IN-VITRO EFFECT OF NON-IONISING RADIATION FROM CELLULAR PHONE ON HUMAN SPERM QUALITY IN MEN

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By

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DECLARATION

With the exception of references and quotations from other sources which have all been credited, I hereby declare that this piece of work is the original research work of mine and that no part of it has been presented elsewhere. Also, I would like to say that any errors of judgment, facts, omissions and style remain my liability.

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ABSTRACT

This systematic randomized case-control study aimed to determine and evaluate the effects of exposure of non-ionizing cellular phone radiation on markers of semen quality, reactive oxygen species levels and DNA damages *in-vitro*, after ejaculation. A total of seventy-three (73) patients attending a fertility center in Sakumono, Tema metropolis were recruited for this study. Structured questionnaire was used to obtain the socio-demographics and the anthropometric measurements were also assessed. About 2-3mls of freshly ejaculated semen neatly collected by masturbation with consent and analyzed initially by the WHO standard semen analysis. Semen was exposed to non-ionizing cellular phone radiation for three hours and superoxide dismutase (SOD) activity levels were determined using SOD assay kit and DNA fragmentation index (DFI) was calculated after the DFI test. Data was entered and analyzed using SPSS version 20.00. The mean age of the participants in this study was 38.81 ± 5.87years. A higher proportion (28.8%) of them was between the ages of 36-40years. The mean height, weight BMI and number of days for abstinence were 1.72m ± 0.048, 76.70kg ±25.91, 25.91kg/m^2± 2.42 and 3.06 days±0.24. DNA fragmentation index (DFI) was significant and linearly positive associated with immotility (R^2 = 0.483; p < 0.0001) and negatively associated with progressive motility (%) (R^2 = 0.299; p <0.0001), total motility (%) (R^2 0.0.483; p <0.0001) and SOD (U/ml) (R^2 = 0.773; p <0.0001). There was a significant positive linear relationship between SOD and progressive motility (R^2 = 0.204; p<0.0001), non-progressive motility (R^2 = 0.296; p<0.0001), vitality (R^2 = 0.725; p <0.0001), total motility R^2 = 0.0.497, p <0.0001). Superoxide Dismutase (SOD) was negatively associated with Immotility (R^2 = 0.497; p<0.0001) exposed to non-ionizing cellular phone radiation. Mean percentage (%) of Superoxide dismutase concentration was significantly lower in order before exposure > after 37^{0}C exposure > after 37^{0}C and RF-EMW exposure (P<.0001). Higher proportional effect of sperm progressive motility (40.5%), non-progressive motility (5.54%), vitality (16.29%), SOD activity (22.2%), total motility (3.82%), immotility (3.82%) and DNA fragmentation index (11.23%) was observed after the exposure to RF-EMW from a mobile phone compare to control and before exposure to non-ionizing cellular phone radiation. This study demonstrated that RF-EMW causes oxidative stress in semen and resulted into a decline in spermatozoa total motility, (progressive and non-progressive motility), viability and SOD and corresponding increase immotility and DFI.

DEDICATION

This work is dedicated to my dear grandmother, Maame Agnes Afua Darkoah, my foundation for education, Dr. Kofi Amaniampong for his fatherhood inspiration, my son Kofi Amaniampong Baah and his sisters Eugenia Adom Bediako Baah and Karen Adjoah Darkoah Baah and beloved wife Stella Baaba Baah. They have been the motivational strength behind this work. Again I dedicate this work to Dr. Christian Obirikorang and Mr. Richard Harry Asmah my wonderful supervisors

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ABREVIATIONS

5-OHdC	5-Hydroxy-2′-deoxycytidine
5-OHdU	5-Hydroxy-2′-deoxyuridine
8-OHdA	8-Hydroxy-2′-deoxyadenosine
8-OHdG	8-Hydroxy-2′-deoxyguanosine
AAB	Acidic Aniline Blue
ATP	Adenosine Triphosphate
AP-site	Apurinic/Apyrumidinic Site
CMA3	Chromomycin A3
DBCP	Dibromo-3-Chloropropane
DBD–FISH	DNA breakage detection– fluorescent *in situ* hybridization assay
DFI	DNA Fragmentation Index
DNA	Deoxyribonucleic Acid
ED	Erectile Dysfunction
FCC	Federal Communication Commission
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophic-Releasing Hormone
HPLC	High-Performance Liquid Chromatography
ICNIRP	International Commission for Non-ionizing Radiation Protection
iOAT	Idiopathic oligoasthenoteratozoospermia
LH	Luteinizing Hormone
NT	Nick Translation
RF-EMW	Radio frequency electro-magnetic waves
ROS	Reactive oxygen species
SAR	Specific Absorption Rate
SCD	Sperm Chromatin Dispersion
SCSA	Flow Cytometric based sperm chromatin structure assay
SOD	Superoxide Dismutase
SPSS	Statistical Program for Social Science
ssDNA	Single-stranded DNA
SSRI	Selective Serotonin Reuptake Inhibition
TB	Toluidine Blue
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
WBC	White Blood Cells
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Infertility is currently defined by World Health Organization (WHO) as the failure of a sexually-active couple (engaging in sexual intercourse at least three times per week), without contraception, to attain pregnancy within one (1) year. Approximately 15% of sexually-active couples are not fertile and 50% of the infertility cases are attributed to the male (Rowe and Comhaire, 2000; Irvine, 2005; Jacob Farhi, 2011). There are both known and unknown causes of male infertility, with idiopathic causes accounting for about 30% - 50% of all the cases of male infertility (Dohle *et al.*, 2005; Irvine, 2005; Rolf *et al.*, 2010). Idiopathic male infertility is also called idiopathic oligoasthenoteratozoospermia (iOAT), which shows that the individual has inexplicable abnormalities in sperm parameters which includes low-sperm concentration (≤ 15×10^6per/mL) decreased spermmotility (≤ 40.0%) and irregular sperm-morphology (≤ 4.0% regular forms)
(Jungwirth *et al.*, 2012).

The list of known causes of male infertility is huge and which mostly includes varicocele, urogenital infections, immunologic factors (e.g., anti-sperm antibodies), sexual or ejaculatory insufficiency, congenital-disorders (e.g., Kallmann's syndrome, Klinefelter's syndrome, etc.), acquired urogenital abnormalities, and disorders of the endocrine system (Dohle *et al.*, 2005; Rolf *et al.*, 2010). There are a few of the known causes of infertility that have a pharmacologic possibility as the first-line of treatment. The known causes of infertility of the male have the tendency to have targeted and active treatment choices, while there are non-specific or empirical treatment choices with questionable efficacy for the idiopathic cases of infertility. This lack of treatment condition turns to have a lot of psychological and social problems for the couple involved (Forrest and Gilbert, 1992).

Presently, cell phone technology is an essential part of every activity of our daily life and its usage is not only limited to voice conversations but also for internet and high resolution pictures. Cellular phones emit radio frequency electro-magnetic waves (RF-EMW), which transmit signals from the cellular phone to the base stations and antennas. Though the frequency of such waves is low (800-2200 MHZ) there is still risk to the human user, because our bodies can act as antennas that absorbs and converts these waves into eddy current (www.privateline.com/ PCS/Frequencies.htm). These cellular devices can affect the general body and more specifically the male reproductive system with higher effects on sperm motility, morphology, counts, sperm functions, Leydig cells and Sertoli cells as a result of too much production of reactive oxygen species (Agarwal *et al.*, 2009; De Iuliis *et al.*, 2009; Fejes *et al.*, 2009). In recent years, it has become clear that high levels of oxidative stress may damage sensitive biological molecules such as DNA, lipids, and proteins, and such damage may play a role in the etiology of several degenerative diseases such as cancer and arthritis (Collins, 1999).

Progressive sperm motility is important in evaluating the fertility potential of spermatozoa, and a prerequisite for fertilization in humans (Amelar *et al.*, 1980). The sperm motility depends on a number of factors, including the temperature at which the semen is kept between the time of ejaculation and the time of analysis. Temperatures lower than 34oC and higher than 37oC have been shown to affect negatively sperm function such as motility and penetration into cervical mucus (Appell *et al.*, 1977; Stumpf *et al.*, 1984; Cohen *et al.*, 1985).

Treatment of infertility is expensive, demanding and time consuming. Prevention and control are more advisable than to contribute to the increasing effect on sperm function before treatment is begun. This research is being undertaken to access how too much exposure to non-ionizing radiation (the use of mobile phone) contribute to poor markers of semen quality as well as how reactive oxygen species (oxidative stress) affects sperms in ejaculated semen sample. Findings may be

useful to fertility specialists, their patients, clinical andrologist and also for the treatment and control of poor markers of semen quality.

1.2 Problem statement

Infertility is on the increase with about 15% of sexually active couples infertile (Rowe and Comhaire, 2000; Irvine, 2005) and male factor infertility contributing to about 50% of the infertility cases, which is largely due to the declining markers of semen quality with less knowledge about the causes. Oligozoospermia, asthenozoospermia and teratozoospermia are on the increase globally (World Health Organisation, 2010).

There has been an increase in mobile phone usage in Ghana with about half of the population using a mobile phone (Mahan *et al.*, 2009; Sey, 2011; Akanlisikum *et al.*, 2014). Life style changes (environmental effects) have led to increasing key reproductive hormonal imbalance and the industrial and technological age is exposing many to occupational hazards (Bonde and Giwercman, 1995; Sharma *et al.*, 2013). Evidence in medical research has shown all these can cause infertility in men. However in Ghana the relationship between the declining of markers of semen quality and key male reproductive hormonal imbalance due to exposure to non-ionizing radiation (mobile phone usage) and other occupational hazard is not yet known. The study hopes to provide a data on these non-ionizing radiation effects and how to manage the growing cases of infertility in men in Ghana.

1.2.1 Hypothesis

Non-ionizing radiation affects the quality of human sperm.

1.3 AIM

The aim of the study was to determine and evaluate the effects of exposure of nonionizing cellular phone radiation on markers of semen quality, superoxide dismutase levels and DNA damages *in-vitro*, after ejaculation.

1.4 Specific objectives

- To compare markers of semen quality of ejaculated semen before and after exposure to non-ionizing radiation using the WHO standard methods for semen analysis.
- To compare the antioxidant superoxide dismutase in ejaculated semen before and after exposure to non-ionizing radiation.
- To assess the effect of non-ionizing cellular phone radiation on the sperm's deoxyribonucleic acid (DNA) damage, using DNA Fragmentation Index (DFI).

1.5 JUSTIFICATION

Problem of infertility affects between 60 million and 168 million people worldwide; as at 2003, generally one in ten couples experience primary or secondary infertility (Vayena *et al.*, 2002; Butler and Khanna, 2003). The majority of those who suffer live in the developing world (Vayena *et al.*, 2002). Infertility interferes with one of the most fundamental and highly prized human activities and thus presents a major life challenge to those who desire children (Fidler and Bernstein, 1999). The condition brings up issues related to the health and well-being of individuals, couples and society as a whole. The burden of infertility includes psychological, social and physical suffering. Documented consequences include: anxiety, depression, lowered life satisfaction, frustration, grief, fear, guilt, helplessness, reduced job performance, marital duress, dissolution and abandonment; economic hardship, loss of social status, social stigma, social isolation and alienation, community ostracism, physical violence and where treatment is available uncomfortable, painful or life-threatening medical interventions (Fidler and Bernstein, 1999; Daar and Merali, 2002; van Balen and Inhorn, 2002; Vayena *et al.*, 2002). The nature and severity of the consequences of infertility differs between developing and developed countries and although effects vary depending on

multiple factors, the consequences appear greater in the developing world (van Balen and Inhorn, 2002). In general, few health conditions affect a person's wellbeing more profoundly or pervasively than infertility in developing countries where the private agony of infertility is transformed into a harsh public stigma with devastating consequences (Daar and Merali, 2002). Despite major attempts over the past century to cure male infertility by both orthodox and traditional medical practitioners it is still on the rise. This rise can be attributed to hormonal imbalance (genetic and environment) temperature, life hazards (mobile phone usage) and occupational hazards (Skakkebaek *et al.*, 2001). Poor markers of semen quality (Oligoasthernoteratozoospermia) are some of the major causes of infertility among most of the men attending the fertility clinic. Presently data available reports that exposure of human semen samples to cell phone radiation under *in vitro* conditions resulted in decreased motility after about five minute of exposure (Erogul *et al.*, 2006). Other researchers found no effect of the radio frequency electro-magnetic wave on mitochondrial membrane potential of spermatozoa and motility at a specific absorbance rate (SAR) of 2W/k (Yan *et al.*, 2007). However at a SAR of 5.7 W/kg there was a decrease in straight-line velocity and beat-cross frequency (Falzone *et al.*, 2008). Data on the causes of oligoasthenoteratozoospermia among infertile men visiting fertility centers in subSaharan Africa are limited. There is thus an urgent need for Ghanaians to know the effects particularly because scanty information exists on the causes of poor markers of semen quality in the country. This study hopes to determine the effects of exposure to non-ionizing radiation on the sperm motility and viability as well as the deoxyribonucleic acid fragmentation index (DFI) of the sperm so that precautionary measures can be taken to reduce male infertility.

CHAPTER TWO

LITERATURE REVIEW

2.1 MALE INFERTILITY

Male infertility can be defined as the male's inability to cause pregnancy in a fertile female. This accounts for 40-50% of infertility (Brugh and Lipshultz, 2004). Male infertility is mainly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity. For many men the cause of their abnormal semen analysis, particularly when the abnormality is mild, is unknown. Clearly, the presence of an abnormal semen analysis does not necessarily lead to an absolute inability for a partner to conceive, though the chance may be reduced (World Health Organisation, 2010; Harris *et al.*, 2011).

2.2 HISTORICAL PERSPECTIVE OF MALE INFERTILITY

Infertility has been a major medical and social problem since the dawn of human existence and women have always been the symbol of fertility. Since the beginning of civilization, couples have been prolific with childbirth and difficulty with conception was seen as a problem. As far back as 1900 BC, there are recorded documents discussing the treatment of gynecologic disorders. Despite the fact that the understanding of anatomy in ancient Egyptian society was sketchy, their references to female reproductive tract (Morice *et al.*, 1998; Morice *et al.*, 1995) and sperm was considered to originate from the bones of males. The practice of medicine was permeated with magic, physicians doubled as priests to the goddess Sekmet and thus gods played a major role in the treatment of childbirth (Morice *et al.*, 1998). Examinations for fertility in both male and females were based on the concept that the genital organs were in continuity with the rest of the body and, especially, with the digestive system. This theory remained in place for hundreds of years and was adopted by Hippocrates and many other medieval physicians (Porter, 1999; Craik, 2009; Morice *et al.*, 1995).

However in Jewish society children were considered to be desired and conceived by the will of God, as Eve said in the Bible "I have gotten a man from the Lord", therefore infertility was considered a divine curse. Male infertility was unrecognized as proven by the statement of Jacob in the Book of Genesis "Am I in God's stead, who hath withheld from thee the fruit of the womb?" This statement was made by Jacob to his wife Rachel. The recognition of infertility as a medical problem in Western medicine started with the works of Hippocrates and his school, he developed a system of medical reasoning based on rational thinking. Hippocrates was well aware of the problems of infertility and theorized a number of causes for it and had formulated numerous treatment options (Morice *et al.*, 1995).

The Renaissance period marks the stage of undeniable scientific progress and advancements in modern day thinking and treatment of infertility. Very instrumental in this was da Vince and others, the mysteries of the female body were gradually resolved and scientific thinking and reasoning replaced magic and the gods. In 1562, St. Bartolomeo recommended that husbands should put their finger in the vagina after intercourse to encourage conception (Haughton, 2014). This is considered as the ancestor of the idea of artificial insemination. De Graaf (1672), refuted Aristotle's theories of fertilization, and described the ovary and follicular function (Short, 2003). The identification of the sperm under the microscope by von Leeuwenhoek was done in 1677 (Birkhead and Møller, 1998). In 1752, Dr. William Smellie was the first to carry out experiments and describe the fertilization process. Despite these progresses made during these times, infertility was almost synonymous with the female; and it was rare that males were considered as a cause (Seibel and Zilberstein, 1995).

The nineteenth and twentieth centuries were marked by tremendous advances in the diagnosis and treatment of infertility. In 1898, fertilization was described as the union of an egg and a sperm. 80 years later, in 1978, the first "test-tube" baby was

born in England and in 1981; her *in-vitro* sister was born (Edwards, 1981; Biggers, 2012). After Antonio Van Leeuwenhoek first described spermatozoa in the 17th century but it was not until 1928 that the sperm count was found to be associated with fertility potential (Seibel and Zilberstein, 1995). Since that time a variety of sperm tests and semen parameters have been developed with the hope of clarifying whether or not a man could impregnate his partner. MacLeod (1942), MacLeod and Gold (1953), Eliasson (1971) and Hellinga (1949,1976) have led the scientific basis of conventional analysis of spermatozoa and the techniques recommended by them are still considered the reference for more advanced methods (Comhaire *et al.*, 1986). In the last 30 years the studies about the qualities and functions of human semen have led to a dramatic increase in the knowledge that health practitioners have about male infertility. Defective sperm function has been found to be the most prevalent cause of male infertility, and a difficult condition to treat (Skakkebaek *et al.*, 2001).

2.3 CAUSES OF MALE INFERTILITY

There are a lot of causes of male infertility, which include from imbalances of hormonal levels, to physical problems, to psychological and/or behavioral problems (Valentine, 1986; Eddy *et al.*, 1996). Moreover, fertility reflects a man's "overall" health. Men who live a healthy lifestyle are more likely to produce healthy sperm (Sandler, 1951).

2.3.1 Hormonal Causes of Male Infertility

A small percentage of male infertility is due to hormonal problems. The hypothalamus-pituitary endocrine system regulates hormonal events that enable testes to produce and effectively disseminate sperm. Several things could arise with the hypothalamus-pituitary endocrine system, thereby interfering with their normal function. For example the brain can fail to release gonadotrophic-releasing hormone (GnRH) properly. This hormone (GnRH) stimulates the hormonal

pathway that causes testosterone synthesis and sperm production; therefore a lack of GnRH release leads to lack of testosterone production and cessation in sperm production (Eddy *et al.*, 1996). The pituitary gland may also fail to produce or may produce inadequate luteinizing hormone (LH) and follicle stimulating hormone (FSH), these are needed to stimulate the testes and testosterone/sperm production. Then also the Leydig cells of the testes' may fail to produce testosterone in response to the LH stimulation. There are many other hormonal disorders that could interfere with male infertility; some of these include factors as below.

2.3.1.1 Hyperprolactinemia

This refers to elevated prolactin concentration. Prolactin is a hormone associated with nursing mothers; this is found in 10-40% infertile males. Mild elevation of prolactin produces no symptoms, but higher concentrations reduce sperm production, reduces libido and may cause impotence (Biller *et al*., 1999).

2.3.1.2 Hypothyroidism

Low levels of thyroid hormone can cause poor semen quality, poor testicular function and may disturb libido. This condition is found in only one percent (1%) of infertile men. It may be caused by high dietary intake of iodine (KrajewskaKulak and Sengupta, 2013).

2.3.1.3 Congenital Adrenal Hyperplasia

This condition occurs when the pituitary gland is suppressed by increased levels of adrenal androgens, resulting in low sperm count, increased number of immature sperm cells and low sperm cell motility. This condition is reported in only 1% of infertile males (Merke and Bornstein, 2005).

2.3.1.4 Hypogonadotropic Hypopituitarism

This is a condition associated low pituitary gland output of LH and FSH. This results in the arrest of sperm development and results in the progressive loss of germ cells in the testes and also leads to the deterioration of seminiferous tubules

and Leydig cells. This condition may result in permanent infertility in males (Ascoli and Cavagnini, 2006).

2.3.1.5 Panhypopituitafism

Complete pituitary gland failure is referred to as panhypopituitafism this condition lowers growth hormone, thyroid-stimulating hormone and LH and FSH levels. It results in lethargy, impotence, decreased libido, loss of secondary sex characteristics and normal or undersized testicles (Glazener *et al.*, 1987).

2.3.2 Physical Causes of Male Infertility

There are a lot of physical problems that may cause male infertility. These may interfere with the sperm production process or disrupt the pathway down which the sperm travel. These usually result in low sperm count and/or abnormal sperm morphology. The following is a list of the most common physical problems that cause male infertility.

2.3.2.1 Varicocele

These are dilated veins in the scrotum (similar to varicose veins in the legs) these veins are dilated because the blood does not drain properly from them. These dilated veins allow extra blood to pool in the scrotum which has a negative effect on sperm production. This condition is the most common reversible cause of male infertility and may be corrected by minor outpatient surgery. Most experts perform this surgery microscopically to preserve the arterial supply and lymphatics. A sub-inguinal incision (about 1 inch) above the penis and (1 inch) from the midline is usually used as this avoids incising the abdominal muscles and creates less post-operative pain (Pryor and Howards, 1987).

2.3.2.2 Damaged Sperm Ducts:

One major cause of infertility is the inability to transport sperm from the testicles to the outer part of the penis; this condition is present in 7% of infertile males. This

pathway can be blocked by a number of conditions: A genetic or developmental mistake may block or cause the absence of one or both tubes, scarring from tuberculosis or STDs may also block the epididymis or tubes and then also an elective or accidental vasectomy may interrupt tube continuity (Jungwirth *et al.*, 2012).

2.3.2.3 Torsion:

This is caused by a supportive tissue abnormality, which allows the testes to twist inside the scrotum characterized by extreme swelling. This condition pinches the blood vessels that feed the testes shut leading to testicular damage. Without emergency surgery this can impair fertility and can permanently result in infertility (Hutson, 1998).

2.3.2.4 Infection and Disease:

Infection of the reproductive tract include; epididymis, orchitis, prostatitis or post pubertal viral infections of the testis and may cause absolute and irreversible infertility. Bacterial infections or sexually transmitted diseases may cause blockage of the sperm duct. The patient may have normal production of sperms, but ducts carrying them are obstructed. Active bacterial or viral production or sperm function. WBC which is the body's response to infection may also have a negative effect on the sperm membrane, making them less healthy. If excessive WBC is seen in a semen specimen, culture should be done. This usually includes cultures of commonly asymptomatic, sexually-transmitted diseases including *Mycoplasma urealyticum* and *Chlamydia*. Also a general genital culture is usually taken. If the infection and the WBC are persistent antibiotics may be considered (Hellstrom, 2012).

2.3.2.5 Klinefelter Syndrome:

This genetic condition results in an additional X chromosome, therefore men with Klinefelter Syndrome have one Y and two X chromosomes. Men with this

condition have peanut sized testicles and an enlarged breasts. However, Klinefelter Syndrome eventually causes all active testicular structures to atrophy. Once testicular failure has occurred, improving fertility is impossible (Kamischke *et al.*, 2003)

2.3.2.6 Retrograde Ejaculation:

In this condition semen is ejaculated into the bladder rather than out through the urethra because the bladder sphincter does not close during ejaculation. Men with this condition have a small ejaculate volume and urine may be cloudy after ejaculation. About 1.5% of infertile men has this condition and may be controlled by medications like decongestants, which contract the bladder sphincter, or surgical reconstruction of the bladder neck can restore normal ejaculation (Yavetz *et al.*, 1994). .

2.3.3 Psychological /Behavioral Causes of Male Infertility:

Several sexual problems exist that can affect male fertility. These problems are most often both psychological and physical in nature: it is difficult to separate the physiological and physical components (Cousineau and Domar, 2007).

2.3.3.1 Erectile Dysfunction (ED):

This condition is also referred to as impotence and reported to affect about 20 million American men (Shindel *et al.*, 2008). ED can be caused by single or multiple factors. Most men who suffer from ED have a secondary psychological problem that can worsen the situation like performance anxiety, guilt, and low self-esteem. Many of the common causes of impotence include: diabetes, high blood pressure, heart and vascular disease, stress, hormonal problems, pelvic surgery, trauma, venous leak, and the side effects of medications such as Prozac and other selective serotonin re-uptake inhibitors (SSRIs) and propecia (Johannes *et al.*, 2000).

2.3.3.2 Premature Ejaculation

Some males are unable to control the ejaculatory response for at least thirty seconds following penetration referred to as premature ejaculation. However this becomes a fertility problem if a man ejaculates before the penis is fully inserted in the partner's vagina. Premature ejaculation can be overcome by artificial insemination or by using a behavioral modification technique called the "squeeze technique" which desensitizes the penis (Hatzimouratidis *et al.*, 2010).

2.3.3.3 Ejaculatory Incompetence:

Another problem with ejaculation is the inability to ejaculate during sexual intercourse even though normal ejaculation occurs during masturbation. This condition sometimes responds well to behavioral therapy; if this technique does not work, artificial insemination can be employed using an ejaculate from masturbation (Riley and Riley, 1981)

2.3.4 Environmental causes of male infertility

Hazardous environmental agents are ever present in the immediate environment. Male fertility is known to be highly susceptible to many chemicals and physical agents, either generated by industrial or agricultural activities (Michal *et al.*, 1993; Bonde and Comhaire, 1996). Such hazardous agents are ever present in the occupational activities and also in the general environment. Toxic damage to the testes can result in many effects, namely, reduced sperm production, the production of defective spermatozoa, and impaired androgen production (Bonde and Comhaire, 1996).

The effect of environmental hazards on the male reproductive system was revealed almost 30 years ago when pesticide manufacturers and agricultural workers in contact with the nematocide, 1,2-dibromo-3-chloropropane (DBCP), suffered from severely impaired spermatogenesis, leading to infertility (Slutsky *et al.*, 1999). Since different chemical classes have been demonstrated to harm the male reproductive

system in animal models (Sundaram *et al.*, 1995). The list of chemicals that have been shown to be deleterious to the male reproductive system is small and observations have been limited to cross-sectional studies on occupational populations that were exposed to these substances at very high concentrations

(Wyrobek *et al.*, 1981; Ratcliffe *et al.*, 1987; Schrader *et al.*, 1988; Ratcliffe *et al.*, 1989). Due to the widespread use of such chemicals, and their potential of leakage into the environment, they constitute a putative hazard to male fertility. In recent times exposure to radiation has become one of the leading causes of health problems in the working environment. Chief amongst the effects of radiation is the cause of infertility especially in males. Research has suggested that Radio-frequency electromagnetic radiation (RF-EMR) emitted by the devices can have a detrimental effect on male fertility (Ratcliffe *et al.*, 1987).

2.4 BIOLOGY OF THE HUMAN SPERMATOZOA

2.4.1 The Human Sperm Cell

The sperm is the male reproductive cell. The human sperm is a haploid cell containing 23 chromosomes, which can join the 23 chromosomes of the female egg to form a diploid cell. Anatomically, the mammalian sperm cell consists of a head, a mid-piece and a tail. The head contains the nucleus with densely coiled chromatin fibers, surrounded anteriorly by an acrosome, which contains enzymes used for penetrating the female egg. The mid-piece has a central filamentous core with many mitochondria spiraled around it, which is used for ATP production for the journey through the female cervix, uterus and uterine tubes. The tail or "flagellum" executes the lashing movements that propel the spermatocyte (Mann and Lutwak-Mann, 2012).

2.4.2 Spermatogenesis and Spermiogenesis

The human sperm is produced during a process referred to as Spermatogenesis. The sperm cells are produced from male primordial germ cells by way of mitosis

and meiosis. The initial cells that begin the pathway of spermatogenesis are referred to as spermatogonia yielding spermatocytes by the process of mitosis. The primary spermatocytes formed divide meiotically into two secondary spermatocytes, which then divide individually by meiotic division again into two spermatids. The spermatids then mature into the spermatozoa. Spermiogenesis is the final stage of spermatogenesis, which sees the maturation of spermatids into mature and motile spermatozoa. Thus at the end of spermiogenesis four spermatozoa (sperms) are produced from a primary spermatocyte (Fishelson *et al.*, 2007). The process of spermatogenesis takes place in the male testes and epididymis in a sequential fashion. This process is highly dependent on optimal conditions; DNA methylation and histone modification play an important role in this process (Song *et al.*, 2011). This process starts at puberty and continues until death, although can be characterized by a slight reduction in quantity with age.

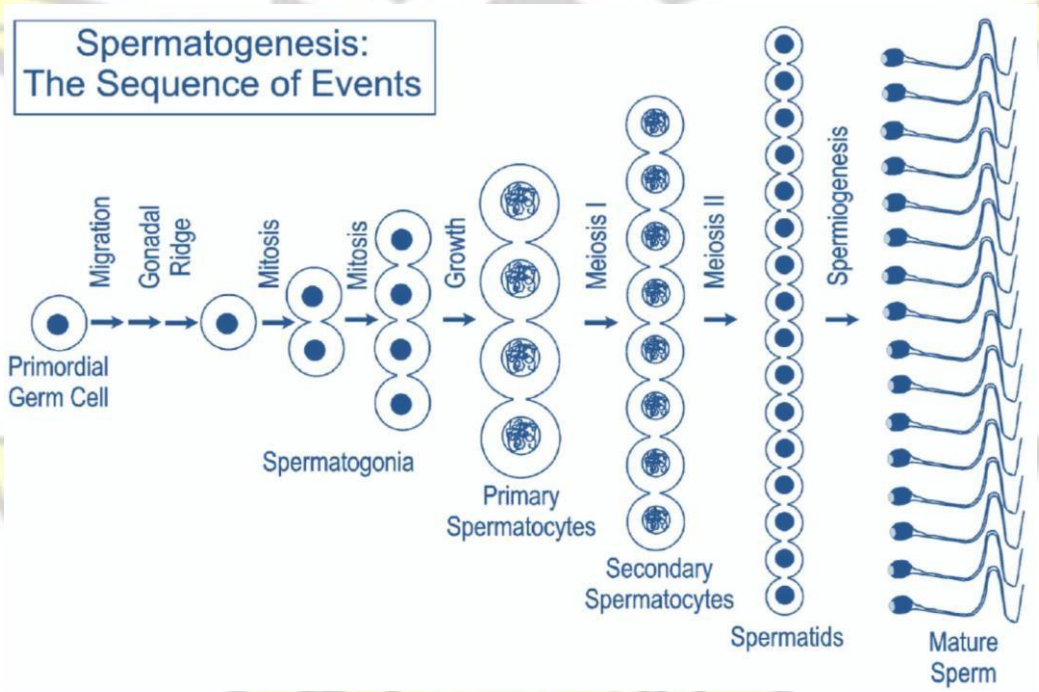


Figure 2.1: The process of Spermatogenesis and Spermiogenesis.

There are sperm cells with abnormal morphology terathos mean monster. It could also be termed globozoospermia. These abnormalities greatly affect fertility (Hellstrom, 2012) The cause of Teratozoospermia are unknown in most cases,

however leukaemia and other conditions contributes to some instance globozoospermia which are sperm cells with round heads and is due to golgi apparatus which is not transformed into the acrosome which is needed for fertilization. The presence of abnormally shaped sperm can negatively affect fertility by reducing sperm motility or preventing sperm from adhering to the ovum (Fedder, 1996).

2.5. REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS

2.5.1 Reactive Oxygen Species and Oxidative Stress

Reactive oxygen species (ROS), such as hydrogen peroxide, super oxide and hydroxyl radicals are products of oxygen metabolism in all aerobic organisms. ROS are generated as a result of energy production from mitochondria (from the electron transport chain), as part of an antimicrobial (Weiss and LoBuglio, 1982) or antiviral (Griot *et al.,* 1989) response, as well as detoxification reactions carried out by the cytochrome P-450 system. Environmental agents such as ultraviolet light, ionizing radiation, redox chemicals, microorganisms and cigarette smoke also readily generate ROS (Mena *et al.*, 2009).

Oxidative stress is a cellular condition associated with an imbalance between the production of free radicals, mainly ROS, and their scavenging capacity by antioxidants. When the production of ROS exceeds the available antioxidant defense, significant oxidative damage occurs to many cellular organelles by damaging lipids, proteins, DNA and carbohydrates, thus ultimately leading to cell death. Sperm is particularly susceptible to oxidative damage due to its unique structural composition. In the process of maturation spermatozoa extrude cytoplasm. Since cytoplasm is the major source of antioxidants, lack of cytoplasm causes a deficiency in antioxidant defence. Ironically, when this process is hindered, residual cytoplasm forms a cytoplasmic droplet in the sperm mid region. The residual cytoplasm contains high concentration of some cytoplasmic enzymes (G6PDH, SOD), which are also a source of ROS (Panagopoulos *et al.*, 2007).

In addition, the sperm plasma membrane is rich in polyunsaturated fatty acids that readily undergo lipid peroxidation by ROS, resulting in a loss of membrane integrity. Immature spermatozoa and seminal leukocytes are the major sources of ROS in semen. In abnormal spermatozoa ROS may be generated at the level of the plasma membrane (nicotinamide adenine dinucleotide phosphate oxidase system) or mitochondria (NADH dependent oxido-reductase). Seminal leukocytes produce hydrogen peroxide through the NADPH oxidase system. H_2O_2 is the most toxic form of ROS for spermatozoa since it is membrane permeable and readily affects the cellular organelles, whereas superoxide and the hydroxyl radical are membrane impermeable and take time to exert their effect (Garrido *et al.*, 2004). Nitric oxide reacts with the superoxide anion to yield the highly reactive metabolites peroxynitrite and peroxynitrous acid, both of which are strong oxidants. Numerous studies now consider oxidative stress to be a real entity that is likely to have a significant impact on normal sperm function, thus affecting reproduction and fertility (fig.4. 2).

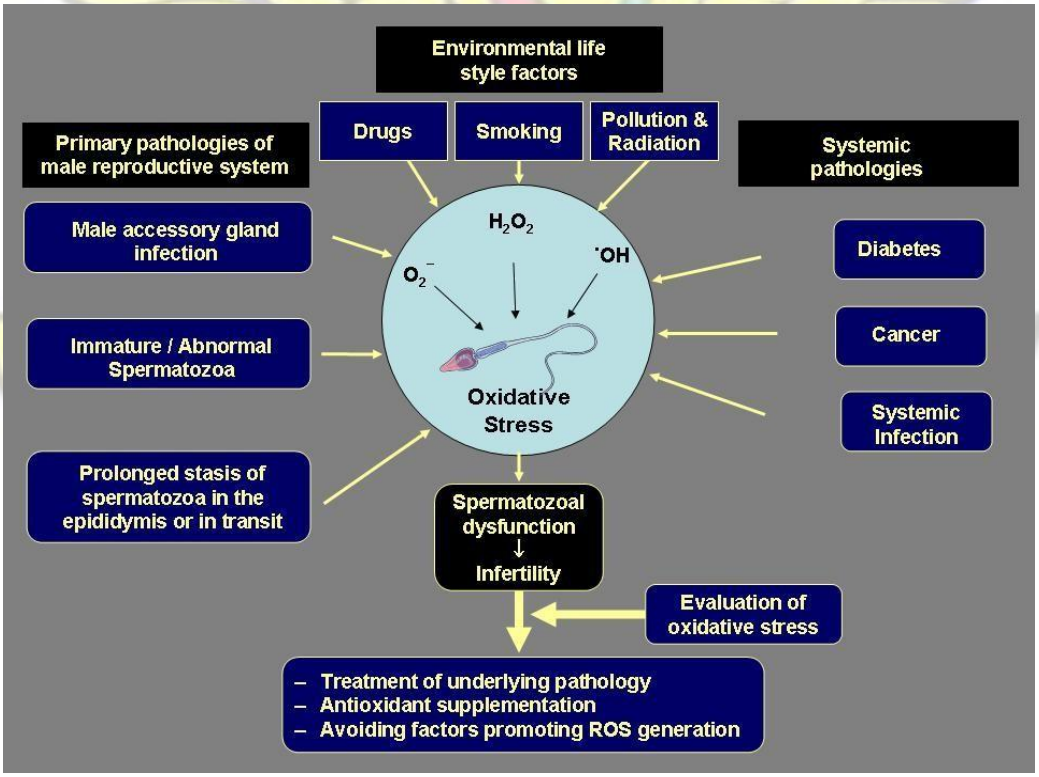


Figure 2.2: Etiology and management of oxidative stress on the sperm

(Adapted from www.clevelandclinic.org)

2.5.2 Oxidative stress And Spermatozoa DNA Fragmentation

Oxidative damage to DNA is a result of interaction of DNA with reactive oxygen species (ROS), in particular the hydroxyl radical. Super oxide and hydrogen peroxide are normally not reactive towards DNA. However, in the presence of ferrous or cuprous ion (the Fenton reaction), both superoxide and hydrogen peroxide are converted to the highly reactive hydroxyl radical. Hydroxyl radical produces a multiplicity of modifications in DNA. Oxidative attack by hydroxyl radical on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple a basic (AP) sites (Kow, 1999). In fact, one of the major types of damage generated by ROS is AP site, a site where a DNA base is lost (Kow, 1999). AP sites are also formed at an appreciable rate from spontaneous depurination. It is estimated that at least 10,000 depurination events occur per cell per day under physiological conditions (Kow, 1999). A similar amount of AP site is thought to be generated by normal aerobic respiration (Kow, 1999). In addition to AP site, a wide spectrum of oxidative base modification occurs with ROS. The C_4-C_5 double bond of pyrimidine is particularly sensitive to attack by OH radical, generating a spectrum of oxidative pyrimidine damage including thymine glycol, uracil glycol, urea residue, 5-OHdU, 5-OHdC, hydantoin and others. Similarly, interaction of hydroxyl radical with purines will generate 8-OHdG, 8-OHdA, formamidopyrimidines and other less characterized purine oxidative products (Kow, 1999).

It has been estimated that endogenous ROS can result in about 200,000 base lesions per cell per day (Kow, 1999). The biological consequences of many of the oxidative products are known. For example, unrepaired thymine glycol is a block to DNA replication and is thus potentially lethal to cells. On the contrary, 8-oxoG, an abundant oxidative damage to dG, is readily bypassed by the DNA polymerase

and is highly mutagenic. Unrepaired 8-oxoG will mispair with dA, leading to an increase in G to T transition mutations (Kow, 1999).

Reactive oxygen species (ROS) generated by spermatozoa play an important role in normal physiologic processes, such as sperm capacitation, acrosome reaction, oocyte fusion, and stabilization of the mitochondrial capsule in the midpiece (Said *et al.*, 2004). ROS produced by spermatozoa and leukocytes are scavenged by various antioxidants in the seminal plasma. Uncontrolled production of ROS that exceeds the antioxidant capacity of the seminal plasma leads to oxidative stress (OS), which is harmful to spermatozoa (Zalata *et al.*, 2004). In addition, ROS is an independent marker of male factor infertility.

2.6. PATHOPHYSIOLOGY OF NON-IONIZING RADIATION

2.6.1 Biophysics behind Cell phones

Cell phones have become indispensable devices in daily life recently (Agarwal *et al.*, 2008a). These phones operate at different frequencies, differing in respect to the frequency usage in different countries and in different continents. Concerns are growing about the possible hazardous effects of radio-frequency (RF) electromagnetic waves (EMW) emitted by these devices on human health. For years the cell phone companies have assured people that cell phones are perfectly safe. However, adverse effects of RF EMW emitted from cell phones on human and animal biological systems have been reported in the literature. Recent studies also suggest that EMW emitted from cell phones can reduce the fertilizing potential of men (Davoudi and Brossner, 2002; Kilgallon and Simmons, 2005; Erogul *et al.*, 2006; Agarwal *et al.*, 2009; Fejes *et al.*, 2009).

Exposure of RF energy depends upon the frequency of the cellular phone used. Most common cell phones (GSM: global system for mobile communication) work at 900–1900 MHz in the USA, whereas in most other parts of world these phones

work at 850–1800 MHz frequencies. The higher the frequency, the more energy they carry. Radiant energy is absorbed into human bodies by three main mechanisms: (i) aerial effect: body receives and absorbs the RF signal depending upon size of the body part and wavelength of signal; (ii) coupling of the RF signal with the tissue; and (iii) resonant absorption (D'Andrea *et al.*, 1985). The amount of RF energy absorbed from phones into local tissues, called the specific absorption rate (SAR), is the most useful quantity for assessing exposure from transmitters located near the body. SAR of cell phones varies from 0.12-to 1.6watts/kg body weight depending upon the model. In the USA, the upper limit of SAR allowed is 1.6 watts/kg (Federal Communications Commission, 1999).

2.6.2 Biological Effects of Radiations Emitted From Cellular

Radiations from several sources have been found to cause different biological effects. The use of cell phones has been demonstrated to cause dose dependent difficulty in concentration, fatigue and headache (Oftedal *et al.*, 2000), it sometimes can lead to increase in reaction time (Preece, 1999), alteration in electroencephalogram pattern and disturbance in sleep (Huber *et al.*, 2000). Exposure to cell phone has also been shown to increase resting blood pressure (Braune *et al.*, 1998). There has been a conflicting report on the effect of RF radiation on melatonin secretion by the pineal gland (Burch *et al.*, 1998; Seze *et al.*, 1999). According to current scientific evidence exposure to RF is unlikely to induce or promote cancer formation or spread. Most studies do not support the incidence of leukemia, brain tumours, testicular cancer, genitourinary and breast cancer with exposure to EMW (Moulder *et al.*, 1999; Colonna, 2005).

2.6.3 Cell phone use and semen quality

A lot of studies have suggested a link between cell phone use and infertility. A study by Agarwal *et al*. (2008a) indicated that the use of cell phones adversely affects the quality of semen by decreasing the sperm counts, motility, viability and morphology, which might contribute to male infertility. In a study by Fejes *et al.*

(2005) the duration of possession and the daily transmission time of cell phones correlated negatively with the proportion of rapidly progressive motile spermatozoa, suggesting that prolonged use of cell phones might have negative effects on the sperm motility. Similarly Davoudi and Brossner (2002) also found that using GSM phones for 6 h a day for 5 days decreased the rapid progressive motility of spermatozoa. Erogul *et al.* (2006) found a decrease in sperm motility in semen samples of 27 men exposed to 900 MHz cell phone in talk mode for 5 min. In a recent study, keeping cell phones close to the waist has been found to decrease sperm concentration as compared with men not using cell phones at all or storing it elsewhere (Erogul *et al.*, 2006).

2.6.4 Cell Phone Radiation and Spermatozoa

The role of cell phone radiation on the male infertility is still unclear although there are a lot of reports suggesting its influence on fertility. High intensities of RF radiation can lead to the generation of heat, an increase in the tissue or body temperature could then lead to the reversible disruption of spermatogenesis (Kandeel and Swerdloff, 1988; Saunders *et al.*, 1991; Jung and Schill, 2000; Wang *et al.*, 2003) suggested that Leydig cells are among the most susceptible cells to EMW and injury to these cells may affect spermatogenesis.

Exposure to 890–915 MHz cell phone, 3 min daily for 30 days, was observed by Dasdag *et al.* (1999) to decrease the mean seminiferous tubular diameter in rats. Ozguner *et al.* (2005) demonstrated a decrease in seminiferous tubular diameter and epithelium thickness after applying a radio-frequency generator of 869–894 MHz. However, a recent study Ribeiro *et al.* (2007) could not find any significant adverse effect of cellular phones (1835–1850 MHz) on rat testis. Several studies have demonstrated an increase in DNA fragmentation in a variety of human and animal cells (Lai, 1996; Stronati *et al.*, 2006; Panagopoulos *et al.*, 2007). Exposure to RF EMW 900MHz for 12 hours a day for 7 days has been found to significantly damage the mitochondria and nuclear genome in the epididymal spermatozoa of

mice (Aitken et al., 2005). Spermatozoa are extremely vulnerable to induction of DNA damage as they lose their cytoplasm, which contains anti-oxidant enzymes and their capacity for DNA repair after spermiation. This induction of DNA damage in spermatozoa has been associated with male infertility, early pregnancy loss and morbidity in the offspring and even childhood cancer (Aitken *et al.*, 2005). Currently there is limited information demonstrating DNA damage in sperm cells as a result of RF radiation exposure, however EMW has been shown to affect sperm motility (Erogul *et al.*, 2006; Fejes *et al.*, 2009) and sperm motility and sperm chromatin damage have a negative correlation

2.6.5 Cell Phone Use and Oxidative Stress

Spermatozoa are highly susceptible to damage induced by oxidative stress, but it is still debatable as to whether RF radiation can lead to oxidative stress. It has been found that high intensity microwave exposure stimulates lipid peroxidation in the hypothalamus of rats (Makker *et al.*, 2009). However, no alteration was found in the concentration of intracellular oxidants, glutathione concentration and antioxidant defences in the interferon and lipopolysaccharides stimulated cells on exposure to RF radiation fields (Hook *et al.*, 2004). Conflicting studies have also been published regarding the effect of EMW exposure on the secretion of an antioxidant melatonin (Lipovac, 2000; Burch *et al.*, 2002). The effects of RF radiation on human sperm cell apoptosis have not yet been evaluated.

2.7 Natural Antioxidants Involved In Spermatozoa Protection

The antioxidant defense system in most cells is composed of two components, the antioxidant enzymes component which includes enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and the low molecular weight antioxidants component that includes vitamins A and E, ascorbate, glutathione and thioredoxin. These substances are the body's natural defense against endogenous generated ROS and other free radicals, as well as ROS generated by external environmental factors.

2.7.1 Superoxide dismutase and its assay

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism (Potts *et al.*, 1999). Three different forms of SODs have been identified according to their metal content: copper/zinc (Cu/Zn), manganese (Mn), and iron (Fe). SOD is widely distributed in both plants and animals. It occurs in high concentrations in brain, liver, heart, erythrocytes, semen and kidney. In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial MnSOD, and extracellular SOD (Ochsendorf, 1999). Extracellular SOD is found in the interstitial spaces of tissues and also in extracellular fluids, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid (Gomez *et al.*, 1996).

The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress. The reaction catalyzed by SOD is extremely fast, having a turnover of $2x10^9$ M-1sec-1 and the presence of sufficient amounts of the enzyme in cells and tissues typically keeps the concentration of superoxide radical very low (El-Tohamy, 2012). However, in a competing reaction, nitric oxide (NO) reacts with O_2^- with a rate constant of 6.7 x 10^9 M-1sec-1 to form the powerful oxidizing and nitrating agent, peroxynitrite.

It is generally supposed that superoxide radical is dismutated spontaneously to hydrogen peroxide due to the high local hydrogen ion concentration, and that the highly permeable peroxide diffuses out of the digesting organelle to be handled by the antioxidant machinery. The mitochondrion's electron transport and possibly other, as yet undefined, processes can produce additional amounts of superoxide radical. But not less importantly, superoxide radical can be produced by the reticulo-endothelial system. Exogenous superoxide radical or hydrogen peroxide are most likely dealt with the host cell Cu, Zn-SOD (that produce hydrogen

peroxide) and by catalase, which reduces the peroxide to water and molecular oxygen. Superoxide radical and hydrogen peroxide are also generated by the endoplasmic reticulum-resident oxidoreductin.

2.8 Sperm Chromatin Dispersion Assay Test

Different methods may be used to evaluate the status of the sperm chromatin for the presence of abnormalities or simply immaturity. These assays include simple staining techniques such as the acidic aniline blue (AAB) and toluidine blue (TB) stains, fluorescent staining techniques such as the sperm chromatin dispersion (SCD) test, chromomycin A3 (CMA3), DNA breakage detection– fluorescent *in situ* hybridization assay (DBD–FISH), *in situ* nick translation (NT), and flow cytometric based sperm chromatin structure assay (SCSA). Some assays employ more than one method for the analysis of their results. Examples of these assays include the acridine orange (AO) and terminal deoxynucleotidyl transferase-mediated fluorescein deoxyuridine triphosphate-nick end labeling (TUNEL) assays. Other methods less frequently used include high-performance liquid chromatography (HPLC).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was done at a fertility center in Sakumono, Tema metropolis. The Tema Metropolis District is one of the ten (10) districts in the Greater Accra Region of Ghana. Its capital is Tema. This metropolis is grouped into twenty-six communities. The most popular and busiest communities are Communities 1, 2, 4, 7, 8, 9, and 13 (Sakumono).

3.2 Study design

This work was an experimental study. The study subjects were both men and their spermatozoa. The test spermatozoa samples were exposed to mobile radiation and the control spermatozoa samples were the non-exposed. All samples were incubated at the same temperature (37ºC) in different cell culture incubators.

3.3 Study Population

3.3.1 Inclusion

All men between 18 to 50 years reporting at the selected fertility centers for semen analysis without any history of diabetes, drug addiction or any S.T.D. infection and any other medical condition that may affect spermiogenesis. Men who presented with normal semen analysis per WHO standards were included.

3.3.2 Exclusion

Ages below 18 and above 50 years, history of diabetes or any S.T.D. infection in the past six month or addicted to drugs and alcohol were excluded from the research.

3.4 Sampling Method

Sampling was conveniently done in the hospital. The systematic random sampling was used base on the average annual population (N) of men coming for semen analysis and the calculated sample size (n) using the formula k=N/n

3.4.1 Sample size calculation

$$n = \frac{\frac{z^2 P(1-P)}{d^2}}{1 + \frac{1}{N}\left(\frac{z^2 P(1-P)}{d^2} - 1\right)}$$

Using the formula: n =

Where n is the sample size, z is the confidence interval (95%= 1.96), p is the proportion of estimate for people exposed to mobile phone radiation with defect in sperm analysis in Ghana (=0.50), q is the proportion of estimate for people who are not exposed to mobile phone radiation with defect in sperm analysis in Ghana, d is the allowable discrepancy between the proportion estimated from the sample and the true population proportion P (= 0.12%). A total of seventy-three (73) men were recruited for the study.

3. 5. Anthropometric and Demographic Assessment by Questionnaire From the administered questionnaire the heights (in meters) were measured without shoes, along with the total body weight (in Kilograms) to obtain the body mass index of the participants. Demographic information about participants' living near a telecom mast, the type (analog or smart) and number of mobile phones used. Information on marital status, occupation, alcohol intake, and smoking and whether their phones were kept in their pocket on were elucidated using a questionnaire (see appendix)

3. 6 Instructions and Semen Sample Collection

The subjects were given clear written and oral instructions concerning the collection. This information was given at least three days prior to the collection of the semen.

The samples were collected after a 2-4 day of sexually abstinence to make enough room for a complete cycle of spermatogenesis to reduce the variability of the results (WHO, 2010). The number of days of sexual abstinence was maintained constant whenever possible. Participants were also educated on how the effects of smoking,

alcohol intake, aphrodisiacs and other stimulants such as caffeine could have on the sperm or semen quality (Zhang *et al.*, 2009; Mann and Lutwak-Mann, 2012). All participants were given verbal as well as written information about the purpose of the investigation and other important facts in order to prevent conditions that could confound the results and final inferences from the investigations. The period of abstinence, the date of collection, completeness of collection, difficulties in producing the samples, the interval between collection and analysis were recorded on the form that accompanied each semen sample for analysis, whenever possible (WHO, 2010). The samples were produced in a private room at the hospital by masturbation and brought to the laboratory immediately after collection.

Participants ejaculated into clean pre-weighed, non-toxic, wide-mouthed sterile plastic semen containers (Shivani Scientific Industries Pvt. Ltd, Mumbai India. Samples were placed in a Galaxy 14 R CO_2 incubator (New Brunswick Scientific, USA) without delay at (37^{o}C) to keep the samples at the normal body temperature after the ejaculated time was noted.

The samples were analyzed after gentle and uniform mixing within 60 minutes after semen liquefaction. For each sample, ejaculate volume, sperm concentration, total sperm count, motility and morphology were evaluated according to WHO guidelines on semen examination (WHO, 2010). The normozoospermic or quality samples were identified and coded as A1, A2 to A73. Letter 'A' for fresh or neat sample was use as a label for those samples that were selected from a pool of samples for the work. Subsequently letters 'B' and 'C' were used respectively for control and exposed samples after each initial baseline analysis on the fresh or neat sample.

3. 6.1 Sample collection/ processing

About 2-3mls of freshly ejaculated semen specimen neatly collected by

masturbation into a sterile semen container after 2-4 days abstinence was used. The standard semen analysis by WHO was used to run the initial analysis on all the selected samples. Each semen sample was aliquot into three micro tubes.

3. 6.2 Sample Analysis

The semen sample was analyzed for concentration, motility, viability, morphology and pH by using; macklar counting chamber, vital stain, morphological stain, and pH strips. Apart from the superoxide dismutase (SOD) activity assay kit from Cayman Chemicals, Michigan- USA, all the other reagents used were commercially prepared from Sperm Processor Private Ltd., Aurangabad India. Products from these companies were all CE marked and ISO certified.

3.7 Macroscopic Examination of Semen

3.7.1 Liquefaction

Each sample was examined for its duration of liquefaction, i.e., liquefaction time. Normal semen sample would liquefy within 60 minutes at a temperature of 37^{o}C, although usually this occurs within fifteen minutes (World Health Organisation, 2010). The liquefaction time was determined by placing the sample in a Galaxy 14 R CO_2 incubator (New Brunswick Scientific, USA) at 37^{o}C. The time a sample was produced till the time it became completely liquefied was noted on the record form. The containers with the specimens were then taken from the incubator and swirled for 20 seconds to ensure that the samples were well mixed. Continuous mixing of the samples was ensured to reduce the errors that could have been created when determining the sperm concentration (World Health Organisation, 2010).

3.7.2 Appearance

The seminal appearance was determined immediately after liquefaction. This was done by first inspection of the colour of the sample at room temperature. Varying visual appearance of the samples such as colour, opalescence or clearness and presence of gel particles or mucous streaks were noted. The observations of the

semen samples were grouped under two main categories: Normal and Abnormal. The abnormally coloured samples were excluded from the work (Potts *et al.*, 1999).

3.7.3 Volume of Semen

Seminal volume was determined for each sample by using a digital weighing scale to weigh the pre-weighed container together with the specimen and the weight in gram (g) converted to volume in milliliters (ml) as in g/ml. Since the weight of the container was known, the volumes were then calculated and recorded with the assumption that the density of semen is 1g/ml (World Health Organisation, 2010).

3.7.4 Viscosity of Semen

A clean dried glass rod was introduced into each of the samples in their original labeled specimen-container after 30 minutes to check the liquefaction. The thread that formed upon withdrawing the rod was observed carefully and recorded for each sample. Based on the viscosity of the samples, they were classified as low, normal, high and very high. A normal sample displayed a thread of length about 2cm upon withdrawal of the glass rod from the sample. Threads that were more than 2 cm were classified as slightly high in viscosity. Samples that formed threads that were more than 3 cm were classified as having high viscosity. Samples that showed threads that were less than 2 cm were classified under low viscous semen (World Health Organisation, 2010).

All samples with very high viscosity were noted on their respective sample record form. High viscosity interferes with determination of sperm motility and concentration (Potts *et al.*, 1999). 1 to 2 ml of phosphate-buffered saline (PBS) at pH of 7.2 was added, depending on the volume of the initial volume of the semen, and carefully mixed with a wide bore pipette to give a homogenous dilution for examination. The original volume was calculated by multiplying it by the dilution factor and used to express the original sperm concentration in undiluted semen.

3.7.5 Semen pH

Special kit; ready to use pH kit

Procedure:

Semen pH strips were labeled with appropriate sample code. The semen pH strips were laid on a flat surface facing yellow circle upwards. A drop (about 10µls) of liquefied semen was placed on the yellow circle with the help of a pipette.

Examination:

Change in colour was observed after about **45-50** seconds of **step - 3**, and was compared with the color chart printed on the strip.

Results' Interpretation:

The compared color match denoted the pH of the sample semen. Normal reference value / range: 7.2 to 8.5

3.8 Microscopic Examination

3.8.1 Motility and concentration using Makler counting chamber

The sperm motility and concentration determination was done using the Makler counting chamber (Sefi medical instruments limited, Haifa Israel). The Makler chamber utilizes undiluted semen sample. The number of spermatozoa counted in any strip of 10 squares of the grid (out of the 100 squares of grids) gives the concentration in millions/ml. The depth of 10 microns prevents blurring and allows free movement of spermatozoa to aid in estimation of motility. The dropped sample is observed in one focal plane. The counting grid is embossed on the cover glass, (which eliminates the need to insert a grid into the microscope eyepiece and remove it when not required). Before assessing the concentration of each of the semen, the samples were allowed to liquefy by allowing them to stand for 10minutes to an hour in the Galaxy incubator at 37^{o}C.

3.8.2 Motility

Sperm motility was the first microscopic examination that was carried out on the semen samples to prevent other unfavorable conditions that might affect sperm motility as a result of sperm metabolism (WHO, 2010). This assessment began immediately after the flow ceased to avoid temperature drop or dehydration of the preparation (WHO, 2010). The slide was focused with the ×10 objective lens and the condenser iris sufficiently adjusted to obtain a sufficiently good contrast. If the preparation under ×10 gave an even distribution, then with the aid of ×40 objective and eyepiece reticule that was used in conjunction with the microscope eyepiece, several fields were assessed for motility. The total motile sperms observed were scored as percentages; Percentages of motile and non-motile fractions were then recorded based on the following classification.

The motility of the spermatozoa were graded A, B and C according to whether it showed;

a) Rapid progressive motility (that is, greater or equal to 25µm/s at room temperature. 25µm is approximately equal to 5 heads lengths and half a tail length)

b) Slow progressive and non-progressive motility (i.e. from 5µm/s to 25µm/s and less than 5µm/s respectively at room temperature).

c) Immotility (IM)- no movement

3.8.3 Concentration

The Makler counting chamber was cleaned gently and a drop of the semen sample approximately 10-20µl using a disposable sterile Pasteur pipette (Sigma-Aldrich, UK) was placed on the chamber and a cover slip placed on it.

The chamber was then placed on the stage of a light microscope CX21 (Olympus corporation, Japan) using the 10x objective the sample was brought into focus by

the adjustment knobs. The number of sperms present in a standard number of grids of 10 squares were counted and calculated as millions/ml in concentration.

3.8.4 Vitality by Vital Staining

Special Kit: Ready to use Eosin-Nigrosin Solution.

Procedure:

Plastic ware & disposable materials were labeled with appropriate sample code. 50µls of Eosin-Nigrosin dye solution was taken and added to 10µls of undiluted, well-mixed, liquefied semen. The dye and the sample were mixed thoroughly with the help of a vortex at a lower speed and allowed to stand for 30 seconds. After **30** seconds, 10µls of sample from step 2 was placed on a clean glass-slide to prepare a smear. The smear was allowed to air-dry with the help of the slide warmer.

Examination:

The smear was examined under the microscope with the X40 and then the oil immersion (X100) lens. At least 200 sperm were examined and the following were counted; unstained/white sperm (indicative of live sperm). Red/dark pink sperm (indicative of dead sperm).

Results' Interpretation:

The percentage of live sperm was calculated. Normal reference value/range: 58% (55%- 63%).

3.8.5 Morphology by Diff-Quick

Special kit: Ready to use staining solution

· Fixative Solution

· Stain - I Solution ·

Stain - II Solution

Procedure:

Plastic wares and disposable materials were labeled with appropriate sample code. Five microliter (sperm concentration greater than 20 millions/ml) or 10µl (sperm concentration less than 20 millions/ml) of liquefied semen sample was placed on pre-cleaned glass slide and a uniform smear was made allowed to air dry. The smear was laid horizontally and the entire smear surface was then covered with 1ml of fixative solution and kept for 15 seconds. The fixative solution was drained off and the smear was allowed to air-dry.

The smear was laid horizontally and the entire smear was then covered with 1 ml of stain - I solution for 10 seconds. Stain - I solution was drained off after the 10 seconds. The smear was laid horizontally and the entire smear was then covered with 1 ml of stain - II solution for 15 seconds. Stain - II solution was drained off after the 15 seconds. The smear was rinsed in distilled water and the back of the slide cleaned with filter paper.he smear was allowed to air-dry.

Examination:

- The smear was examined under the microscope with the help of 100× lens.
- At least 200 sperm were examined and the following were counted as per Kruger's strict criteria / WHO criteria:

- Normal Sperm

- Abnormal sperm

In abnormal sperm, detailed abnormality (defects) pertaining to head, mid piece and tail were noted down.

Results' Interpretation:

The percentages of normal and abnormal sperm were calculated.

3.9 Incubating the Semen Samples (control and Exposed)

The Galaxy 150i CO_2 incubators (New Brunswick Scientific, USA) were used to incubate the control and the exposed samples. The incubators were placed in

different rooms with a distance of about 20 feet apart and also with concrete walls partitioning the rooms. Prior to the beginning of the work, incubators were tested with different set of trial semen samples of known motility, vitality and superoxide dismutase activity (SOD) as well as the DNA fragmentation index (DFI). No significant differences were observed between the two incubators in the results obtained from the different samples for both unexposed and exposed, which were aliquot into two parts for each incubator. Initial samples were incubated in each incubator without the mobile phone for three hours. The same was repeated for different set of trial samples with the mobile phone for the same incubator 1 and 2.

Samples B and C were incubated in incubator 1 and 2 respectively at 37°C for three hours after the baseline analysis, measurement of superoxide dismutase (SOD) and DNA Fragmentation Index (DFI) were estimated on the neat or coded sample A.

Sample B was without phone whereas sample C was with a mobile phone with known specific absorption rate (SAR), maximum power, frequency and power density at a distance of 2.5cm from the samples in the standard CO_2 incubator. Samples B and C were analyzed again after the three hour incubation as was done for the initial samples.

3.9.1 Source of Radiofrequency Electromagnetic Waves (RF-EMW)

3.9.1.1 Exposure of Semen Samples to Electromagnetic Waves

One aliquot of each divided semen sample was exposed to EMW emitted from a commercially available mobile telephone in standby and data mode. The Samsung galaxy Y cell phone operating on the Vodafone frequency was used. The measured frequency was 947.6MHz with average receiving and transmitting powers of 24.78 **(**μW) and 21.74 (μW) respectively. The SAR for both receiving and transmitting were also 3.29 (W/Kg) and 2.89 (W/Kg) respectively. The distance between the phone antenna and specimen was kept at 2.5cm. The duration of exposure was 180 minutes.

1.9.1.2 Power Density (μW/cm2)

According to the International Commission for Non-ionizing Radiation Protection (ICNIRP 1998) and the Federal Communication Commission (FCC 1998), the reference level for exposure of RF-EMW is peak power density. It is a commonly used term for characterizing an RF electromagnetic field. Power density was monitored in control condition (no cell phone radiation) and experimental condition (cell phone in standby and data mode) in the laboratory throughout the experiment using a power meter (Anritsu Power Sensor, model MA 24126A, Anritsu Corporation, Kanagawa Japan) at the Ghana Atomic Energy Physics Laboratory. Power density in the control condition was 0.01 to 0.1μW/cm^2. Power density in the experimental condition (cell phone in data mode and the samples at 2.5cm from cell phone antenna) was 1 to 40μW/cm^2.

3.9.1.3 Frequency and Temperature

The frequency emitted by the cell phone was confirmed to be 947.6MHz with the help of a radio frequency spectrum analyzer (Kaltman Creations LLC, Georgia, USA). Both specimens (aliquots) were kept at 37^{o}C to mimic the normal reactions in the human body taking into consideration the effect of temperature on ROS formation and semen parameters as baseline analysis on all samples were measured.

3.9.2 DNA Fragmentation Index (DFI) test

Technique used:

Aliquots of either raw or washed semen samples should be adjusted to concentrations ranging between 5 and 10 million/ml. Fifty microliters of the suspensions are mixed with 1% low-melting-point aqueous agarose (to obtain a 0.7% final agarose concentration) at 37°C. Aliquots of 50μl of the mixture should be pipetted onto a glass slide pre-coated with 0.65% standard agarose dried at 80°C, covered with a coverslip, and left to solidify at 4°C for 4 minutes. The coverslips are then carefully removed, and the slides are immediately immersed

horizontally in a tray of freshly prepared acid denaturation solution (0.08N HCl) for 8 minutes at 22°C in the dark, which generates restricted single-stranded DNA (ssDNA) motifs from DNA breaks. Denaturation is then stopped, and the proteins are removed by transferring the slides to a tray with neutralizing and lysing solution 1 (0.4 mol/l Tris, 0.8 mol/l DTT, 1% SDS, and 50 mmol/l EDTA, pH 7.5) for 10 minutes at room temperature. The slides are then incubated in neutralizing and lysing solution 2 (0.4 mol/l Tris, 2 mol/l NaCl, and 1% SDS, pH 7.5) for 5 minutes at room temperature. The slides are thoroughly washed in Tris-borate–EDTA buffer (0.09 mol/l Tris-borate and 0.002 mol/l EDTA, pH 7.5) for 2 minutes, dehydrated in sequential 70%, 90%, and 100% ethanol baths (2 minutes each), and air-dried. Cells are stained with DAPI (4′, 6-diamidino-2-phenylindole) (2 μg/ml) or Wright's stain for fluorescence microscopy and light microscopy respectively.

3.9.2.1 Principle of Sperm Chromatin Dispersion Assay Test

If spermatozoa with non-fragmented DNA are immersed in an agarose matrix and directly exposed to lysing solutions, the resulting deproteinized nuclei (nucleoids) show extended halos of DNA dispersion as monitored by fluorescent microscopy or light microscopy depending on the type of stain used. The presence of DNA breaks promotes the expansion of the halo of the nucleoid (Fernández *et al.*, 2011). The SCD test is based on the principle that when sperm are treated with an acid solution prior to lysis buffer, the DNA dispersion halos that are observed in sperm nuclei with non-fragmented DNA after the removal of nuclear proteins are either minimally present or not produced at all in sperm nuclei with fragmented DNA (Fernández *et al.*, 2011).

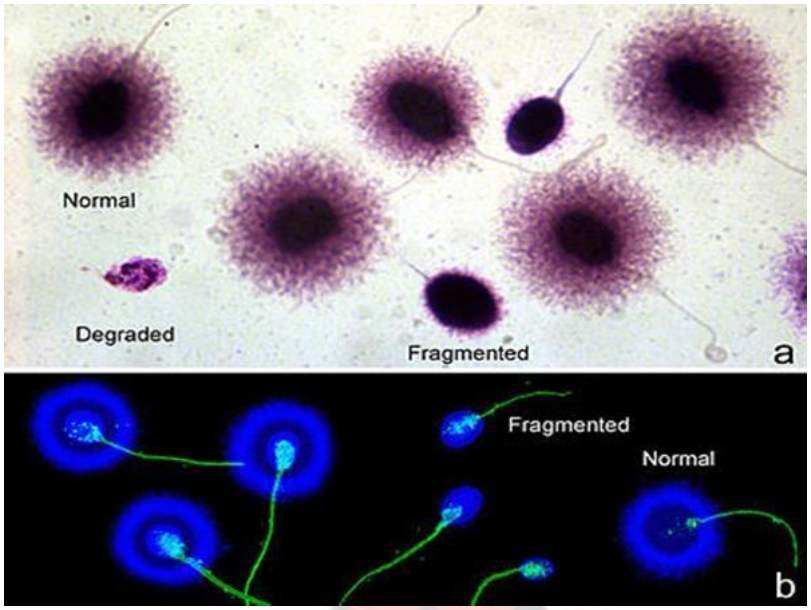


Figure 3.1: Sperm Chromatin Dispersion Assay Test showing normal and fragmented sperm cells

Advantages

The major advantage of the SCD test is that it does not require the determination of color or fluorescence intensity. Rather, the percentage of spermatozoa with nondispersed (very small halos or none at all) or dispersed nuclei is determined, which

can be easily and reliably accomplished by the normal light microscope. Furthermore, the test is simple, fast, and reproducible, and its results are comparable to those of the SCSA.

Special Reagents:

Ready to use sperm chromatin dispersion assay kit

Pre-coated glass slides (agarose gel)

Coverslips

· Tubes with low melting Agarose gel

· Reagent – I: denaturing solution

· Reagent – II: lysing and neutralizing solution

· Reagent – III: ethanol (70%, 90% and 100)

· Reagent – IV: Wright's staining solution and buffer

Procedure:

Plastic wares and disposable materials were labeled appropriately and reagents were brought to room temperature before use. The semen samples were prepared by diluting them with normal saline to achieve a sperm concentration of 10-20 millions/ml. Agarose tubes and pre-coated slides were prepared based on the manufacturer's instructions. The pre-coated slides with the embedded spermatozoa were exposed to the reagent-I by dipping the prepared slides in the reagent-I for 8 minutes. The slides were then dipped in reagent II for 20 minutes and then distilled water for 5 minutes. For each step, the backs of the slides were cleaned with a filter paper. The smears were then dehydrated and fixed using ethanol in a progressive manner (70%, 90%, 100%) for 2 minutes at each concentration. The fixed smears were then stained using the Wright staining technique.

The smears were then allowed to air-dry with the help of the slide warmer.

Examination;

About 200-400 sperms were examined and the following counted; sperm without fragments DNA (large hallo), sperm with fragments DNA (small or no hallo) and degraded sperm

Results' Interpretation:

The following were calculated;

· Percentage of Sperm with Fragmented DNA

· Percentage of Sperm without Fragmented DNA

$$\text{DFI \%} = \frac{100 \times (\text{No. of sperm with fragmented DNA} + \text{No. of Degraded Sperm})}{\text{No. of sperm observe}}$$

Normal reference value / range: DFI % < 25%.

3.9.3 Control Slides for DNA fragmentation

Control slides were prepared by using H_2O_2 to induce single stranded DNA breaks at different concentrations on three different slides. A known normal semen sample with DFI of <25% was used to prepare three different slides and each was treated and incubated for 30 min at room temperature with a different concentration of hydrogen peroxide (0%, 0.03% and 0.15%) prepared from a 30% concentration of stocked hydrogen peroxide (Sigma Aldrich). After treating the three slides respectively with the specific diluted concentration of the hydrogen peroxide, the lysing, denaturing and dehydration steps including the Wright staining procedure were done to obtained the control slides.

3.9.4 Superoxide Dismutase Assay

The SOD activity levels were determined by a colorimetric method, using SOD Assay kit (Cayman Chemicals, Michigan- USA) and ELISA reader machine URIT660 (URIT Medical Electronic Group Co. Ltd., Guilin-China) The seminal plasma (SP) were obtained from each of the aliquot samples from the neat, unexposed and exposed sample (A, A1, A2) after centrifugation with the spermfuge (a special temperature regulated centrifuge for semen preparation from Shivani Scientific Pvt. Ltd., India) at 1500rpm, 10mins at 4^{o}C). The SP of each sample were carefully separated and stored at -80^{o}C which were to be stable for at least one month according to the manufacturer. The supernatant fluid was then diluted by a factor of 30 with sample buffer, and 10 µl of the diluted solution used to measure all the SOD (Cu/Zn-SOD (SOD1), Mn-SOD (SOD2) and extra cellular-SOD (SOD3)) activities per sample as described by the manufacturer (Cayman Chemicals, Michigan, USA).

3.9.4.1 Principles for Measurement of Superoxide Dismutase

Cayman's Superoxide Dismutase Assay Kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (see scheme 1, below).

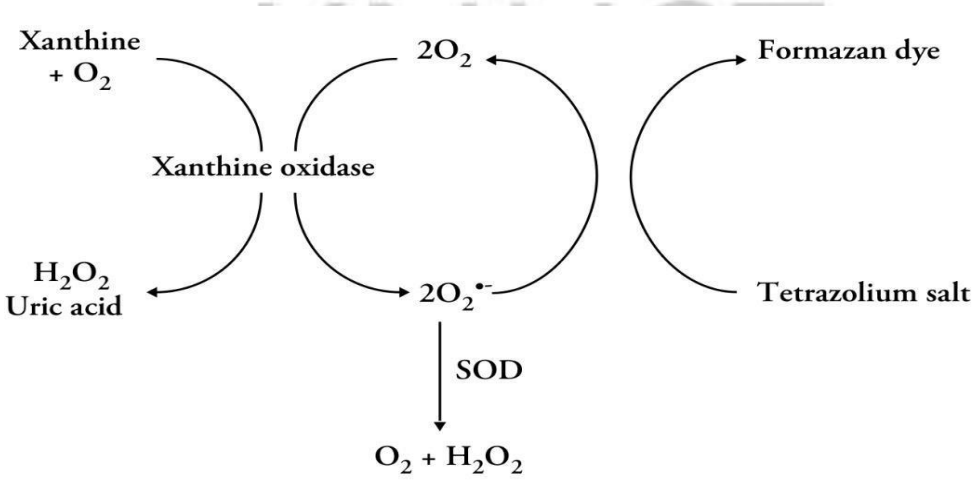


Figure 3.2: Principle for measurement of SOD3.9.4.2 Superoxide dismutase (sod) protocol for seminal plasma assay

a) Reagents

i. Assay Buffer (10X) ii. Sample Buffer (10X) iii. Radical Detector iv. SOD standard

b) Reagent Preparation Assay Buffer (10x)

Dilute 3mls of assay buffer concentrate with 27mls of HPLC-grade water (or de-ionized distilled water) for assaying 96 wells. This final assay buffer was used to dilute the radical detector. Stored at 4°C and was to be stable for at least two months.

 Sample Buffer (10x)

Dilute 2mls of sample buffer concentrate with 1.8mls of HPLC-grade water (or de-ionized distilled water) for assaying 96 wells. This was used to prepare the SOD standard and dilute the xanthine oxidase and SOD samples prior to assaying. Stored at 4°C and was to stable for at least two months.

 Radical Detector

Prior to use, 50 µl of radical detector was transferred to another vial and dilute with 19.95mls of diluted assay buffer for 96 wells. Cover with foil. The diluted radical detector was stabilized for two hours. Unused radical detector was stored at -20°C.

 SOD Standard

20µl of the SOD Standard was diluted with 1.98ml of sample buffer (dilute) to obtain the SOD stock solution. Seven clean glass test tubes were taken and marked them A-G. Addition of the amount of SOD stock and sample buffer (dilute) to each tube was done as described in manufacturers manual.

 Xanthine Oxidase

Prior to use, one vial was thawed and 50µl of the supplied enzyme was transferred to another vial and diluted with 1.95ml of sample buffer (dilute) for 96 wells. It was stored on ice to stabilize for one hour.

c) Sample preparation (Seminal Plasma)

The semen samples were aliquot into 1.5ml eppendorf tubes and labeled accordingly.

The samples were centrifuged at 1,000rpm for 10 minutes at 4°C. Seminal Plasma (SP) were stored on ice until assaying. The SP samples were to be stable for at least

one month when stored at -80°C. The SP was diluted 1:30 with Sample buffer before assayed for SOD activity.

d) Performing the Assay

SOD Standard Wells: 200 µl of the diluted Radical Detector was added and 10 µl of Standard (tubes A-G) per well in the designated wells on the plate. Sample Wells: 200 µl of the diluted Radical Detector was added and 10 µl of Sample to the wells. The reaction was initiated by adding 20 µl of diluted Xanthine Oxidase to all the wells used.

The 96- well plate was carefully shaken for a few seconds to mix and covered with the plate cover. The plate was on a shaker for 20 minutes at room temperature. The absorbance was taken at 440- 460 nm using a plate reader.

e) Calculations

The average absorbance of each standard was calculated and sample (if assay was done in duplicates). After assayed, sample absorbance was subtracted from the sample.

Standard A's absorbance was divided by itself and standard A's absorbance was divided by all the other standards and samples absorbance to yield the linearized rate (LR)

The linearized SOD standard rate (LR) (from step 2 above) was plotted as a function of final SOD activity (U/ml).

The SOD activity of the samples was calculated using the equation obtained from the linear regression of the standard curve substituting the linearized rate (LR) for each sample.

$$\text{SOD (U/ml)} = \left[\left(\frac{\text{sample LR - y-intercept}}{\text{slope}}\right) \times \frac{0.23\ \text{ml}}{0.01\ \text{ml}}\right] \times \text{sample dilution}$$

3.9.5 Standard Preparation

The standards were prepared to calibrate the ELISA reader for direct concentration reading after the incubation period. The same standards were used as control samples alongside the test samples since their concentrations were known.

20 µl of the SOD standard was diluted (provided by manufacturer) with 1.98 ml of Sample Buffer (provided by manufacturer) to obtain the SOD stock solution. Seven clean glass test tubes were labeled A-G, and the concentrations were prepared as described in Table1 below.

Table 3.1: Prepared SOD Standards for Calibration of ELISA Plate Reader

Tube	SOD Stock (µl)	Sample Buffer (µl)	Final SOD Activity (U/ml)
A	0	1000	0
B	20	980	0.025
C	40	960	0.05
D	80	920	0.1
E	120	880	0.15
F	160	840	0.2
G	200	800	0.25

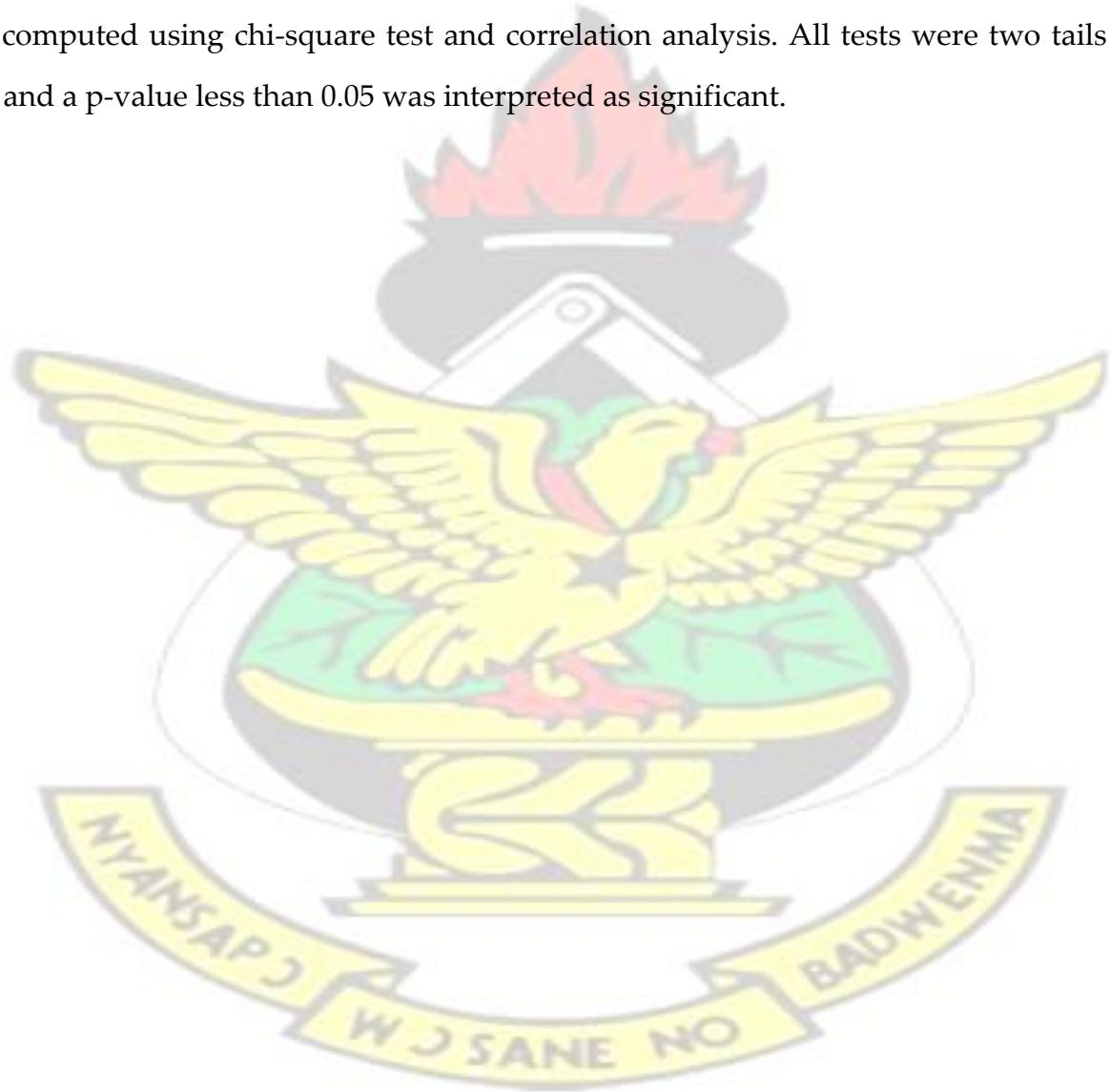
3.9.6 Ethical Consideration

Ethical Clearance was obtained from the committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Science (SMS), Kwame Nkrumah University of Science and Technology (KNUST), and Jubail Specialist Hospital. The consent of all research participants (i.e. clients who participated in the study) was obtained. Information was presented to enable persons to voluntarily decide whether or not to participate as a research participant. Consent forms were given to all research participants to sign, and they were informed that they had the option of opting out if they so wished. A questionnaire covering some specific demographic parameters and participant's state of health were

administered to enable us obtain the true status of their health.

3.9.7 Data Analysis

Data obtained was cleaned and analyzed using Statistical Program for Social Science (SPSS) version 20.0. Data was summarized as frequencies, percentages, means and standard deviations. The effects of the independent variables on the dependent variable were assessed using generalized linear models and logistic regression. Association between dependent and independent variables was computed using chi-square test and correlation analysis. All tests were two tails and a p-value less than 0.05 was interpreted as significant.



CHAPTER 4

4.0 RESULTS

4.1 Socio-Demographic Characteristics and Anthropometric of the Participants

Table 4.1 shows socio-demographic characteristics and anthropometric of the participants. A total of seventy-three participants were enrolled. The mean age of the participants in this study was 38.81 ± 5.87years. A higher number (28.8%) of the participants were between the ages of 36-40years. The mean BMI, height, weight and number of days for abstinence were 1.72m ± 0.048, 76.70Kg ±25.91, 25.91Kg/m^2 ± 2.42 and 2.973 days ±0.572. All participants (100%) in this study were married and majority of them (86.3%) had completed tertiary education. A higher proportion of participants (78.08%) had one (1) phone and none of them (100%) resided close to a telecom mast.

Table 4. 1: Socio-Demographics characteristics anthropometrics of the participants

Variable	Frequency	Percentage (%)
Age (years) (Mean±SD)	38.81 ± 5.87	
Age Groups (years)		
25 – 30	5	6.85%
31 – 35	16	21.92%
36 – 40	21	28.77%
41 – 45	15	20.55%
46 – 50	16	21.92%
Height (m) (Mean±SD)	1.72 ± 0.048	
Weight (Kg) (Mean±SD)	76.70 ± 25.91	
BMI (kg/m^2) (Mean±SD)	25.91 ± 2.42	
Educational Level		
Primary	2	2.74%
Secondary	8	10.96%
Tertiary	63	86.30%
Marital Status		
Married	73	100%
Single	-	
Proximity of Telecom Mast (50 meters radius)		
Yes	-	
No	73	100%
Type of Phone		
Analog	2	2.74%
Smart	71	97.26%
Keeping Phones in Front, Pockets of Pants		
No	12	
Yes	61	
No. of Phones in Pockets		
1	57	78.08%
2	13	17.81%
3	3	4.11%

No. of Days of Abstinence (Mean±SD)	3.06±0.24

SD = Standard Deviation, BMI= Body Mass Index

Table 4.2 shows comparison of progressive motility, non-progressive motility, immotility, Total motility, vitality, SOD and DFI of semen between before exposure, after exposure at 37^0C and after exposure to RF-EMW and 37^0C from a mobile phone. There was a significant reduced median percentage sperm progressive motility and vitality in the order: before exposure> exposure after 37^0C >after exposure 37^0C and RF-EMW ($p < 0.0001$). Median percentage (%) of Superoxide dismutase concentration was significantly lower in order before exposure> exposure after 37^0C >after exposure 37^0C and RF-EMW ($P<.0001$). An increase in mean percentage (%) DFI was observed in the order: before exposure> exposure after 37^0C >after exposure 37^0C and RF-EMW. The difference between median percentage DFI in control and before exposure was statistically significant ($p = 0.002$). There was a significant increase of median progressive percentage in order before exposure> exposure after 37^0C >after exposure 37^0C and RF-EMW from mobile phones ($p<0.0001$).

Table 4.2: Comparison of progression, Non-progression, vitality, SOD, DFI of semen between Neat (A), Control (B) and after exposure to RE-EMW at 37°C from a mobile phone (C)

Variables	Neat (n=73) (A)	37°C (n=73) (B)	RE-EMW +37°C (n=73) (C)	Significant Pairs			
				P-value	B vs. C	A vs. B	A vs. C
Progression (%)	3 (9.0, 16.65)	10.5 (6.10, 14.50)	7.2 (3.00, 11.10)	**<0.0001**	<0.0001	0.0001	<0.0001
Non-Progression (%)	53 (48.15, 57.50)	55 (50.90, 59,10)	56.70 (48.14, 57.51)	**0.0083**	ns	ns	0.0057
Vitality (%)	70.4 (65.00, 70.40)	68.7 (63.30, 76.55)	66 (61.90, 75.00)	0.2123	0.0001	ns	ns
Total Motility (%)	66.0. (60.70, 74.00)	65.40 (60.25, 72.40)	63.80 (58.50, 70.80)	**<0.0001**	<0.0001	0.0001	<0.0001
Immotility (%)	34.00	34.60	36.20	**<0.0001**	<0.0001	0.0001	<0.0001

	(26.00, 39.30)	(27.60, 39.75)	29.20, 4.50)				
SOD (U/ml)	5.58	5.23	4.18	**<0.0001**	<0.0001	<0.0001	<0.0001
	(4.49, 6.50)	(4.02, 6.23)	(3.21, 5.315				
DFI (%)	33.55	34.85	36.83	**<0.0001**	<0.0001	< 0.0001	<0.0001
	(25.90, 42.90)	(27.78, 45.03)	(30.40, 46.95)				

ns = not significant, SOD = Superoxide Dismutase, DFI = DNA Fragmentation Index

Table 4.3 shows Correlation between SOD, DFI, pH, Progressive motility (%), Non-progressive motility (%), Total Motility (%), Immotility, Vitality, and Age of participants before exposure to RF-EMW from a mobile phone. There was a significant positive correlation between SOD and progressive motility ($r = 0.439$; $p<0.0001$), non-progressive motility ($r = 0.578$; $p<0.0001$), morphology ($r =0.578$ $p < 0.0001$). DNA fragmentation index (DFI) was negatively significantly correlated with progressive motility (%) ($r = -0.434$; $p <0.0001$), non-progressive motility (%) ($r = -0.529$ $p < 0.0001$), vitality (%) ($r = -0.528$; $p <0.0001$) and SOD (U/ml) ($r = -0.900$; $p <0.0001$). Sperm counts (($\times 10^6$sperm/ml) is positively associated with progressive motility ($r = 0.390$, $p = 0.001$), non-progressive motility (%) ($r = 0.287$ $p = 0.014$) and morphology ($r = 0.390$; $p = 0.001$).

Table 4.3: Correlation between PH, Progressive, Non-progressive, Total Motility, Immotility, Vitality, SOD, DFI and Age in the neat sample (A) (before exposure to RF-EMW from a mobile phone and incubating at 37°C

	pH	Count (×10⁶sperm/ml)	Progressive (%)	Non Progressive (%)	Total motility	immotility	Morphology	Vitality (%)	SOD (U/ml)	DFI (%)
Age (years)	-0.089	-0.052	-0.257	0.214	-0.047	0.047	0.042	-0.021	0.062	-0.082
p-value	0.454	0.659	**0.028**	**0.07**	0.693	0.693	0.727	0.857	0.604	0.489
PH		-0.075	-0.078	0.001	-0.058	0.058	-0.063	-0.026	-0.044	-0.021
p-value		0.529	0.515	0.997	0.627	0.627	0.596	0.826	0.711	0.861
Count (×10⁶sperm/ml)			0.39	0.287	0.498	-0.498	0.391	0.108	0.227	-0.198
p-value			**0.001**	**0.014**	**<0.0001**	**<0.0001**	**0.001**	0.362	0.054	0.094
Progressive (%)				-0.028	0.731	-0.731	0.678	0.214	0.439	-0.434
p-value				0.814	**<0.0001**	**<0.0001**	**<0.0001**	0.069	**<0.0001**	**0.0001**
Non Progressive (%)					0.661	-0.661	0.387	0.082	0.578	-0.559
p-value					**<0.0001**	**<0.0001**	**0.001**	0.488	**<0.0001**	**<0.0001**
Total motility						-1.00	0.773	0.217	0.724	-0.707
p-value						**<0.0001**	**<0.0001**	0.065	**<0.0001**	**<0.0001**
Immotility							-0.773	-0.217	-0.724	0.707

p-value		**<0.0001**	0.065	**<0.0001**	**<0.0001**
Morphology			0.079	0.578	-0.528
p-value			0.506	**<0.0001**	**<0.0001**
Vitality (%)				0.003	0.043
p-value				0.982	0.717
SOD (U/ml)					-0.9
p-value	DFI (%)				**<0.0001**

r = Correlation coefficient, SOD = Superoxide Dismutase, DFI = DNA fragmentation Index, P <0.0001 significant

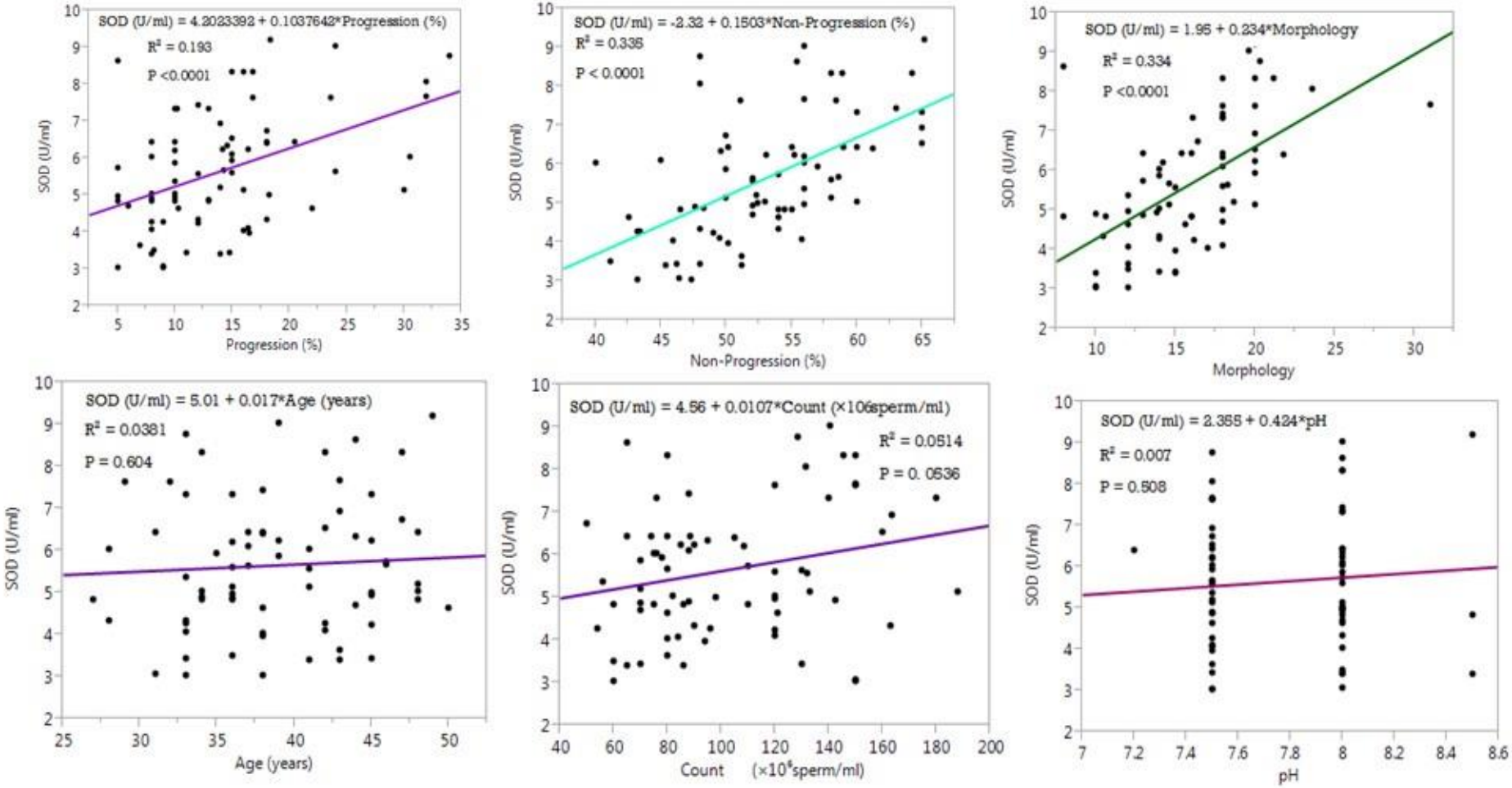


Figure 4.1: Regression graphs between SOD and pH, Progressive (%), Non progressive (%), Counts (x10^{6}sperm/ml), Morphology, Vitality, DFI and Age before incubating at 37oC and exposure to RE-EMW at 37^{o}C from a mobile phone

Figure 4.1 shows regression analysis between SOD and pH, Progressive motility (%), Non-progressive motility (%), Counts (x 10^6sperm/ml), Morphology, Vitality, DFI and Age of before exposure to RF-EMW from a mobile phone. There was a significant positive linear relation associated with SOD and progressive motility (R^2 = 0.193; p<0.0001), non-progressive motility (R^2 = 0.335; p<0.0001), morphology (R^2 =0.334 p < 0.0001).

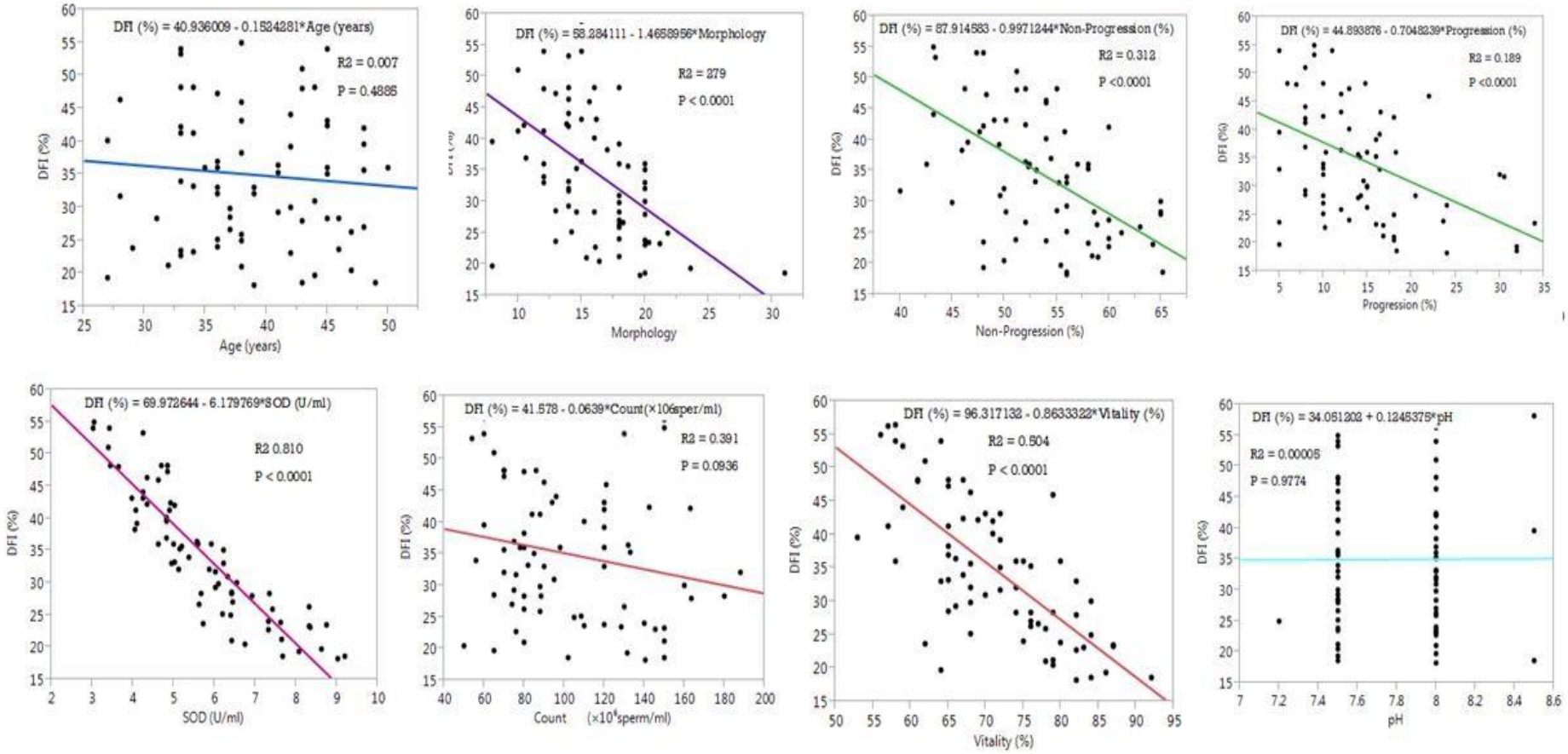


Figure 4.2: Regression analysis between DFI and Progressive motility (%), Non-progressive motility (%), counts (x106sperm/ml)(%), Morphology, Vitality, and Age of before exposure to RF-EMW from a mobile phone

Regression graphs between SOD and pH, Progressive (%), Non progressive (%), Counts (x10^{6}sperm/ml), Morphology, Vitality, DFI and Age before incubating at 37^{o}C and exposure to RE-EMW at 37^{o}C from a mobile phone

Figure 4.2 shows the Regression analysis between DFI and Progressive motility (%), Nonprogressive motility (%), counts (x10 sperm/ml) (%), Morphology, Vitality, and Age of before exposure to RF-EMW from a mobile phone. DNA fragmentation index (DFI) was negatively significant and linearly associated with progressive motility (%) (R^2 = 0.189; p <0.0001), non-progressive motility (%) (R^2= 0.318 p < 0.0001), vitality (%) (R^2 = 0.504; p <0.0001) and SOD (U/ml) (R^2 = 0.801; p <0.0001).

Table 4.4 shows correlation between SOD, DFI, pH, Progressive Motility (%), Nonprogressive Motility (%), Total Motility (%), Immotility, Vitality, and Age of the participants after exposure to RF-EMW from a mobile phone at 37^{0}C. There was a significant positive correlation between SOD and progressive motility (r = 0.451; p<0.0001), non-progressive motility (r = 0.544; p<0.0001), vitality (r= 0.725; p <0.0001), total motility r = 0.705, p = 0.049). Superoxide Dismutase (SOD) was negatively associated with Immotility (r = -0.705; p<0.0001). DNA fragmentation index (DFI) was positively significantly correlated with Immotility (r = 0.233; p = 0.049) and negatively associated with progressive motility (%) (r = -0.258; p = 0.028), total motility (%) (r -0.233; p =0.049) and SOD (U/ml) (r = -0.270; p =0.022). There was a significant negative correlation between pH and non-progressive

motility (%) (r = -0.292; p = 0.012), total motility (r = -0.237; p = 0.044) vitality (%) (r = - 0.25;

p = 0.028). pH is positively associated with immotility (r = 0.237; p = 0.044). Progressive motility (%) is positively associated with total immotility (r = 0.720, p < 0.0001) and vitality (%) (r = 0.713 p < 0.0001) but negatively correlated with immotility (%) (r = 0.720, p < 0.0001). There was a significant positive correlation between non-progressive motility (%) and total motility (%) (r = 0.068, p < 0.0001), vitality (r = 0.594, p < 0.0001). Non-progressive motility (%) is negatively associated with immotility (%) (r = -0.688; p < 0.0001).

Table 4.3: Correlation between pH, Progressive, Non-progressive, Total Motility, Immotility, Vitality, SOD, DFI and Age of participants after exposure to RF-EMW from a mobile phone at 37°C

		pH	Progressive (%)	Non Progressive (%)	Total Motility (%)	Immotility (%)	Vitality (%)	SOD (U/ml)	DFI (%)	Age (years)
							-0.258	-0.207	0.006	
pH	R		-0.047	-0.292	-0.237	0.237				0.192
	p-value		0.693	**0.012**	**0.044**	**0.044**	**0.028**	0.080	0.960	0.104
Progressive (%)	R			-0.008	0.720	-0.72	0.713	0.451	-0.258	-0.24
	p-value			0.944	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.028**	**0.041**
Non Progressive (%)	R				0.688	-0.688	0.594	0.544	-0.066	0.219
	p-value				**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.579	0.062
Total Motility (%)	R					-1	0.929	0.705	-0.233	-0.022
	p-value					**<0.0001**	**<0.0001**	**<0.0001**	**0.049**	0.854
Immotility (%)	R						-0.929	-0.705	0.233	0.022
	p-value						**<0.0001**	**<0.001**	**0.049**	0.854
Vitality (%)	R							0.725	-0.178	-0.025
	p-value							**<0.0001**	0.134	0.832
SOD (U/ml)	R								-0.270	0.043

	p-value	**0.022**	0.72
DFI (%)	R		0.155
Age (years)	p-value		0.193

r = Correlation coefficient, SOD = Superoxide Dismutase, DFI = DNA fragmentation Index, P <0.0001 significant

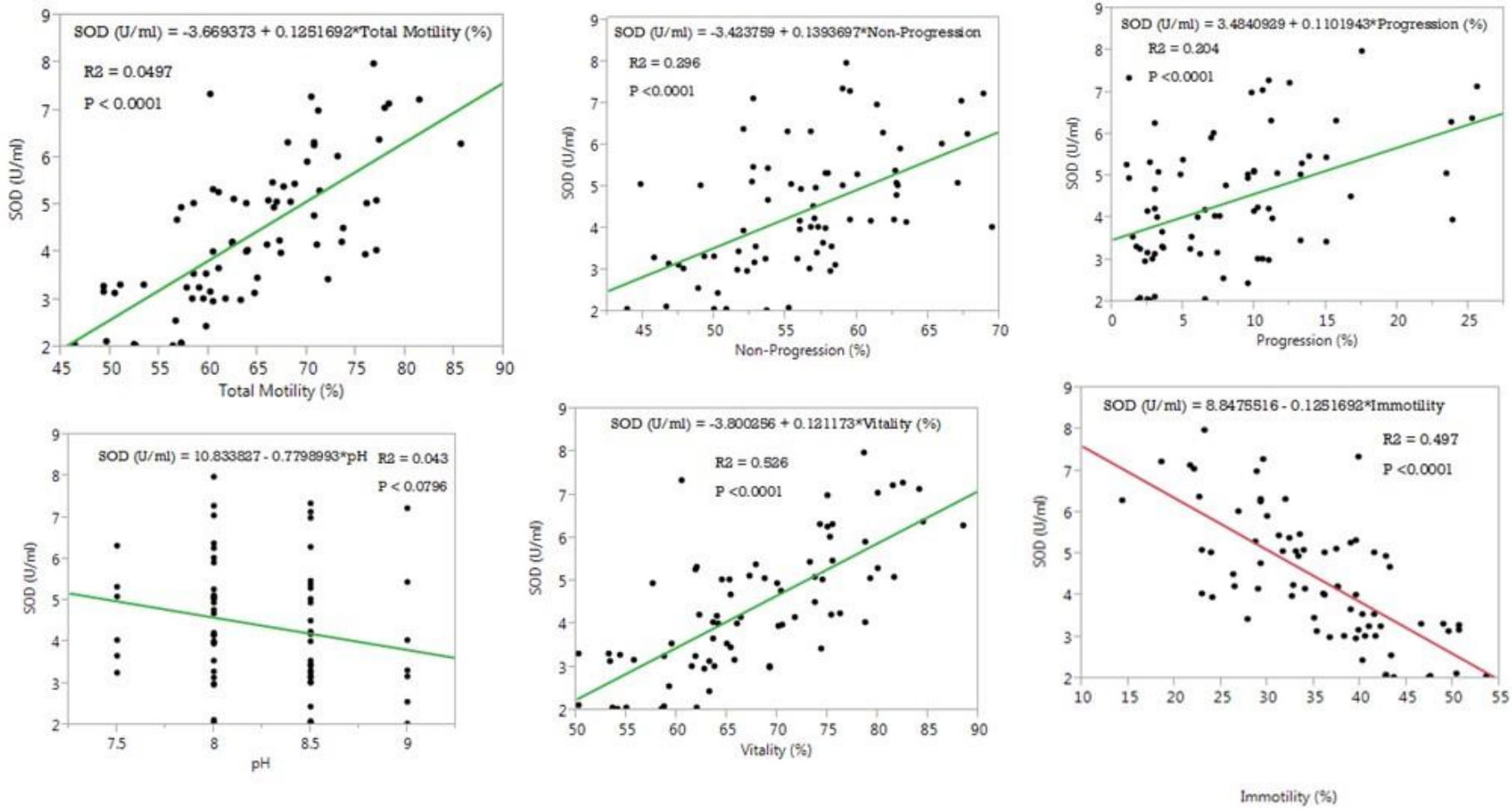


Figure 4.3: shows the regression line graphs between SOD and pH, progressive, immotility, vitality, n=no progressive and total motility after exposure to RF-EMW from a mobile phone at 37oC

Figure 4.3 shows the regression analysis between SOD and pH, progressive motility, immotility, vitality, non-progressive motility and total motility after exposure to RF-EMW from a mobile phone at 37°C. There was a significant positive linear relationship between SOD and progressive motility (R^2 = 0.204; p<0.0001), non-progressive motility (R^2 = 0.296; p<0.0001), vitality (R^2 = 0.725; p <0.0001), total

motility R^2 = 0.0.497, p <0.0001). Superoxide Dismutase (SOD) was negatively associated with Immotility (R^2 = 0.497; p<0.0001).

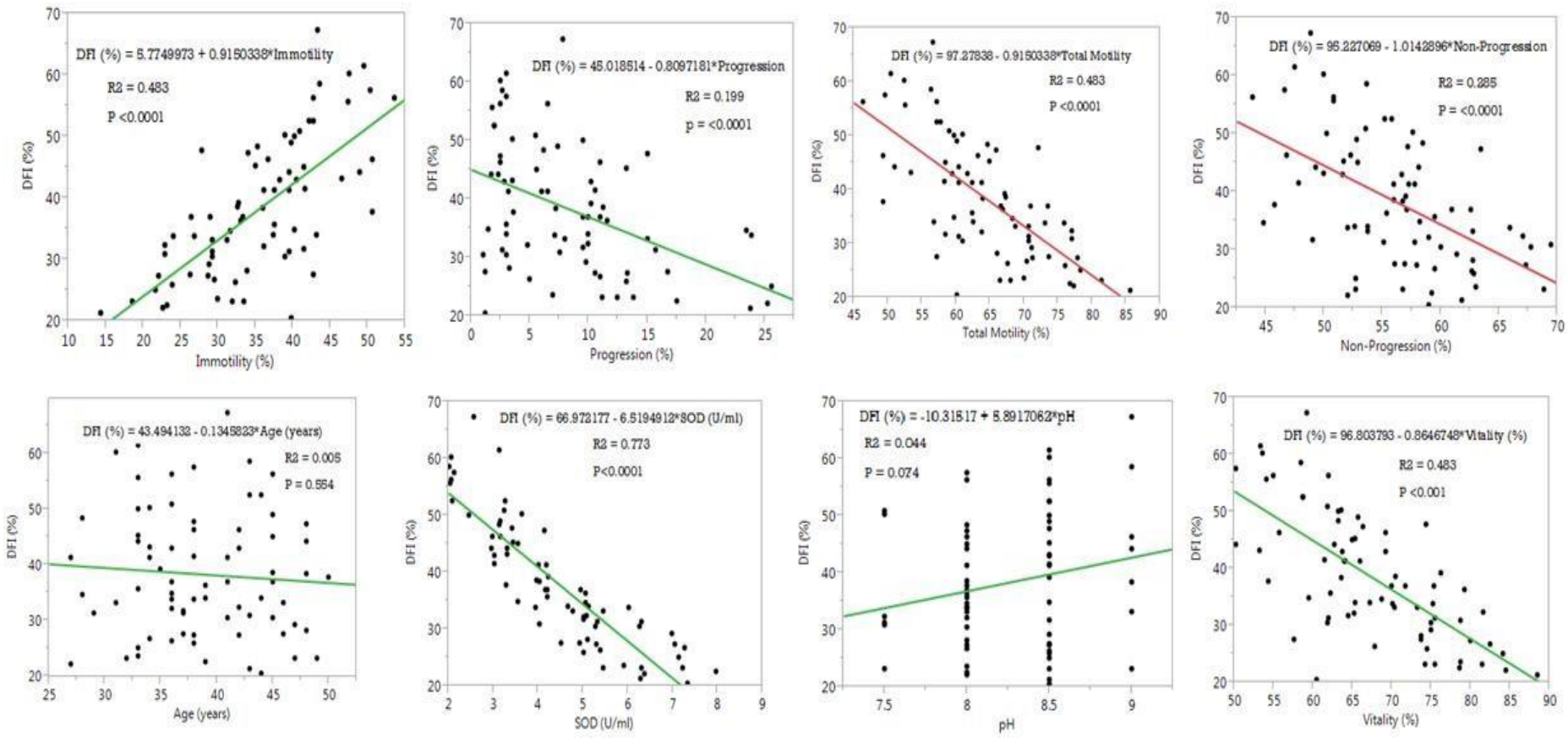


Figure 4.4: shows regression graphs between DFI (%) and pH, progressive, non-progressive, SOD, total motility, immotility, vitality, and age of participants

Figure 4.4 shows regression analysis between DFI (%) and pH, progressive motility, non-progressive motility, SOD, total motility, immotility, vitality, and age of participants. DNA fragmentation index (DFI) was significant and linearly positive associated with immotility (R^2 = 0.483; p < 0.0001) and negatively associated with progressive motility (%) (R^2 = 0.299; p <0.0001), total motility (%) (R^2 0.0.483; p <0.0001) and SOD (U/ml) (R^2 = 0.773; p <0.0001).

Table 4.5 shows correlation between SOD, DFI, pH, Progressive motility (%), Nonprogressive motility (%), Total Motility (%), Immotility, Vitality, and Age of participants after 37°C and unexposed (Sample B) to a mobile phone. There was a significant positive correlation between SOD and progressive motility ($r = 0.407$; $p<0.0001$), non-progressive motility ($r = 0.615$; $p<0.0001$), vitality ($r= 0.728$; $p <0.0001$), total motility ($r = 0.711$; $p < 0.0001$). Superoxide Dismutase (SOD) was negatively associated with Immotility ($r = -0.711$; $p<0.0001$). DNA fragmentation index (DFI) was positively significantly correlated with Immotility ($r = 0.695$; $p <0.0001$) and negatively associated with progressive motility (%) ($r = -0.430$; $p <0.0001$), total motility (%) (r -0.695; $p <0.0001$) and SOD (U/ml) ($r = -0.85$; $p <0.0001$). There was a significant negative correlation between pH and non-progressive

motility (%) ($r = -0.289$; $p = 0.013$), total motility ($r = -0.243$; $p = 0.038$) vitality (%) ($r = - 0.251$; $p = 0.032$). pH is positively associated with immotility ($r = 0.243$; $p = 0.038$). Progressive motility (%) is positively associated with total immotility ($r = 0.725$, $p < 0.0001$) and vitality (%) ($r = 0.722$ $p < 0.0001$) but negatively correlated with immotility (%) ($r = 0.722$, $p < 0.0001$). There was a significant positive correlation between non-progressive motility (%) and total motility (%) ($r = 0.689$, $p < 0.0001$),

vitality (r = 0.595, p < 0.0001). Non-progressive motility (%) is negatively associated with immotility (%) (r = -0.689; p < 0.0001).

Table 4. 4: Correlation between PH Progressive, Non-progressive, Total Motility, Immotility, Vitality, SOD, DFI and Age participants of after 37°C and unexposed (Sample B)

		PH	Progressive (%)	Non Progressive (%)	Total Motility (%)	Immotility (%)	Vitality (%)	SOD (U/ml)	DFI%	Age (years)
pH	R		-0.062	-0.289	-0.243	0.243	-0.251	-0.227	0.184	0.192
	P		0.605	**0.013**	**0.038**	**0.038**	**0.032**	0.055	0.118	0.104
Progressive (%)	R			0	0.725	-0.725	0.722	0.407	-0.43	-0.255
	P			0.998	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.029**
Non Progressive (%)	r				0.689	-0.689	0.595	0.615	-0.556	0.229
	P				**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.052**
Total Motility (%)	R					-1	0.933	0.711	-0.695	-0.028
	P					**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.816
Immotility (%)	R						-0.933	-0.711	0.695	0.028
	P						**<0.0001**	**<0.0001**	**<0.0001**	0.816
Vitality (%)	R							0.728	-0.697	-0.032

	P		**<0.0001**	**<0.0001**	0.787
SOD (U/ml)	R			-0.885	0.07
	P			**<0.0001**	0.56
DFI%	R				-0.078
	P				0.51
Age (years)					

r = Correlation coefficient, SOD = Superoxide Dismutase, DFI = DNA fragmentation Index, P <0.0001 significant

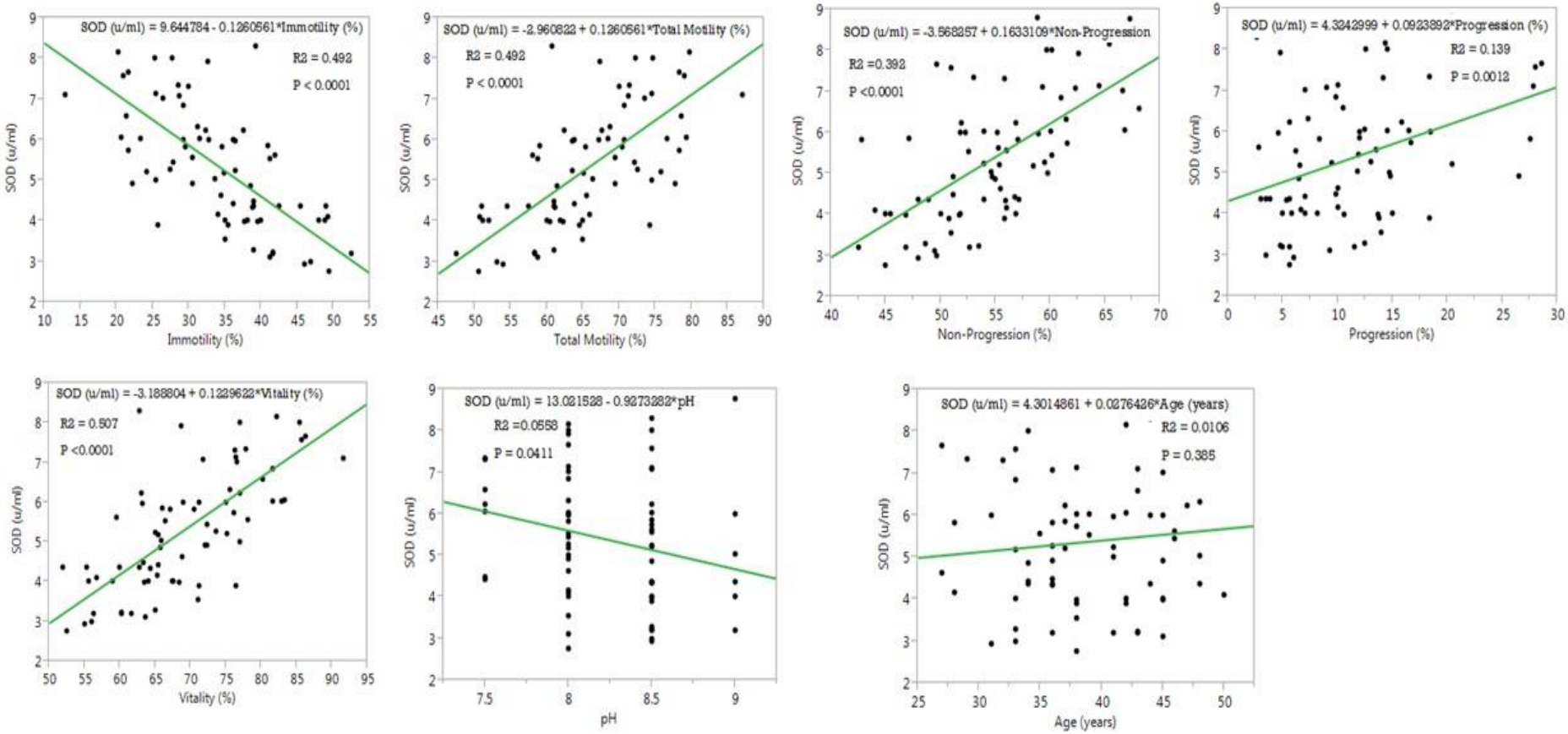


Figure 4.5 shows regression analysis between SOD and progressive motility (%), non-progressive motility (%) pH, total motility, immotility, vitality, DFI and age of participants after 37oC and unexposed (Sample B).

There was a significant positive linear relationship between SOD and progressive motility (R^2 = 0.139; p = 0.0002), non-progressive motility (R^2 = 0.392; p<0.0001), vitality (R^2 = 0.507; p <0.0001), total motility (R^2 = 0.492; p < 0.0001). Superoxide Dismutase (SOD) was negatively associated with Immotility (R^2 = 0.492; p<0.0001).

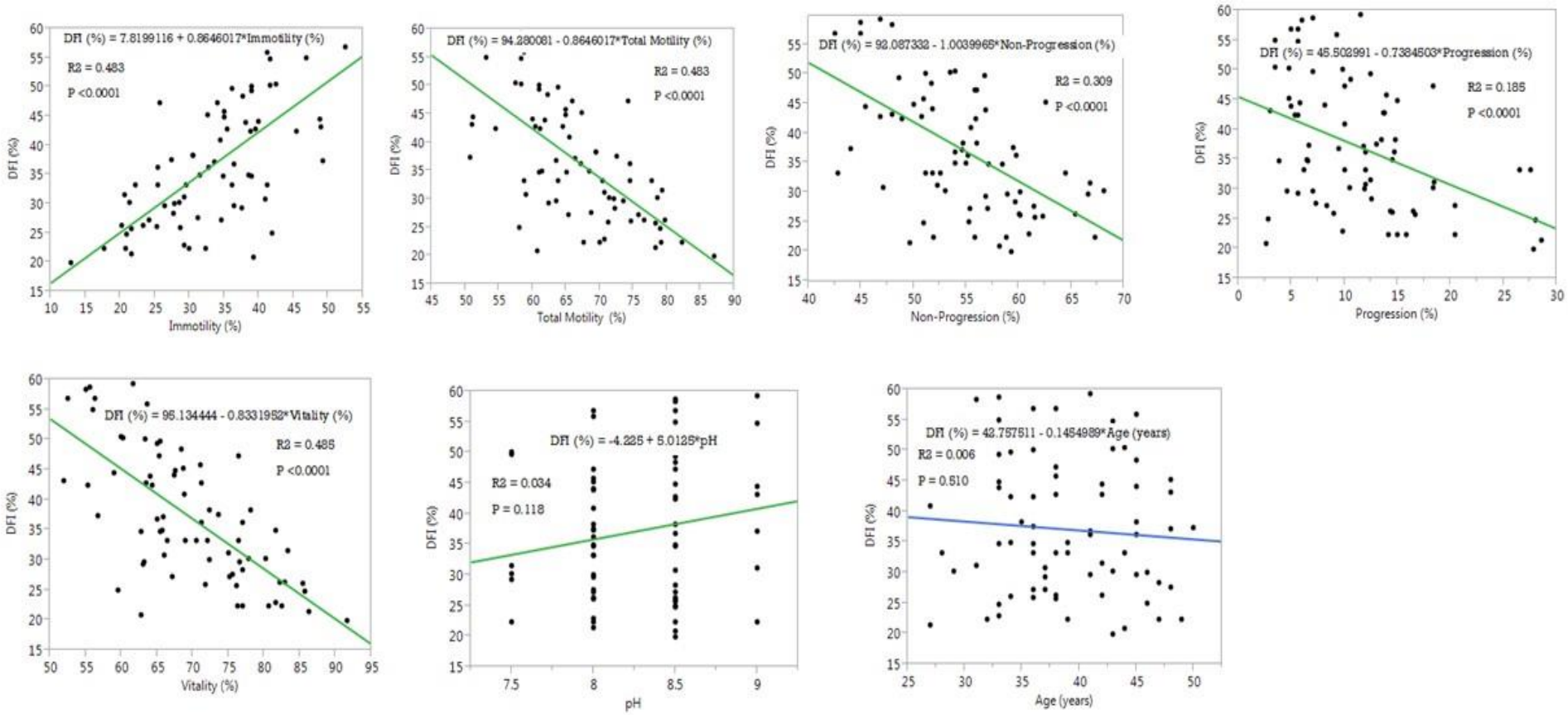


Figure 4.10: shows regression graphs between DFI and pH, progressive, non-progressive, age, total motility, immotility and vitality.

Figure 4.10 shows regression analysis between DFI and pH, progressive motility, non-progressive motility, age, total motility, immotility, vitality and SOD. DNA fragmentation index (DFI) was positively significant and linearly related to Immotility (R^2 = 0.483; p <0.0001) and negatively associated with progressive motility (%) (R^2 = 0.185; p <0.0001) and total motility (%) (R^2 = 0.485; p <0.0001)

Table 4.6 shows percentage (%) effect of Mean difference of semen before exposure, exposure after 37^0C and after 37^0C and REMF exposure from a mobile phone. Higher proportional effect of sperm progressive motility (40.5%), nonprogressive motility (5.54%), vitality (16.29%), SOD activity (22.2%), total motility (3.82%), immotility (3.82%) and DNA fragmentation index (11.23%) was observed after the exposure to REMF from a mobile phone compare to control (26.70%, 3.12%, 2.80%, 17.45%,1.39%, 1.39% and 5.53%respectively) and before exposure (18.92%, 2.48%, 13.88%, 5.75%, 2.43%, 2.43% and 11.23% respectively

Table 4. 5: Percentage (%) effect of Mean difference of semen Neat (A), Control (B) and after exposure to RF-EMW 37oC from a mobile phone (C)

Variables	Mean Difference A and B	% Effect before exposure and Control	Mean Difference A and C	% Effect before and after exposure	Mean Difference B and after C	% Effect of samples B and C
Progression (%)	2. 65	18.92%	5.68	40.50%	3.03	26.70%
Non -Progression	1.39	2.48%	3.11	5.54%	1.71	3.12%
Vitality (%)	11.22	13.88%	13.17	16.29%	1.96	2.80%
Total motility	0.93	1.39%	2.56	3.82%	1.63	2.43%
Immotility	0.93	1.39%	2.56	3.82%	1.63	2.43%
SOD (U/ml)	0.33	5.75%	1.26	22.20%	0.93	17.48%
DFI (%)	3.69	8.12%	6.74	11.23%	2.05	5.53%

CHAPTER 5

5.0 DISCUSSION

In this study the effect of RF-EMW radiation on sperm motility, viability and deoxyribonucleic acid fragmentation index (DFI) and progression of the sperm before and after exposure were determined. The findings indicated that sperm progression (progressive and non-progressive motility), viability and SOD were significantly reduced with corresponding increase in DFI after exposure to RFEMW radiation compared to unexposed at 37 degree Celsius and pre-exposure (neat samples). A significantly higher percentage effect of deficiency in total motility (progressive and non-progressive motility) and viability, SOD with increased DFI was observed after radiation exposure (Table 4.2). SOD correlated positively with total motility (progressive and non-progressive motility) and viability but negatively with immotility of sperm and DFI. DNA fragmentation (DFI) was directly associated with immotility but inversely associated with total motility (progressive and non-progressive motility), viability and SOD.

5.1 REACTIVE OXYGEN SPECIS PRODUCTION (OXIDATIVE STRESS INDUCED RF-EMW RADIATION) AND SPERM QUALITY

Increase reactive oxygen specie (ROS) production in sperms has been implicated in radiation exposure in several animal studies. For the first time in Ghana, this study used ejaculated semen and after exposure to RF-EMW radiation a significantly reduced SOD activity was observed in their semen compared to their participants without radiation exposure. This finding concurs well with an in-vitro pilot study on human ejaculated semen in Ohio, USA by Agarwal *et al.* (2009). The increase ROS after exposure of semen to RF-EMW radiation was depicted by the reduced SOD. The probable explanation for the reduced SOD is increased oxidative stress due to stimulation of the spermatozoa's plasma membrane redox system by RF-EMW and or the effect of RF-EMW on macrophage present in the neat semen (Agarwal *et al.*, 2009).

A current study by Friedman *et al.* (2007) indicated that RF-EMW stimulate plasma membrane NADH oxidase in mammalian cells and cause production of ROS and thus may also be the implicating factor to an increase in the activity of NADH oxidase in semen after RF-EMW exposure. The human spermatozoa possess a multiple plasma membrane redox system that shares correspondences with trans membrane NADH oxidase as demonstrated by Aitken *et al.* (2003).

Disturbance in free radical metabolism or production of oxidative stress by cell phone radiation have also been proven in animal studies (Ozguner *et al.*, 2005; Meral *et al.*, 2007). While some studies explained that chronic exposure to RF-EMW can decrease total antioxidant capacity depicted by a reduce catalase, superoxide dismutase (SOD), and glutathione peroxidase activity, other animal study designed to measure malondialdehyde (MDA) level and SOD activity observed inconsistency in findings (Irmak *et al.*, 2002). The production of ROS is a normal physiological process in sperm formation and production but the state of this stress is neutralized by antioxidant system present in the semen (Sharma *et al.*, 1999; Agarwal *et al.*, 2008b). However, when the increase ROS production override the antioxidants capacity an oxidative stress state is formed.

Another finding of this study was a significantly declined in SOD after exposure to 37°C (Controls) compared to pre-exposure (neat sample) 37°C. The finding is consistent with an *in-vitro* study Esfandiari *et al*. (2002) who observed significantly increased levels of ROS in semen exposure to 37°C but inconsistent with (Kobayashi *et al.*, 2005) who observed a decline in level of ROS in semen stored at 37°C. The probable explanation for this disparity could be due to the difference in duration of temperature exposure. In this study the percentage decline in antioxidant activity of SOD after 37°C exposure was lower compared to RF-EMW exposure at 37°C (5.57% v 22.20%). This indicates that RF-EMW from phone shows

a significantly higher effect on semen quality and may culminate into infertility compared to 37 degrees however; this effect largely depends on the duration of exposure.

5.2 RF-EMW-INDUCED ROS PRODUCTION AND SPERM CELL QUALITY

In this study, the mean levels of sperm progressive motility, total motility and viability were significantly reduced after exposure to RF-EMW radiation while immotility was increased. These findings are consistent with an in vitro study by Veerachari and Vasan (2012) who observed a significantly reduced viability, progressive and non-progressive motility (total motility) and corresponding elevated immotility.

A reduction in sperm motility and viability is associated with an increased in concentration of superoxide anion in semen. Increased production of superoxide anion in extracellular compartments can oxidize membrane phospholipids and cause a decrease in viability and sperm motility (Henkel *et al*., 2005) as observed in this study. The probable explanation to increased immotility could be linked to increased RF-EMW-induced oxidative stress. Another study by Agarwal *et al.* (2008a) reported that the use of cell phone had adverse effect on the quality of semen by reducing the sperm count, total motility, viability and morphology in 361 men attending the fertility clinic. The effect of these sperm parameters was significantly associated with longer duration of exposure.

Several other mechanisms relating increase in ROS to the decline in sperm motility, viability and acrosomal integrity. Firstly, the diminution of adenosine triphosphate (ATP) in sperm mitochondrion which subsequently affect sperm axonemes and inversely influence enzyme activity (De Lamirande and Gagnon, 1991; De Lamirande and Gagnon, 1992). Secondly, decline in sperm motility could also be attributed to oxidation of phospholipid in mitochondrion by reactive oxygen species (De Lamirande and Gagnon, 1992). All these approach may

explain the relatively decline in sperm motility.

This study demonstrated a statistically significant and positive correlation between SOD activity and progressive and non-progressive motility (total motility) and viability of sperm but a negative correlation with immotility of sperm. These findings are clear and supported by the earlier findings in this study, which observed that increased exposure of semen to RF-EMW reduces the antioxidant activity of sperm. A study by Erogul *et al.* (2006) have also reported a significant negative correlation between electromagnetic radiations and sperm motility which is consistent with this study.

5.3 EFFECT OF TEMPERATURE ON SPERM CELL QUALITY

Previous study have demonstrated that temperature may inhibit sperm motion and thus affect motility and progression (Esfandiari *et al.*, 2002). Consistent with study by Esfandiari *et al.* (2002), this study also observed a significantly reduced spermatozoa total motility (progressive and non-progressive motility) and viability. The effects of temperature on sperm cell motion have received conflicting arguments. Some study reported that abnormal spermatozoa produces high ROS during an *in-vitro* incubation which has been implicated in infertility due to increase lipid peroxidation activity of the sperm plasma membranes (Esfandiari *et al.*, 2002). However, the major confounder of temperature effect on sperm motion is duration of incubation. A study by Esfandiari *et al.* (2002) found no statistically significant difference in sperm motility at 25^{o}C compared to 37^{o}C although the ROS produced at 37^{o}C was significantly lower and thus conclude that 37^{o}C is an optimal temperature for semen collection.

5.4 RADIATION EXPOSURE AND DNA DAMAGE IN SPERM CELL

Studies regarding DNA damage are complicated due to variations in methodology and interpretations. In this study there was RF-EMW-induced DNA damage as depicted by the increased DFI after RF-EMW exposure. This finding is in accordance with an in-vitro study by Veerachari and Vasan (2012) on human

ejaculated semen. In an animal experimental study by Aitken *et al.* (2005) an exposure of mice to RF-EMW, 900 MHz, 12 hours/day for 7 days led to damage to the mitochondrial genome and nuclear beta-globin locus of epididymal spermatozoa. Exposure to electromagnetic radiations and mild scrotal heating can induce DNA damage in mammalian spermatozoa; thought the mechanism underlying this pathogenesis is not well-understood.

Spermatozoa are extremely vulnerable to induction of DNA damage as they lose their cytoplasm which contains antioxidant enzymes and thus exposure to radiation may have alternated the DNA and cause it to damage (Aitken *et al.*, 2005). This may be probable explanation for the high DFI observed after exposure to the radiation. Aside RF-radiation which may induced DNA damage, DNA induced damage may also be associated with male infertility (Aitken and Baker, 2006).

This study also observed that semen stored at 37^{o}C resulted into a mild DNA damage compared to pre-exposure semen (Neat samples). However, the proportional effect DNA damage after RF-EMW exposure was 11.23% compared to 8.12% when stored at 37^{o}C. Previous studies by Agarwal *et al.* (2009) and Veerachari and Vasan (2012) have demonstrated a relationship between DNA damage and sperm cell quality by radiation exposure from mobile phone use. This study observed as significant negative correlation between DFI and sperm total motility (progressive and non-progressive motility) and viability. Another *in-vitro* study by Giwercman *et al.* (2003) also reported a significantly negative correlation between sperm motility and sperm chromatin damage. Men carrying their cell phones in their front trouser pocket while using data or running application or clipping phone close to their belt at the waist makes them susceptible to RF-EMW exposure which might affect semen quality through oxidative stress induceddamage and subsequently result into infertility.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study demonstrated that RF-EMW causes oxidative stress in semen and resulted into a decline in spermatozoa total motility, (progressive and nonprogressive motility), viability and SOD and corresponding increase immotility and DFI. SOD correlated inversely to DFI and immotility but directly to total motility, viability and progressive motility of spermatozoa.

6.2 LIMITATIONS

It was difficult to get the calculated sample size due to;

- A lot of men were not willing to be enrolled in the work even though they were eligible to be enrolled
- Some could not produce the sample by the prescribed method of producing the sample (masturbation)
- It was also difficult to get a good number of men with good semen parameters for the normal standard semen analysis.

6.3 RECOMMENDATION

Based on the findings of this study;

- It is suggested that men must avoid carrying a cell phone in a pocket or clipping it on their waist belts while applications are running as the electromagnetic radiation from the phones might negative effect on their sperm quality as seen in the *in-vitro* study.

- Further studies are therefore needed to assess the effect seen *in-vitro* in *in-vivo.*

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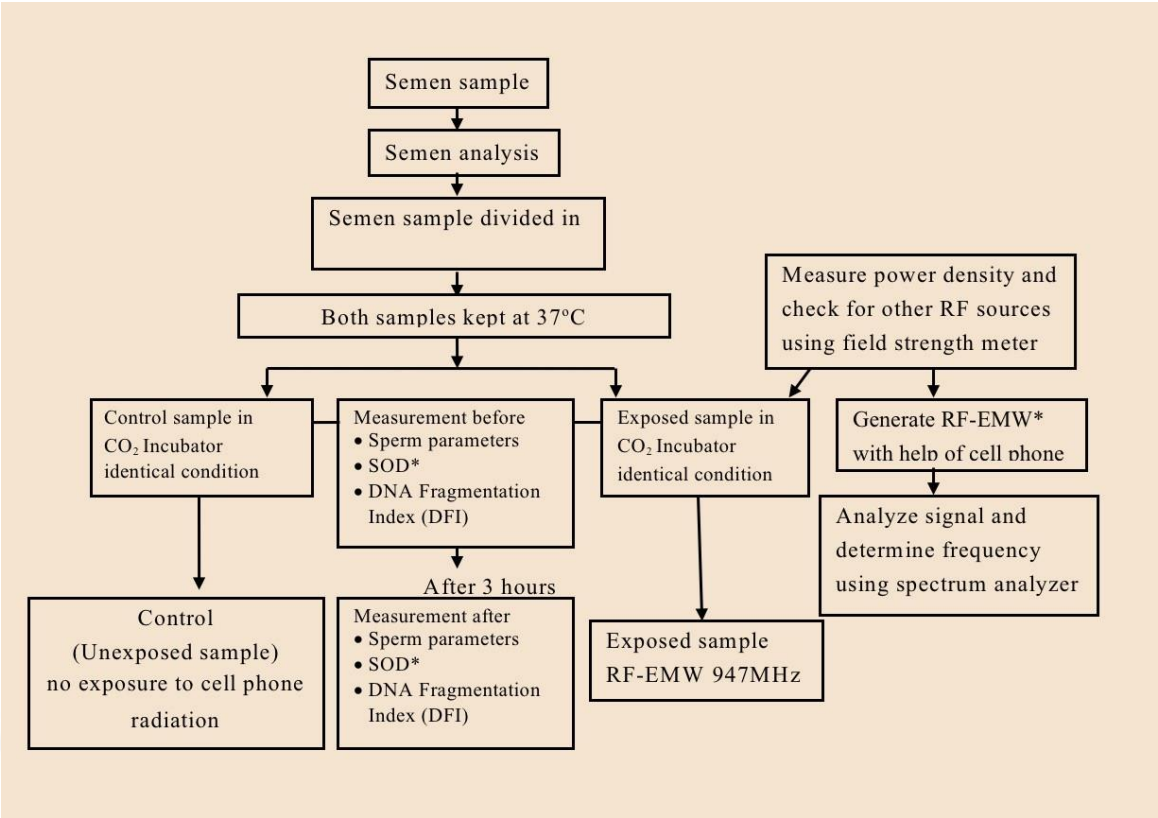
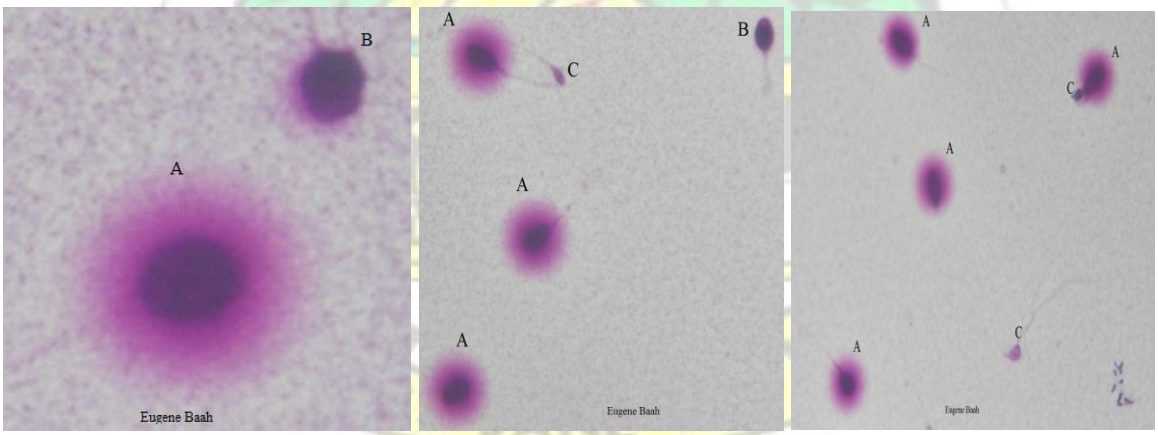
APPENDIX

Summary of study design set-up methods for the exposure of semen samples to RF-EMW



Key: *RF-EMW: Radiofrequency Electromagnetic Waves

*SOD: Superoxide Dismutase



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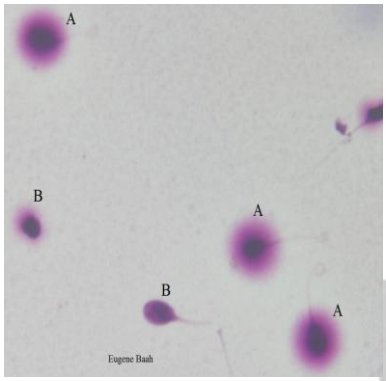

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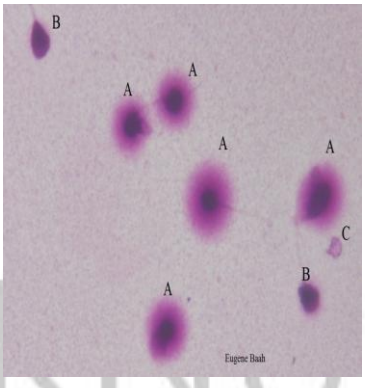

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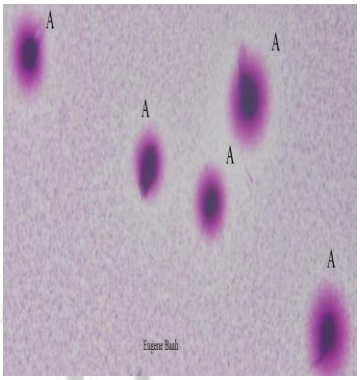

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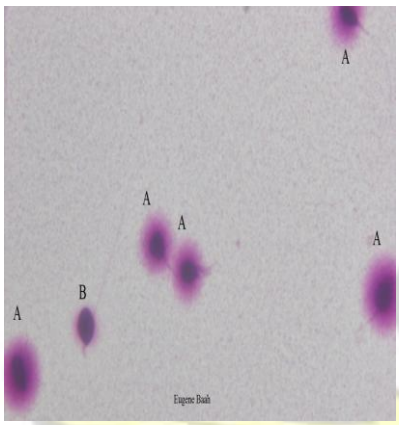

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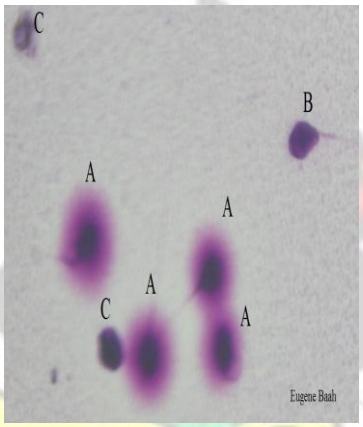

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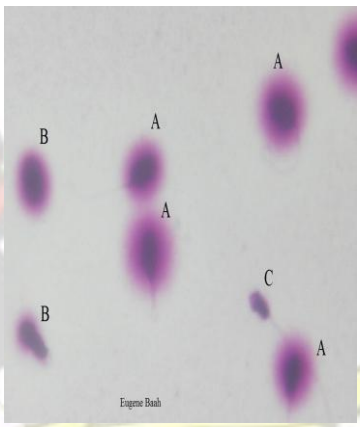

9

Key

A- Non-Fragmented sperm,

B- Fragmented sperm,

C- Degraded sperm.

PATICIPANT'S QUETIONAIRE

Date: …….. Participant No.:…… Age: ……

Marital Status: [] Single / [] Married

Occupation:…… Abstinence Days:.....

Collection Time:……. Receiving Time:……

Weight (Kg):…… Height (m):…… BMI:……

**Please tick the correct answer

1. Do you live closer to a Telecom Mast? [ ] Yes / [ ] No
2. What type of phone do you use? [ ] Simple phone, [ ] Smart phone [ ] Both
3. How many phones do you have? [ ] One, [ ] Two [ ] More than Two
4. Do you put your phone(s) in your front pocket? [ ] Yes / [ ] No
5. Have you done Semen Test before? [ ] Yes / [ ] No
6. Have you had any S.T.D. infection in the last six month? [ ] Yes / [ ] No
7. Are you diabetic? [ ] Yes/ [ ] No
8. Are you on any fertility treatment drug? [ ] Yes / [ ] No
9. Are you addicted to any harmful drug? [ ] Yes / [ ] No
10. Do you smoke? [ ] Yes/ [ ] No
11. Do you take alcoholic beverages? [ ] Yes / No

Comments:……
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