

A limitation of this study was the impossibility in obtaining semen samples from men with proven fertility, even among the enlightened groups in our society, including health care professionals. Our people and culture attaches much to bodily parts, not to mention a sensitive thing like human semen. This study would have been able to evaluate and compare results obtained with this group of men with proven fertility, if they had agreed to provide semen samples for analysis.

In this study, assay of zinc level in seminal plasma was carried out once in each sample. A better assay method that limits intraassay coefficients of variation of assay, would have been to repeat the measurement of zinc levels in each sample at least thrice and obtaining 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. We assumed that the participants would have been on the same prevalent diet available in this part of the country. Some participants invariably may have been on diet rich or deficient in zinc, which could have affected the levels of zinc in seminal plasma.
REFERENCES


18. van Pelt AM, de Rooij DG. Retinoic acid is able to reinitiate spermatogenesis in vitamin A-deficient rats and high replicate doses support the full development of spermatogenic cells. Endocrinology 1991; 128:69–704.


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APPENDICES

APPENDIX A

DATA EXTRACTION FORM

Patient code
Sample code

Section A:  Sociodemographic detail

Age

Wife level of Education

Occupation

Marital Status

Social class

Ethnic group

Types of infertility

Primary

Secondary

Section B:  Semen Parameters

Method of Collection ( ) Masturbation ( ) Coitus Interruptus

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

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

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

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

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

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

WBC (Pus cells) ..............10^6 cells/ml  Fructose test ---------------------------------

Section C:  Seminal Plasma Zinc level-----------------------------------------------(Mm)
APPENDIX B

SCORING SYSTEM FOR SOCIAL CLASS

A. Husband’s occupation

Score.
1. Professional, top civil servants politicians and businessmen
2. Middle-level bureaucrats, technicians, skilled artisans and well to-do traders
3. Unskilled workers and those in general whose income would be at or below the minimum wage.

B. Wife level of educational attainment

Score: 0  Education up to university level.
1. Secondary or tertiary level below the University level (e.g. College of education, School of nursing etc).
2. No Schooling or up to primary level only

Social Class = Score A + Score B

APPENDIX C

INFORMED CONSENT FORM

TITLE OF STUDY: CORRELATION OF SEMINAL PLASMA ZINC LEVELS AND SEMEN PARAMETERS IN NORMOSPERMIC AND OLIGOSPERMIC MEN ATTENDING AN INFERTILITY CLINIC.

PRINCIPAL INVESTIGATOR: Dr. Omu Patrick; Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital.

FINANCIAL SPONSORSHIP: This research project is self-sponsored.

PURPOSE OF THE RESEARCH

It is important you read and understand the following explanation of the proposed study procedures before agreeing to participate. This information describes the purpose, procedures, benefits, discomforts, risks and precautions associated with this study. It also describes your right to refuse to participate or withdraw from the study at any time. In order to decide whether you wish to participate in this research study, you should understand enough about its risks if any and benefits to be able to make an informed decision. This is known as the informed consent process. Please ask that any word(s) you do not understand be explained to you before signing this consent form. Make sure all your questions have been answered to your satisfaction before signing this document.

About the study: Abnormal sperm count and poor sperm quality are common causes of male factor infertility among infertile couples in our environment. Normal formation and maturation of sperm depends on the presence in seminal plasma of some very important substances called essential trace element which are readily provided by some diet consumed. Zinc is one of such essential trace element and its abnormal levels could result in abnormal sperm count and poor sperm quality. Checking the levels of zinc in seminal plasma could help in deciding if appropriate replacement therapy will be of benefit in infertile men with abnormal semen parameters.

PROCEDURES INVOLVED IN THE STUDY

What is required? Apart from this information sheet, you will be given a Consent Form in which you will formalize your willingness to participate. That is required to assure that your participation is entirely voluntary. Thereafter, you will be required to complete a questionnaire prior to production of semen for analysis. This questionnaire has questions about your general health and treatment. You will be required to produce small sample of semen which will be tested for the level of your sperm count and a substance called zinc in the semen.
COMPENSATION
There shall be no financial compensation for participation in this study.

VOLUNTARY PARTICIPATION
Please note that your participation in this research is entirely voluntary. If you are unwilling at any point to participate, you are completely at liberty to refuse to, and this will not be held against you in any way, now or in future, in your management in the University of Benin Teaching hospital or any of its affiliated institutions.

RISKS
You face no additional risks participating in this study. Sperm produced during investigation of infertility will also be the same sample that will be used for sperm count and to check for zinc levels.

BENEFITS
You may not benefit directly from the study. However, you will receive standard clinical care and you are not facing any additional risks on account of this study.

CONFIDENTIALITY
All information obtained will be known only to the investigator. Data will be coded and put in a data bank. There will be no identification of the patient by name while the data is being analyzed. The study result may be published in scientific journal without identifying the subjects by name.

CONTACT INFORMATION
Dr. Omu Patrick
Department of Obstetrics and Gynaecology,
University of Benin Teaching Hospital,
Benin City,
Phone Number: 08035461958
Email: patrickomu@yahoo.com

Chairman, Ethics and Research Committee,
University of Benin Teaching Hospital
Benin City.
Phone Number: 08181940459
Telegram: UNITECHOS, BENIN
Telex: 41120 NG
CERTIFICATE OF CONSENT

I have read the above information (or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction.

(A) I consent voluntarily to take part as a participant in this research.
(B) I do not consent to participate in this research

Name of Participant:______________________________

Signature of Participant:________________________

Date:__________________________________________
APPENDIX D

ETHICAL AND RESEARCH COMMITTEE CLEARANCE CERTIFICATE FORM

UNIVERSITY OF BENIN TEACHING HOSPITAL
P.M.B. 1111 BENIN CITY NIGERIA

Telephone: 052-600418
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CHAIRMAN:
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DIRECTOR OF ADMINISTRATION: F.B. KOVENIKAN Esq.
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E-mail: officeofthed@ubth.org

ETHICS AND RESEARCH COMMITTEE CLEARANCE CERTIFICATE

PROTOCOL NUMBER: ADM/E 22/A/VOL. VII/755

PROJECT TITLE: "CORRELATION OF SEMINAL PLASMA ZINC LEVELS AND SPERM PARAMETERS IN NORMOSPERMIC AND OLIGOSPERMIC MEN ATTENDING AN INFERTILITY CLINIC"

PRINCIPAL INVESTIGATOR(S): DR. OMU PATRICK

DEPARTMENT/INSTITUTION: DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY, UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY

DATE CONSIDERED: SEPTEMBER 26, 2011

DECISION OF THE COMMITTEE: APPROVED

REMARK:
CHAIRMAN: PROF. M.N. OKOBA
SUPERVISORS: PROFESSOR A.A.E. ORHUE, DR. M.E. AZIKEN

DECLARATION BY INVESTIGATOR(S)

PROTOCOL NUMBER (please quote in all enquiries)
To be completed in four and three copies returned to the secretary, Ethics and Research committee, Clinical services and Training Division. University of Benin Teaching Hospital Benin City.

I/We fully understand the conditions under which I am/we are authorized to conduct the above mentioned research and I/We undertake to resubmit the protocol to the Ethics and Research Committee.

Signature............................................ Date.............................................
APPENDIX E

LETTER FROM HEAD OF DEPARTMENT OF MEDICAL MICROBIOLOGY

DEPARTMENT OF MEDICAL MICROBIOLOGY
UNIVERSITY OF BENIN TEACHING HOSPITAL
P.M.B.1111, Benin City, Nigeria

Our Ref: UBT/MED/2017/124
Your Ref: 

10th Feb., 2012

The secretary,
National Postgraduate Medical College of Nigeria,
Faculty of Obstetric & Gynaecology.

Dear Sir/Madam,

RE: DR. OMU PATRICK ORUERAKPO

I wish to refer to the request of the assessor of the above named resident doctor's dissertation study proposal; that the head of Medical Microbiology of his institution (U.B.T.H) be involved in the study.

I hereby agree to participate in this study, titled: Correlation of Seminal Plasma Zinc levels and Sperm Parameters in normospermic and Oligospermic men attending an infertility clinic.

Thank you.

Yours sincerely,

[Signature]

DR. E. O. YUSUF, MSc, FMCpath
Head of department