THE RELATIONSHIP BETWEEN SEMINAL PLASMA CREATINE KINASE ACTIVITY AND SPERM COUNT IN MEN EVALUATED FOR INFERTILITY IN BENIN CITY

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MAY, 2025

CERTIFICATION

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DEDICATION

This work is dedicated to God almighty and those with fertility concerns.

ACKNOWLEDGEMENT

I give thanks to almighty God for his grace and mercy over my life and completion of this undergraduate project.

My profound gratitude goes to my wonderful supervisor PROF. M.A. EMOKPAE for his immense support, concern and patience towards me which aided my project work

Special thanks to the Head of department Medical laboratory science DR. MRS. Z. OMORUYI also to the entire staff (scientists, lecturers,doctors,professors) of the department for investing so much in my academic development.

Special appreciation to my family, I could not have done this without my family, thanks for the immense love and support I have been shown.

Thanks to my friends and classmates for the support.

God bless you all.

ABSTRACT

Male infertility is a prevalent and distressing condition affecting millions of couples globally, with significant psychosocial consequences. While conventional semen analysis, particularly sperm count, remains the cornerstone of male fertility evaluation, it frequently fails to fully explain reduced fertility or predict successful conception, especially in idiopathic male infertility. The aim of the study was to determine the relationship between seminal plasma creatine kinase (CK) activity and sperm quality indices among males investigated for infertility in Benin City. The seminal plasma CK activity, and semen analysis were evaluated in 75 men investigated for infertility and 50 men with proven fertility using spectrophotometric and microscopic techniques respectively. Chi square, Student –t-test and Pearson correlation coefficient were used compare and correlate the variables. The mean age of men investigated for infertility (40.32 ±1.51)years was not significantly different from the control subjects (41.20 ± 1.20)years ($p>0.05$). When the age was stratified, a significant difference was observed between the two groups (X^2=0.036). Seminal plasma CK activity of men investigated for infertility (756.45 ± 23.7)IU/L was markedly higher ($p=0.001$) compared with the controls (412.60 ±19.56)IU/L. The

sperm count (18.30 ± 11.04 vs 38.18 ± 9.60)x106/ml, volume (2.20 ±0.02 vs 2.75 ±0.06)ml, total motility (14.30 ± 3.10 vs 42.20±2.40)%, viability (18.32 ± 0.20 vs 59.18 ± 2.5)%, progressive motility (11.60 ±2.20 vs 34.22 ±2.60)% were markedly lower in men investigated infertility compared with controls. Conversely, the abnormal morphology (27.89 ±1.50 vs 7.30±2.50)% was markedly higher in infertile men than controls. Sperm count, total motility, viability and progressive motility correlated negatively with CK, while abnormal morphology correlated positively with creatine kinase activity ($p<0.05$). High CK activity suggests poor sperm quality among men with infertility.

1.0 INTRODUCTION

1.1 Background of study

Infertility, defined as the inability of a couple to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse, is a significant global health issue affecting an estimated 15% of couples worldwide (*WHO, 2023).* This complex condition carries substantial emotional, psychological, and social burdens for affected individuals and families, particularly in societies where childbearing holds immense cultural and personal significance. While infertility has historically been viewed as primarily a female issue, it is now well-established that male factors contribute to approximately 50% of all cases, either solely or in combination with female factors (*Sharlip et al., 2011; Agarwal et al., 2018).*

The evaluation of male fertility largely hinges on semen analysis, a fundamental diagnostic tool that assesses various parameters of sperm quality. Among these, sperm count (concentration) is a critical and widely used indicator of a man's reproductive potential. Oligozoospermia (low sperm count) and azoospermia (absence of sperm) are frequently encountered abnormalities that strongly correlate with reduced fertility

(*WHO, 2010*). However, it is equally recognized that semen analysis, including sperm count, does not always perfectly predict fertility outcomes, and men with seemingly normal conventional semen parameters can still experience infertility (idiopathic infertility). This highlights the need for additional, more sensitive markers that can provide deeper insights into sperm function, integrity, and the overall physiological health of the male reproductive system.

Creatine Kinase (CK), an ubiquitous enzyme, plays a pivotal role in cellular energy metabolism, particularly in tissues with high and fluctuating energy demands such as skeletal muscle, brain, and spermatozoa. It facilitates the reversible transfer of a phosphate group between ATP and creatine, thereby rapidly regenerating ATP from phosphocreatine. This critical role in maintaining cellular energy homeostasis underscores its importance in sperm function, which is highly energy-dependent for processes like motility, capacitation, and fertilization (*Wallimann et al., 1992; Huszar and Sikka, 1994*). CK exists in different isoenzymic forms (CK-MM, CK-MB, CK-BB), with the CK-BB isoenzyme being the predominant form found in the male reproductive tract and spermatozoa (*Huszar et al., 2000*).

The presence of CK in seminal plasma – the fluid component of semen – is of particular interest in the context of male infertility. Seminal plasma is a complex mixture of secretions from the testes, epididymis, seminal vesicles, and prostate gland, containing a myriad of enzymes, proteins, and metabolites that reflect the functional status of these reproductive organs and the quality of spermatozoa (*Sharma et al.,* 2009). Elevated levels of enzymes in seminal plasma are often indicative of cellular leakage due to damage or dysfunction of the cells lining the reproductive tract or the spermatozoa themselves. Specifically, increased seminal plasma CK activity has been

proposed as a biochemical marker of sperm membrane damage, impaired sperm metabolism, oxidative stress, or compromised mitochondrial function (*Huszar et al., 2000; Bilgen et al., 2004)*. Such cellular damage can directly impact sperm viability, motility, and overall fertilizing capacity.

Previous research has explored the relationship between seminal plasma CK activity and various semen parameters. Some studies have reported an inverse correlation between CK activity and sperm motility, suggesting that higher CK levels may indicate poorer sperm movement (*Bilgen et al., 2004*). Others have linked elevated CK to increased levels of reactive oxygen species (ROS) and DNA fragmentation in sperm, further indicating its potential as a marker of cellular stress and damage *(Sharma et al., 2009*). However, the direct and specific relationship between seminal plasma CK activity and sperm count has been less consistently or comprehensively investigated across diverse populations. While a high CK activity might suggest damage leading to reduced overall sperm quality, its precise correlation with the quantitative aspect of sperm production (sperm count) remains an area that warrants further exploration.

In Nigeria, and specifically in regions like Benin City, male infertility is a growing concern, contributing significantly to the overall burden of infertility. Local studies on male infertility are crucial to understand the unique etiological factors and biological markers prevalent in the population, which may differ from findings in other geographical contexts due to varying environmental, genetic, and lifestyle factors. Limited local data exist on the utility of biochemical markers like seminal plasma CK activity in the diagnostic workup of male infertility.

Therefore, this study aims to bridge this knowledge gap by meticulously investigating the relationship between seminal plasma creatine kinase activity and sperm count in

men evaluated for infertility in Benin City. Understanding this relationship could potentially establish seminal plasma CK activity as a valuable, simple, and cost-effective biochemical adjunct to conventional semen analysis, aiding in the more precise diagnosis and prognostication of male infertility in our local clinical setting. This research holds the promise of contributing significant insights to male reproductive health, ultimately benefiting infertile couples in the region.

1.2 Statement of the Problem

Male infertility is a prevalent and distressing condition affecting millions of couples globally, with significant psychosocial consequences, particularly in communities like Benin City, Nigeria, where societal expectations surrounding procreation are immense. While conventional semen analysis, particularly sperm count, remains the cornerstone of male fertility evaluation, it frequently fails to fully explain reduced fertility or predict successful conception, leading to a substantial proportion of cases classified as "idiopathic male infertility." This diagnostic gap underscores the limitations of relying solely on traditional sperm parameters.

Current clinical practice in Benin City, and indeed in many parts of Nigeria, heavily relies on basic semen analysis, often lacking access to advanced functional sperm assays or comprehensive biochemical markers. This limitation can hinder the precise diagnosis and tailored management of male infertility. Seminal plasma creatine kinase (CK) activity has been proposed as a marker reflecting cellular damage, membrane integrity, and metabolic dysfunction within the male reproductive tract and spermatozoa. Elevated CK levels could signal underlying issues not immediately apparent from routine sperm count. However, despite its potential as a simple and cost-effective biochemical indicator, the specific relationship between seminal plasma CK activity and sperm count, as well as its overall clinical utility in men evaluated for

infertility, remains inadequately explored, especially within the context of the Nigerian population.

The absence of localized data on this relationship means that clinicians in Benin City lack specific evidence to determine if seminal plasma CK activity could serve as a valuable complementary tool to conventional semen analysis, particularly for men presenting with various degrees of oligozoospermia or even normozoospermia but unexplained infertility. Without this understanding, the opportunity to identify subtle biochemical dysfunctions associated with sperm quantity or quality, and thus improve diagnostic accuracy and potentially guide therapeutic strategies, is missed.

1.3 Justification

The limitations of conventional semen analysis in fully elucidating the causes of male infertility necessitate the exploration of additional, accessible biochemical markers. Seminal plasma CK, being involved in energy metabolism and indicative of cellular integrity, holds promise in this regard. Identifying a significant relationship between CK activity and sperm count could provide a practical and objective means to assess sperm health beyond mere quantification.

In resource-constrained settings like Benin City, expensive or complex diagnostic tests are often prohibitive. Seminal plasma CK activity can be measured using widely available and relatively inexpensive enzymatic assays on standard laboratory equipment. If proven to be a reliable indicator of sperm count deficiencies or related sperm quality issues, it could become a readily implementable tool, enhancing diagnostic capabilities without imposing significant financial burden on patients or healthcare systems.

There is a dearth of research specifically investigating the relationship between seminal plasma CK activity and sperm count in the Nigerian male population,

particularly in Benin City. This study will contribute vital indigenous data, providing context-specific insights that may differ from findings in other populations due to unique genetic, environmental, lifestyle, and dietary factors prevalent in this region. This local data is crucial for developing relevant diagnostic and management protocols tailored to the Nigerian context.

A clearer understanding of the relationship between seminal plasma CK and sperm count could assist clinicians in potentially identifying subtle biochemical abnormalities in semen that correlate with reduced sperm count, even in cases where the cause of infertility is otherwise obscure and prognostication. A strong correlation might allow CK levels to be used as a prognostic indicator for certain male factor infertility cases.

It may also guide therapeutic decisions. While not a direct therapeutic target, understanding biochemical markers can inform management strategies, research into underlying causes, or even aid in counseling regarding assisted reproductive techniques.

1.4 Aim

The aim of the study is to investigate and characterize the relationship between seminal plasma creatine kinase activity and sperm count in men undergoing infertility evaluation in Benin City, Nigeria.

1.4.1 Specific Objectives

The specific objectives are to:

1. determine the seminal plasma creatine kinase (CK) activity levels in a cohort of men evaluated for infertility in Benin City.

2. establish if a statistically significant correlation exists between seminal plasma CK activity and sperm count.
3. To compare seminal plasma CK activity levels across different World Health Organization (WHO) defined categories of sperm count (e.g., normozoospermia, oligozoospermia, azoospermia) within the study population.

1.5 Research Questions

1. What are the seminal plasma creatine kinase (CK) activity levels in men evaluated for infertility in Benin City?

2. Is there a statistically significant correlation between seminal plasma creatine kinase activity and sperm count in men evaluated for infertility in Benin City?
3. Do seminal plasma creatine kinase activity levels differ significantly across various World Health Organization (WHO) defined sperm count categories (normozoospermia, oligozoospermia, azoospermia) in men evaluated for infertility in Benin City?

1.6 Hypotheses

1.6.1 Null Hypotheses (H0):

1. The seminal plasma creatine kinase (CK) activity levels in men evaluated for infertility in Benin City are low

2. There is no statistically significant correlation between seminal plasma creatine kinase activity and sperm count in men evaluated for infertility in Benin City.

3. There is no significant difference in seminal plasma creatine kinase activity levels across various WHO-defined sperm count categories (normozoospermia, oligozoospermia, azoospermia) in men evaluated for infertility in Benin City.

1.6.2 Alternative Hypotheses (H1):

1. The seminal plasma creatine kinase (CK) activity levels in men evaluated for infertility in Benin City are high.

2. There is a statistically significant correlation between seminal plasma creatine kinase activity and sperm count in men evaluated for infertility in Benin City.

3. There is a significant difference in seminal plasma creatine kinase activity levels across various WHO-defined sperm count categories (normozoospermia, oligozoospermia, azoospermia) in men evaluated for infertility in Benin City.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Infertility

Infertility has been a concern throughout history and remains a significant clinical issue today, affecting 8–12% of couples globally. Approximately 40–50% of all infertility cases can be attributed to "male factor" infertility, with as many as 2% of men displaying suboptimal sperm parameters. Male infertility is often linked to deficiencies in semen, and the quality of semen serves as a surrogate indicator of male fecundity (*Cooper, et al., 2010*).

Men with sperm parameters that fall below the WHO normal values are classified as having male factor infertility (*Plachot, et al., 2002*). The most critical factors include low sperm concentration (oligospermia), reduced sperm motility (asthenospermia),

and abnormal sperm morphology (teratospermia). Other factors that are less strongly associated with infertility encompass semen volume and various seminal markers indicative of epididymal, prostatic, and seminal vesicle function (*Harris, et al., 2011*). As much as 90% of male infertility issues are related to sperm count, and there exists a positive correlation between abnormal semen parameters and sperm count (*Sabra & Al-Harbi, 2014*). In Nigeria, male factor infertility accounts for up to 50% of all cases (*Patrick et al., 2005*). The primary causes include inadequately treated STIs and hormonal imbalances (*Patrick et al., 2005*).

There are currently no dependable statistics regarding the global prevalence of infertility (*WHO, 1991*). However, estimates indicate that approximately 72.4 million couples around the world face fertility challenges (*Thomas et al., 1995*). According to WHO estimates, between 60 and 80 million couples globally are presently affected by infertility (*Sule et al., 2008*). The prevalence varies by region and is estimated to impact 8-12% of couples worldwide (*Umezulike & Efetie, 2004; Okonofua et al., 2005*). This issue tends to be most pronounced in countries with high fertility rates, a phenomenon referred to as "barrenness amid plenty" (*Ikechebelu et al., 2003; Emokpae et al., 2009*). In recent years, infertility has increasingly affected couples. In the United States, around 10% of couples are classified as infertile due to their inability to conceive after 12 months of unprotected intercourse. Although semen analysis is an imperfect tool, it remains fundamental for investigating male infertility (*Sikka & Wang, 2008*). It must be conducted to a consistently high standard to assess the descriptive parameters of the ejaculate (*Araoye, 2003; Emokpae and Adobor, 2015*). While this assay provides valuable information for the initial assessment of the infertile male, it does not serve as a definitive test of fertility (*Abell et al., 2000*). It does not offer insights into the functional capacity of the spermatozoon to undergo the

subsequent maturation processes necessary for fertilization. It is crucial to understand that although the results may correlate with "fertility," the assay does not directly measure fertility (*Welch, 1990; Stewart and Kim, 2011; Dunson et al., 2004*).

Given that male infertility in many cases remains unknown. Therefore, it is necessary to introduce new key factors and diagnostic and noninvasive biomarkers. There is a constant need to identify cellular markers of sperm quality so that in addition to their diagnostic value, they may facilitate the identification of specific deficiencies of sperm-function (*WHO, 1999*). Therefore the activity of creatine kinase (CK), the key enzyme in synthesis and transport of energy (Huszar *et al.,* 2000; Guerin *et al.,* 1979; *Huszar&Vigue, 1990*), was studied in correlation with sperm concentration, and lipid peroxidation. Studies on the enzymatic status of spermatozoa are specialized and are limited to a few enzymes. The fertilizing capacity of a sperm sample is usually estimated by the concentration of spermatozoa and their motility and morphology. There are, however, some samples which while having normal spermiograms are infertile. It would thus appear that the classical, biological criteria of fertility are sometimes insufficient and might be overcome by some biochemical criteria. The enzymatic profile of spermatozoa should constitute a good indication of functional metabolic activity. The enzymes present in seminal fluid are shown to be derived from secretions of seminiferous tubules, spermatozoa, epididymis, seminal vesicles and prostate gland (Huszar *et al.,* 1994). Thus, the estimation of different enzymes in semen permits one to obtain markers of seminal quality (*Walliman& Hemmer, 1994*).

Human Sperm: CK lsoenzyme Ratios as an Indicator for Infertility.

2.2 Semen Analysis

Semen analysis is a fundamental step in investigating various disorders affecting the male genital tract. The examination of seminal parameters yields crucial clinical

insights into spermatogenesis and the functional capability of spermatozoa, as well as the secretory behavior of the accessory genital glands. This analysis is especially beneficial for couples undergoing fertility assessments, as it helps identify genital infections and pathologies, and evaluates the impact of environmental factors, medications, lifestyle choices, chemical substances, and occupational activities on numerous conditions that influence male reproductive health. The assessment of semen quality is of significant importance for diagnoses in clinical urology, andrology, and gynecology (Laboratory Practice: An Overview of Routine Tests 2003 Journal of Clinical Laboratory Analysis).

2.2.1 Parameters Measured in Semen Analysis

The parameters measured in semen analysis are among the most critical predictive indicators of fertilization and pregnancy success rates in in-vitro fertilization and embryo transfer (*Moazzam et al., 2015*). These parameters are evaluated differently depending on the clinical context, such as during infertility investigations, monitoring infertility treatments, selecting suitable assisted reproduction methods, conducting reproductive toxicology studies, or in contraception research (*Trine et al., 2006*).

Data indicates that there are slight variations in semen parameters among men from different geographic regions and even among samples from the same individual. Seasonal fluctuations in semen parameters have been observed in both fertile and infertile men (*Ashok and Tamer, 2011*). Key semen parameters, including appearance (color), volume, pH, motility, morphology, viability, concentration, liquefaction, and the presence of motility, have been identified as significant determinants of the functional competence of spermatozoa.

2.2.2 Seminal Plasma

Seminal plasma refers to the liquid component of semen, produced by both the epididymis and the accessory glands prior to and during ejaculation. This fluid is a complex mixture that includes various elements such as proteins, enzymes, macro- and microelements, lipids, and nutrients, playing a crucial role in the motility, viability, and fertilizing capacity of spermatozoa. Furthermore, the proteins present in seminal plasma may affect the fertilization process (*Anel-López, et al. 2017*). The impact of the biochemical constituents in human seminal plasma remains a topic of discussion (*Zhang, et al. 2015; Vickram, et al. 2016*). Seminal plasma is a sophisticated blend of carbohydrates, proteins, minerals, vitamins, and enzymes. It is a fluid that combines with sperm during their passage through the epididymis as part of the ejaculation process and is generated by the epididymis and other related sexual glands (*Campanholi, et al. 2017*). This seminal plasma is involved in the creation of the ejaculate, facilitates sperm transport during ejaculation, and safeguards sperm from the various effects encountered in the female reproductive tract. Although the physiological roles of seminal plasma and its constituents have not been completely clarified, it is acknowledged that it significantly contributes to sperm maturation and metabolism, sperm activation, and pre-fertilization activities (*Rodriguez-Martinez et al., 2011*).

2.2.3 Sperm Count

Sperm count assesses the concentration of sperm within a man's ejaculate, which is distinct from total sperm count, defined as the sperm count multiplied by the volume. A normal sperm count is considered to be over 15 million sperm per milliliter, as per the WHO guidelines established in 2010 (*Cooper, et al., 2010*).

2.2.4 Motility

Sperm motility refers to the capability of sperm to move effectively. Reduced motility can hinder the sperm's ability to navigate through the female reproductive tract to fertilize the egg. The World Health Organization (WHO) establishes a threshold of 50%, which must be assessed within 60 minutes post-collection. Additionally, WHO sets a vitality parameter with a lower reference limit of 60% live spermatozoa (*Cooper, et al., 2010*). If over 60% of the observed sperm cells exhibit good forward movement, it is advantageous, as nature prioritizes quality over quantity. Semen samples with more than 32% progressive motility are classified as normozoospermia, while those below this threshold are categorized as asthenozoospermia according to WHO standards.

2.2.5 Consistency

The consistency, often referred to as viscosity, of the liquefied semen sample can be assessed by gently aspirating it into a 5-ml pipette and allowing it to drop by gravity, observing the length of the thread formed. A normal sample will release small, discrete drops from the needle, whereas samples with abnormal consistency will produce a thread longer than 2cm. An alternative method for estimating consistency does not involve needles; it entails inserting a glass rod into the sample and observing the thread that forms upon withdrawal of the rod. Again, the thread should not exceed 2cm (*Comhaire and Vermeule, 1995).*

2.2.6 pH

The pH level is influenced by the acidic secretions from the prostate and the alkaline secretions from the seminal vesicles. Typically, it should fall within the range of 7.2 to 8.0. Recently, a researcher has indicated that the average pH values are consistently above 8.0, irrespective of the analytical method or timing of the examination, and has proposed that the normal value range should be further revised.

To measure pH, pH paper with a range of 6.1 to 10.0 is utilized. Regardless of the type of pH paper employed for this analysis, its accuracy must be verified against established standards prior to its application in routine semen analysis *(Comhaire and Vermeulen, 1995*).

If the pH level exceeds 8.0, it is advisable to suspect an infection, which may be linked to a reduced secretion of acidic products from the prostate, such as citric acid. Abnormal pH levels may also be observed in instances of incomplete ejaculation. A highly acidic pH (less than 6.5) is typically found in cases of agenesis or occlusion of the seminal vesicles.

2.2.7 Oligospermia

Sperm counts can range from 10 to 20 x 10^6/mL in mild cases, 5 to 10 x 10^6/mL in moderate cases, and less than 5 x 10^6/mL in severe oligozoospermia. Functional disturbances of the testes, such as endocrine disorders and varicocele, along with non-testicular factors like drug toxicity, environmental pollutants, mumps, orchitis, radiation, and exposure to chemical substances, contribute to the development of mild and moderate oligozoospermia. Severe oligozoospermia is often linked to genetic abnormalities, including Y chromosome microdeletions. Oligozoospermia is associated with abnormal sperm morphology and reduced sperm motility, thereby impairing semen quality and its fertilization potential. Nevertheless, these males may still possess some capacity for natural fertilization, even under severe oligozoospermic conditions (*Moazzam et al., 2015*).

2.2.8 Appearance

Semen normally has a whitish-gray color. It tends to get a yellowish tint as a man ages. Colour A normal liquefied semen sample is homogeneous, grey-opalescent in

appearance. it may also appear less opaque if the sperm concentration is very low the colour may appear red-brown when red blood cells are present (haematospermia), or yellowish in a subject with jaundice or taking certain vitamins or drugs (*WHO, 2010*)**.**

2.3 Creatine Kinase

Creatine kinase found in human sperm serves as an indicator of cytoplasmic retention, which correlates with reduced sperm maturity. It is an enzyme that plays a crucial role in the synthesis and utilization of sperm. Creatine kinase (CK), classified as enzyme commission number 2.7.3.2, is produced by various tissues and cell types that demand high energy levels. This enzyme facilitates the reversible conversion of creatine and adenosine triphosphate (ATP) into phosphocreatine and adenosine diphosphate (ADP). CK is responsible for regenerating ATP through the chemical exchange between creatine and creatine phosphate, which is vital for sperm functionality (*Banihani and Abu-Alhayjaa, 2016*). Its biological function is to establish an ATP buffering system for tissues that require substantial energy (*Ellington, 2007*). Research indicates that ATP and the phosphoryl creatine shuttle are critical energy sources for sperm *(Miyaji et al., 2001; Bessman & Carpenter, 1985; Raga et al., 1985*).

Creatine kinase facilitates the production of ATP from creatine phosphate and ADP. In the subsequent step, ATP is employed for the synthesis of glucose-6-phosphate in the presence of hexokinase. The third step involves the oxidation of glucose-6-phosphate to 6-phosphogluconate, accompanied by the reduction of NADP to NADPH, which is quantified by a change in optical density at 340 nm. In this process, glucose-6-phosphate dehydrogenase (G6PDH), an oxidoreductase, catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate. This step is crucial in the hexose monophosphate shunt, as it is through this pathway that dihydronicotinamide adenine dinucleotide phosphate (NADPH) is produced by spermatozoa. NADPH

serves as the primary source of electrons responsible for the generation of free radicals (O2) by human spermatozoa (*Aitken & Clarkson, 1988*).

The disorders of spermiogenesis, which lead to the retention of excess residual cytoplasm by differentiating spermatozoa, are significantly influenced by an important factor. Numerous independent studies have provided evidence for this hypothesis, indicating that sperm function is often linked to elevated activities of specific key enzymes, including creatine kinase (*Huszar & Vigue, 1993*). While these enzymes are not believed to directly cause the loss of sperm function, they serve as biochemical markers indicating the normality of sperm differentiation. Errors in spermiogenesis can lead to oxidative stress, as confirmed by a positive correlation between the CK content in human spermatozoa and the induction of peroxidative damage (*Huszar, 1994*). Creatine kinase (CK), which is produced from creatine phosphate, acts as an energy reservoir for the rapid buffering and regeneration of ATP, playing a crucial role in sperm motility. Huszar and Vigue (1994) discovered a significant correlation between morphological irregularities in sperm and high creatine kinase (CK) activity, an enzyme involved in the synthesis and utilization of energy within sperm. The levels of CK reflect sperm maturity, with higher levels found in immature spermatozoa that retain their cytoplasmic droplets. Elevated CK levels are inversely correlated with the fertilizing potential of spermatozoa, indicating a degree of cellular immaturity (*Agarwal et al., 2003*). CK activity serves as a measure of cellular maturity and fertilizing potential in human spermatozoa. Furthermore, recent studies have identified an inverse relationship between CK levels and various sperm morphological forms, suggesting that CK levels can be utilized as a reliable marker for sperm quality and fertilizing potential in subfertile men (*Hallak et al., 2001*). Oxidative stress has been indirectly linked to the clinical implications of

sperm DNA damage, such as an increased risk of miscarriage (*Benchaib et al., 2007; Borini et al., 2006; Virro et al., 2004*).

2.4 Oxidative Damage

Oxidative DNA damage has been associated with inadequate embryonic development (*Sakkas et al., 1998*) and may even elevate the risk of childhood cancer (*Lewis and Aitken, 2005*).

The implications of oxidative DNA damage in sperm on the health and welfare of offspring remain incompletely understood; however, there exists a possibility that spermatozoa with significant DNA damage can still achieve fertilization and lead to full-term pregnancies (*Ahmadi and Ng, 1999; Gandin et al., 2004; Twig et al., 1998c*). A positive correlation was observed between CK activity and the rate of lipid peroxidation, as indicated by malondialdehyde (MDA) formation in sperm fractions (*Huszar et al., 1994*). Recent developments in reproductive medicine have drawn the attention of numerous researchers to consider reactive oxygen species (ROS) as one of the mediators of infertility that leads to sperm dysfunction. Although ROS plays a role in various physiological functions of human spermatozoa, excessive production leads to oxidative stress. Mitochondria and sperm plasma membranes are the two primary sites of ROS production, which involves intricate enzyme systems such as creatine kinase and diaphorase. ROS inflicts damage on spermatozoa DNA, resulting in increased apoptosis of these cells. The generation of ROS is significantly heightened by various environmental and lifestyle factors, including pollution and smoking. An effective scavenging system is crucial to mitigate the effects of ROS. Various endogenous antioxidants, which belong to both enzymatic and non-enzymatic categories, can eliminate excess ROS and avert oxidative stress. Since ROS is vital for normal sperm physiology, a judicious application of antioxidants is recommended

(*Ashok et al., 2005*). The male factor is regarded as a significant contributor to infertility. In addition to the traditional causes of male infertility, which include varicocele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma, and tumors, a new and critical cause has been recognized: oxidative stress (*Makker et al., 2009*). Oxidative Stress (OS) arises from an imbalance between Reactive Oxygen Species (ROS) and antioxidants within the body. It serves as a primary etiological factor for sperm damage, deformity, and ultimately, male infertility (*Makker et al., 2009; Agarwal et al., 2009*). OS is a prevalent pathology observed in nearly half of all men experiencing infertility. An increase in Radical Oxidative Species (ROS) generation, coupled with a decrease in antioxidant capacity, is inversely related to sperm concentration and motility in infertile men (*El-Taiebet al., 2009*).

2.5 Oxidative stress

Oxidative Stress is triggered by Reactive Oxygen Species (ROS), or free radicals. While ROS are essential for certain critical functions of sperm, excessive amounts can adversely affect sperm quality (*Kefer et al., 2009*). ROS significantly influence both the quality and quantity of sperm. Oxidative Stress (OS) manifests when the production of potentially harmful ROS surpasses the body's natural antioxidant defenses, leading to cellular damage (*El-Taiebet al., 2009*). Aydemir et al. (2008) proposed that heightened oxidative damage could contribute to the hyperviscosity of seminal plasma in infertile males. OS is a crucial factor in male factor infertility. Evaluating OS provides vital insights that can inform treatment strategies aimed at enhancing male reproductive potential.

Furthermore, reactive oxygen species lead to a reduction in intracellular ATP levels. Consequently, insufficient protein phosphorylation results in a decline in beat frequency and an increase in axonemal damage, ultimately causing sperm

immobilization (*de Lamirande and Gagnon, 1992a; de Lamirande and Gagnon, 1992b; Griveau and Le Lannou, 1997*). The exposure of mitochondria to ROS triggers an apoptotic process through the release of apoptosis-inducing factor (AIF), which also contributes to DNA fragmentation (*Cande et al., 2002*). Elevated levels of ROS disturb mitochondrial membrane polarization and initiate the release of cytochrome-C protein, acting as a trigger for apoptosis (*Wang et al., 2003*).

The function of creatine kinase (CK) in mitochondria is to facilitate the phosphorylation of creatine into creatine phosphate (*Wallimann & Hemmer, 1994; Huszar & Vigue, 1990*). In the neck of sperm cells, creatine kinase catalyzes the rephosphorylation of adenosine diphosphate (ADP) into adenosine triphosphate (ATP). Spermatozoa, which require substantial energy, exhibit high levels of creatine kinase activity. The energy utilized by spermatozoa is closely linked to its production, and in certain species, creatine kinase is regarded as the principal enzyme responsible for the generation, transport, and utilization of energy (*Wallimann and Hemmer, 1994*).

Sperm cell CK consists of two subunits: CK-B and CK-M. Additionally, there are mitochondrial forms of the enzyme known as mi-CK, which differ from serum CK derived from the cytosol. This distinction is evident in the electrophoregram obtained by separating CK isoenzymes in a gel (*Huszar et al., 1998*). Previous research has indicated a negative correlation between the number of sperm cells and CK activity. The metabolic characteristics of sperm cells in men with oligozoospermia differ from those in normozoospermia. A cytochemical study (*Huszar & Vigue, 1993*) revealed higher CK values in ejaculate samples that contained cytoplasmic residues, indicating

the presence of immature sperm cells. Huszar and Vigue (1994) discovered that the morphological irregularities of sperm are significantly associated with elevated creatine kinase (CK) activity, an enzyme crucial for energy synthesis and utilization in sperm. CK levels serve as an indicator of sperm maturity, with higher levels observed in immature spermatozoa that retain their cytoplasmic droplets. Elevated CK levels are inversely correlated with the fertilizing potential of spermatozoa and reflect the extent of cellular immaturity (*Agarwal, et al., 2003*).

Reactive oxygen species (ROS) can exert both positive and negative effects on sperm functions, depending on the type and concentration of ROS, as well as the duration and location of exposure (*Agarwal & Saleh, 2002*). During their transit through the epididymis, sperm gain the ability to move progressively. However, they acquire the capacity to fertilize within the female reproductive tract through a series of physiological changes known as "capacitation" (*Visconti and Kopf, 1998*). Under normal physiological conditions, spermatozoa produce minimal amounts of ROS, which are essential for capacitation and the acrosomal reaction. The superoxide anion appears to play a significant role in this process (*Agarwal, et al., 2003*). Research has shown that male germ cells at different stages of differentiation possess the ability to generate ROS. Excessive production of free radicals or reactive oxygen species (ROS) can harm sperm, and ROS have been extensively investigated as a mechanism of infertility. Superoxide anion, hydroxyl radical, and hydrogen peroxide are among the primary ROS found in seminal plasma. CK activity serves as a measure of cellular maturity and fertilizing potential in human spermatozoa. Likewise, recent studies have identified an inverse relationship between CK levels and sperm morphological forms, suggesting that CK levels may serve as a reliable marker for sperm quality and

fertilizing potential in subfertile men (*Hallak, et al., 2001*). Spermatozoa are susceptible to reactive oxygen species (ROS) due to the high concentrations of polyunsaturated fatty acids present in their plasma membrane and cytoplasm (*Alvarez and Storey, 1995).*

3.0 MATERIALS AND METHODS

3.1 Research design and methods

3.1.1 Study design

This is a cross sectional study involving male partners of infertile couple presenting at the fertility clinic with established diagnosis of infertility after review by a clinician. Males with proven fertility served as controls.

3.1.2 Area of study

The study was conducted at the Fertility Clinic of the University of Benin Teaching Hospital, Benin City, Nigeria.

3.1.3 Selection of participants

Participants were selected on the basis of detailed history, clinical examination and laboratory investigations.

3.1.4 Sample size determination

The minimum sample size was determined using Sample determination size in health studies Lwanga and Lemeshow (1991) formula;

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

Where

n= minimum sample size

Z= Standard normal deviate that corresponds to 95% confidence limit (1.96)

p = Infertility prevalence rate in a previous study 5-8% (Okonofua, 2003).

q= (1-p)

I = the alpha level of significance (10%).

$$n = \frac{(1.96)^2 \times 0.05 \times (1-0.05)}{0.0025} = 72.99$$

A total of 75 participants were enrolled in the study.

3.1.5 Inclusion criteria

Apparently healthy males with infertility but normozoospermic who had regular unprotected sex without conception for 1 year were recruited in the study. Subjects who have had history of infertility for more than a year, sperm count $<15 \times 10^6$ cells/mL and few or no leukocyte count per field were enrolled. Individuals with no history of infertility and normal semen analysis, with at least 50% motility and >30%

normal sperm morphology and count of ≥15 x 10^6cells/mL were enrolled as controls.

3.1.6 Exclusion criteria

Individuals with poor semen quantity and quality were excluded from the study. The subjects who are already using supplementary antioxidants or any other medication for male infertility was not included in the study. In addition, subjects with testicular varicocele, genital infection, leukocytospermia, sexually transmitted diseases, chronic illness and serious systemic diseases, alcohol abusers, or smoking history were excluded from the study due to high seminal reactive oxygen species levels that decrease antioxidant activity, which results in decreased motility and abnormal morphology.

3.1.7 Ethical considerations

Ethical approval was sought and obtained from the Ethics Review Committee of the Edo Ministry of Health, Benin City, Nigeria. Informed consent was duly obtained from the participants

3.1.8 Sample collection

The semen samples was obtained by masturbation and collected in a clean, sterile, and wide-mouthed container made up of plastic that was confirmed as nontoxic for spermatozoa. The sample container was kept at ambient temperature (37 °C), thus avoiding the large changes in temperature that may affect the spermatozoa after ejaculation. After collection, the specimen was labeled with identification number, date, and time of collection. The semen container was placed in an incubator at 37 °C while the semen liquefies. The subjects were told to collect the semen after atleast three days of sexual abstinence.

3.1.9 Semen analysis

Liquefied sample was taken for further analysis on semen parameters. Semen parameter analysis includes physical appearance, volume, viscosity, pH, and microscopic analysis. Microscopic analysis of the semen included sperm concentration, count of motile sperms, and count of morphologically normal sperm (WHO, 1999).

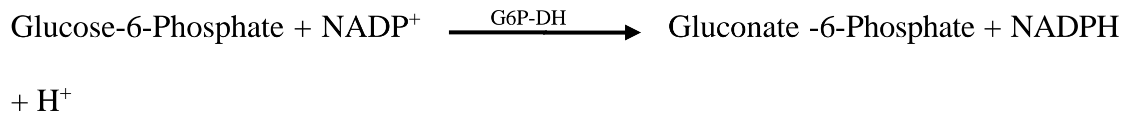
3.1.10 Sample Preparation and Laboratory Analysis For Creatine Kinase Estimation

After liquefaction, the semen sample was centrifuged at 4000 rpm for 5 min and the supernatant seminal fluid was separated into another clean and sterile plastic container prior to the assay of CK activity.

3.1.11 Assay Method for Creatine Kinase Activity (*Wicks and Usategui, 1982; Gerhardt and waldenstrom, 1972*).

Principle

A specific antibody inhibits both M subunits of CK-MM (CK-3), and the single M subunit of CK-MB (CK-2), and thus allow determination of the B subunit of CK-MB,(assuming the absence of CK-BB or CK-1). CK-B catalytic concentration, which corresponds to half of CK-MB concentration, is determined from the rate of NADPH formation, measured at 340nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) coupled reaction.



Glucose-6-Phosphate + $NADP^+$ $\xrightarrow{\text{G6P-DH}}$ Gluconate -6-Phosphate + NADPH + H^+

Procedure:

The working reaction and the instruments was brought to 37^0C centigrade, an automatic pipette was used to transfer 40µL of seminal plasma into labeled test tube containing 1mL working reagent. This was mixed thoroughly and incubated immediately at 37^0C.The stopwatch was started immediately and the absorbance (A) was read at 340 nm after exactly 5minutes (A_5) and 10minutes (A_{10}) of incubation.

Calculations

The CK-MB concentration in the sample was calculated using the following formula:

$$(A_{10} - A_5) \quad X \quad \frac{\text{CK-B}}{V_t \ x\ 10^6} \quad X \quad 2 \quad = \quad U/L$$

$$E \ \ x\ I\ x\ V_s\ X\ 5min$$

The molar absorbance (E) of NADPH at 340nm is 6300, the light path (I) is 1cm, the total reaction volume (V_t) is 1.04, the sample volume (V_s) is 0.04, and 1 U/L , are 0.0167 ukat/L. the following formulas are deduced for the calculation of the catalytic concentration :

<table>
<tr><td rowspan="2">$A_{10} - A_5$</td><td>X 1651 = U/L</td></tr>
<tr><td>X 27.5 = Ukat/L</td></tr>
</table>

Data analysis

Data generated was analyzed using Statistical Package for the Social Sciences software (version 20.0, IBM SPSS, Armonk, NY, USA). Comparison between means was done using the Student t- test, ANOVA (Analysis of variance) and Pearson correlation coefficient was used to determine the association between the measured variables and clinical condition. The level of significance was set at p-value of 0.05.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 represents the demographic data of study participants. The mean age of men investigated for infertility (40.32 ±1.51)years was not significantly different from the control subjects (41.20 ± 1.20:($p>0.05$). When the age was stratified, a significant difference was observed between the two groups (X^2=0.036). The educational attainment of the study participants was not significantly different from the controls (X^2 =0340). All study participants were either civil servants or businessmen and the different between the groups was insignificant. Similarly, majority of the study group

had normal body mass index, with only 2.7% and 6.7% were underweight and overweight respectively.

Table 4.2 indicates that creatine kinase activity in the seminal plasma of men investigated for infertility (756.45 ± 23.7)IU/L was markedly higher (p=0.001) compared with the controls (412.60 ±19.56). The sperm count (18.30 ± 11.04 vs 38.18 ± 9.60), volume (2.20 ±0.02 vs 2.75 ±0.06), total motility (14.30 ± 3.10 vs 42.20±2.40), viability (18.32 ± 0.20 vs 59.18 ± 2.5), progressive motility (11.60 ±2.20 vs 34.22 ±2.60) were markedly lower in men investigated infertility compared with controls. Conversely, the abnormal morphology (27.89 ±1.50 vs 7.30±2.50) was markedly higher in infertile men than controls.

Table 4.3 shows the relationship between creatine kinase activity and sperm quality indices among men investigated for infertility compared with controls. All sperm quality indices except abnormal morphology correlated negatively with creatine kinase, while abnormal morphology correlated positively with creatine kinase activity (p<0.05).

Table 4.1: Demographic Characteristics of study Participants

Age group (Years)	**Infertile men N = 7**	**Fertile Men N = 5**		**P-value**

	5	0		
Mean Age	40.32±1.51	41.20±1.20		0.890
25 - 34	19(25.3)	4(8.0)	6.672	0.036
35 - 44	38(50.7)	28(56.0)		
45 and above	18(24.0)	18(36.0)		
Educational Qualification				

Primary	18 (24.)	10 (20.0)	1.513	0.340
Secondary	41 (54.7)	25 (50.0)		
Tertiary	16 (21.3)	15 (30.0)		
Occupation				
Civil servant	30 (40.0)	8 (16.0)	0.880	0.348
Business	45 (60.	42 (84.		

	0)	0)		
BMI				
Under weight	02(2.7)	1(2.0)	0.683	0.711
Normal	68(90.7)	48(96.0)		
Overweight	05(6.7)	1(2.0)		

Table 4.2: Mean levels Creatine Kinase and Sperm indices among men investigated for infertility and fertile control group

	Infertile males (n = 75)	Controls (n = 50)	t-value	P-value
Creatine kinase (IU/L)	756.45 ± 23.70	412.60 ± 19.56	12.448	0.001
Sperm Count ($x10^6$/mL)	18.30 ± 11.04	38.18 ± 9.60	-5.675	0.001
Volume (mL)	2.20 ± 0.	2.75 ±	-4.5	0.01

	02	0.006	04	
Total motility (%)	14.30 ± 3.10	42.20 ± 2.40	-48.212	0.001
Viability (%)	18.32 ± 0.20	59.18 ± 2.50	-20.012	0.001
Progressive motility (%)	11.60 ± 2.02	34.22 ± 2.60	-26.200	0.001
Abnormal morphology	27.89 ± 1.50	7.30 ± 2.5	9.765	0.001

(%)		0		

Table 4.3: Relationship between Creatine kinase and Sperm indices among infertile male subjects

Correlation		
	R	P
Sperm count/creatine kinase activity	-.582	0.001
Volume/creatine kinase activity	-0.049	0.584
Total motility/cre	-.690	0.001

atine kinase activity		
Viability (%) / creatine kinase activity	-.488	0.001
Progressive motility (%) /creatine kinase activity	-.222	0.001
Abnormal	.33	0.0

morphology (%) / creatine kinase activity	8	01

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CHAPTER FIVE

5.0 DISCUSSION AND RECOMMENDATIONS

The purpose of this research was to determine the relationship between seminal plasma creatine kinase activity and sperm count, in men evaluated for infertility compared to a control group of fertile men in Benin city. Male factor infertility accounts for up to 50% of all cases (*Uadia and Emokpae, 2015*). This study aimed to fill gaps in knowledge existing in literature such as Is there a statistically significant correlation between seminal plasma creatine kinase activity and sperm count in men

evaluated for infertility in Benin city?. The major findings of the research are as follows: The demographic characteristics (such as age, education, occupation ,BMI) of the infertile and fertile men groups were comparable. This implies that the observed difference between the 2 groups is not likely to be responsible for the infertility. Seminal plasma creatine kinase (CK) activity was significantly elevated in the infertile group compared to the controls (fertile groups). All standard semen parameters (sperm count, motility, viability, progressive motility except from volume which is not statistically significant) were significantly poorer, and abnormal morphology was significantly higher in the infertile group. Strong negative correlation was observed between CK activity and all sperm quality indices in the infertile group (count, motility, viability, progressive motility) while a positive correlation was found with abnormal morphology.

The markedly higher level of creatine kinase in the seminal plasma of infertile men, compared to the fertile controls is a central finding of this study. As CK is an intracellular enzyme, its presence in the seminal plasma is likely due to leakage from damaged sperm cells or immature sperm cells. This suggests that the infertile group had a higher proportion of spermatozoa with compromised cellular integrity. The high CK levels could be a biomarker reflecting a failure in the final stages of sperm maturation (spermiogenesis), where excess cytoplasm which contains CK is not properly extruded, leading to immature, dysfunctional sperm that are prone to leaking their contents (*Huszar and vigue, 1993*).

The data from this study depicts impaired spermatogenesis and function in the infertile group. The significantly lower sperm count, volume, total and progressive motility, and viability, coupled with the higher percentage of abnormal forms, are consistent with a diagnosis of male factor infertility. The particularly low motility

parameters suggest a severe defect in the energy-dependent mechanisms essential for sperm movement, which aligns with the role of CK in cellular energy metabolism, CK biologic function is to establish an ATP buffering system for tissues(in this case, sperm) (*Ellington, 2007*).

The correlation analysis provides powerful evidence linking high CK activity is directly associated with poor sperm quality. The strong negative correlations between sperm quality indices and CK activity indicate that as CK levels in the seminal plasma rise, sperm count, motility, and viability consistently fall. Conversely, the positive correlation with abnormal morphology confirms that higher CK is associated with more structurally defective sperm. This inverse relationship strongly supports the hypothesis that elevated seminal plasma CK is a reflection of underlying sperm pathology, where cellular damage and immaturity lead to both the leakage of CK and the observed functional deficiencies. (*Agarwal et al., 2003*).

The findings of this research are consistent with existing research. For instance, (*Huszar, et al., 1990*) first established the link between CK activity and sperm maturity. Our results confirming that elevated CK is associated with poor motility and abnormal morphology align with their work. Similarly, a study by (*Agarwal, et al., 2003*) established that elevated CK levels are inversely correlated with the fertilizing potential of spermatozoa and reflect the extent of cellular immaturity, which is consistent to our observation in low viability and overall motility. Another study (*Huszar and Vigue, 1994*) reported a significant correlation between morphological irregularities in sperm and high CK activity, an enzyme involved in the synthesis and utilization of energy within sperm, our results showed that elevated CK was associated with an increase in abnormal sperm morphology, this agrees with the

theory. Furthermore, recent studies have identified an inverse relationship between CK levels and various sperm morphological forms, suggesting that CK levels can be utilized as a reliable marker for sperm quality and fertilizing potential in subfertile men (*Hallak et al., 2001).*

Limitations

Despite clear results, there were few limitations or constraints in the research which may have affected my results

- Firstly, the sample size was small for an analysis that involved Benin city as a whole, though adequate, samples were limited to a specific population i.e fertility clinic in UBTH ; a larger, multi-center research would enhance generalizability of the findings
- Secondly, the research did not investigate the potential causes of elevated CK, such as specific genetic defects, inflammatory conditions, or obstruction in the reproductive tract, which can be explored in future research and possible treatments for infertility.
- Also, the analysis for CK activity was limited to seminal plasma and not the sperm cell itself, this should be explored in future research.

Conclusion and Recommendations

In conclusion, this research demonstrates that seminal plasma creatine kinase activity is significantly elevated in infertile men and is strongly and inversely correlated with key sperm quality indices except abnormal morphology. This suggests that CK acts as a useful biochemical marker for detecting sperm cellular immaturity and damage.

Based on these findings, the following recommendations are made:

1. The assay of seminal plasma CK should be considered as a supplementary diagnostic tool in the evaluation of male infertility.

2. Future research should focus on measuring intracellular CK in purified sperm samples to confirm this relationship more directly.

3. Investigations into the clinical utility of CK levels in predicting outcomes of assisted reproductive techniques (like IVF) are warranted.

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APPENDIX II

Informed Consent Form

Title of research; The Relationship Between Seminal Plasma Creatine Kinase Activity and Sperm Count in Men Evaluated for Infertility in Benin City

Principal Investigator: EWEKA OSAZE MAUREEN
Phone number: 08143206043

Name of supervisor: PROF. M.A EMOKPAE
Phone number: 08034511182

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Introduction and Invitation
You are invited to participate in a research study. This form provides information about the study, which will help you decide if you wish to participate. Please read this carefully. Ask the researcher if there is anything that is not clear or if you need more

information. Your participation is entirely voluntary, and you are free to withdraw at any time without affecting your medical care.

Purpose of the research

Infertility is a significant challenge affecting many couples. This study aims to investigate a potential link between a biochemical marker (Creatine Kinase enzyme activity) in semen and sperm count. The goal is to determine if measuring this enzyme can provide a reliable and additional diagnostic tool for evaluating male fertility in our population. This could lead to better understanding and management of male infertility in the future.

Reason for invitation

You have been invited to participate because you are an adult male currently undergoing evaluation for infertility at this clinic.

Do I have to take part?

No. Your participation is completely voluntary. It is your choice. If you decide to participate, you will be asked to sign this consent form. You are free to withdraw from the study at any time, without giving a reason, and without any penalty or impact on the quality of your current or future medical care at this facility.

What will happen if I take part?

If you agree to participate:

- The procedure will be explained to you in detail.
- You will be asked to provide a semen sample by masturbation into a sterile container, which is the standard procedure for a semen analysis you are already undergoing for your fertility evaluation.
- A small portion of this sample (which would otherwise be discarded after the routine analysis) will be used for this research to measure Creatine Kinase activity.
- The results of your standard semen analysis (sperm count, motility, morphology) will be recorded anonymously for correlation with the research data.
- You may also be asked to provide some basic non-identifying information (e.g., age, duration of infertility) on a separate form. Your name or hospital ID will not be used.

Possible benefits of participating

You will not receive any direct personal benefit from participating in this study. However, the information obtained may contribute to a better scientific understanding of male infertility, which could help improve diagnosis and treatment for men in Benin City and beyond in the future.

Risks or discomforts involved

The process of providing a semen sample is the same as for your standard medical care. There are no additional physical risks beyond those associated with the routine semen analysis. We understand that discussing and undergoing fertility testing can be emotionally sensitive. All information you provide and the results of your tests will be handled with the utmost confidentiality.

Confidentiality

Your privacy is very important to us.
Your name and any personal identifiers will not be recorded on the research data sheets. Instead, you will be assigned a unique code number (e.g., RSP001).
The list linking your name to this code will be kept separately in a password-protected file or a locked cabinet, accessible only to the principal investigator.
All research data (semen analysis results, enzyme activity levels) will be stored and analyzed using only this code number. The findings of this study may be published in a scientific journal or presented at conferences, but no information that could identify you personally will ever be disclosed.

Results of the study
The results will be compiled into a research thesis or scientific publication. The overall findings will contribute to medical knowledge. You will not receive individual results from the research-specific tests (Creatine Kinase activity), as these are for research purposes only and are not yet established clinical tests. You will receive the results of your standard semen analysis from your doctor as part of your routine care.

Financial sponsors
Parents and Self

Contact Information for Further Questions
If you have any questions about the research now or in the future, you may contact:
The Principal Investigator: EWEKA OSAZE MAUREEN, 08143206043, impossiblegirl002@gmail.com
contact address: Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin city.

Supervisor : PROF. M.A EMOKPAE Phone number: 08034511182

Statement of Consent

By signing below, you confirm that:
- You have read and understood the information sheet above.
- You have had the opportunity to ask questions.
- You understand that your participation is voluntary and you can withdraw at any time.
- You agree to participate in the research study described.

Participant's Signature: ___________________________
Printed Name: ___________________________
Date: ___________________________

Researcher's Signature: ___________________________
Printed Name: ___________________________
Date: ___________________________

Witness's Signature (Optional but Recommended): ___________________________

Printed Name: ________________________
Date: ________________________

One copy of this form will be given to you, and one will be kept by the research team.

APPENDIX III

Participant Data Questionnaire

Research Title: The Relationship between Seminal Plasma Creatine Kinase Activity and Sperm Count in Men Evaluated for Infertility in Benin City

Participant ID: ___________ (For office use only)
Date of Sample Collection: ___//_______

Section A: Demographic Information

(Please tick [√] the appropriate box or write your answer in the space provided)

1. Age: ……......…… years
2. Educational qualification: Primary [ ] Secondary [ ] Tertiary [ ]
3. Weight(kg) ……………..
4. Height(m^2)………………..

5. Occupation:
   [ ] Skilled Manual (e.g., mechanic, welder, technician)
   [ ] Unskilled Manual (e.g., labourer, driver)
   [ ] Professional/White-collar (e.g., banker, teacher, civil servant)
   [ ] Other (please specify): ________________________

6. Duration of Infertility:……......…… years

Section B: Medical & Lifestyle History

7. Do you have a history of any of the following? (Tick all that apply)
   [ ] Undescended testis (as a child)
   [ ] Mumps infection after puberty
   [ ] Testicular injury or surgery
   [ ] Genital tract infection (e.g., STI, prostatitis)
   [ ] Varicocele
   [ ] Diabetes
   [ ] Hypertension
   [ ] None of the above

8. Do you currently smoke cigarettes?
   [ ] Yes
   [ ] No
   If yes, how many per day? ……....…… cigarettes

9. Do you consume alcohol?
   [ ] Yes
   [ ] No
   If yes, how often?
      [ ] Daily
      [ ] Weekly
      [ ] Occasionally

10. How long was the abstinence period before providing this semen sample?
   [ ] 2-3 days
   [ ] 4-5 days
   [ ] 6 days or more

Section C: Semen Analysis Results (To be completed by Lab Technician)

11. Semen Volume: ……....…… mL

12. Sperm Concentration (Count): ……....…… x 10^6 / mL

13. Total Sperm Count: ……....…… x 10^6 / ejaculate

14. Total Motility (Progressive + Non-progressive): ……....…… %

15. Sperm Morphology (Normal forms): ……....…… %

16. Sample for CK Analysis Processed?[ ] Yes [ ] No

Thank you for your participation.