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SEMEN PROFILE OF MALE PATIENTS ATTENDING UROLOGY CLINIC FOR INFERTILITY: ANALYSIS OF LABORATORY RECORDS AT SURGICAL RESEARCH LABORATORY, IBADAN, NIGERIA.

BY

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DECLARATION

I hereby declare that this thesis entitled "SEMEN PROFILE OF MALE PATIENTS ATTENDING UROLOGY CLINIC FOR INFERTILITY: ANALYSIS OF LABORATORY RECORDS AT SURGICAL RESEARCH LABORATORY, IBADAN, NIGERIA" is a genuine work carried out by me and that data abstraction was done at the Surgical Research Laboratory, Surgery Department, College of Medicine/University College Hospital, Ibadan, Nigeria under the supervision of Dr. Alexander Ansah Manu (School of Public Health, Legon) and Prof. Linus Okeke (College of Medicine, Ibadan).

The works of others which served as sources of information were duly referenced.

Adediji Julius A. (Student)

Signature 

Date: 09/12/2021

Dr. Alexander Ansah Manu	Prof. Linus Okeke
Supervisor	Co-supervisor
Signature 	Signature 
Date: 09/12/2021	Date 9/12/2021

DEDICATION

This piece of work is dedicated to the Almighty GOD, the source of all wisdom, power and knowledge.

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"If I have seen further, it is by standing upon the shoulders of giants" Sir Isaac Newton. It therefore implies that this academic achievement would not have been possible without the support, cooperation and positive criticisms from my supervisor Dr. Alexander Ansah Manu and co-supervisor Prof. Linus Okeke, as well as other faculty members in the School of Public Health, Legon. I say many thanks for the knowledge you have imparted on me.

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ABSTRACT

Infertility is a disease of male or female reproductive system and is one of the major public health problems. Male infertility is the inability to impregnate a woman after 12 months of regular, unprotected sexual intercourse. Male factor is reported to be the cause of about 40-50% of cases of infertility worldwide. Deficiencies in semen due to many factors such as environmental, genetics, hormonal, infections etc have been strong indicators of infertility in males. However, in Nigeria there is a dearth of systematic evidence on the semen profiles of already infertile men. Thus, this research aims to investigate the semen profile of adult male patients attending urology clinic for infertility.

A retrospective record review was conducted on 286 male patients aged between 18 and 65 years. A multi-stage sampling method was employed. The first stage involved was the purposive selection of both the urology clinic and the surgical research laboratory. In the second stage, laboratory registers were screened for eligibility and registers covering September 2016 – August 2018 were selected. Patients' results in the selected registers were screened for inclusion criteria, sampling interval was calculated and simple random sampling was performed. Simple tabulations and cross tabulations were done to determine proportions. Associations were tested with chi-squared, or Fisher's exact tests and significance was tested at the 5% level.

Out of 286 patients reviewed, 159 (55.6%) of the patients had never achieved pregnancy (primary infertility) and 127 (44.4%) had at least achieved one prior pregnancy (secondary infertility). We found that the enlargement of the veins that hold the testicles (varicocele) accounted for 141 (49.3%) cases of infertility and it was responsible for 79 (27.6%) of primary and 62 (21.7%) of secondary infertility. Inability to keep/sustain erection (erectile dysfunction) was second accounting for 42 (14.7%) while physical injury (trauma) to the testicle/scrotum accounted for 39 (13.6%) cases. It was observed that 143 (50.0%) of the patients had low sperm

count (oligospermia) in one ejaculate and this accounted for the cause of 79 (34.5%) primary and 64 (28.0%) secondary infertility cases. No/absence of sperm cell (azoospermia) in semen ejaculated was found in 57 (19.9%) patients. The percentage of low quantity of semen (hypospermia) per ejaculate in this study was 22.4% while high quantity of semen (hyperspermia) per ejaculate was 3.2%. In this study, semen with acidic pH was 24 (8.4%), neutral pH was 205 (71.7%) and basic pH was 57 (19.9%). The age of patients ranged from 18 to 64 years with mean age of 38.0 ± 7.6 years and the modal age group was 30-50 years 243 (85.0%).

The clinical condition most reported among the patients under review was the enlargement of the veins that hold the testicles (varicocele) which was the most common cause of primary infertility. Low sperm count per ejaculate (oligospermia) was common among the patients during the period under reviewed. More than six out of ten attendees had low sperm count per ejaculate, this was commoner among those who had never achieved pregnancy than those who had at least achieved one prior pregnancy and in more than four of the six, the low sperm cells count was severe.

Therefore, this study observed that varicocele, erectile dysfunction, low volume of ejaculate and low sperm count are commonest presentations among males during the period under review and were also strongly associated with infertility in males.

Keywords: Semen. Sperms, Motility, pH, Urology Clinic

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ABBREVIATIONS

SFA-	Seminal Fluid Analysis
WHO-	World Health Organization
NSFG-	National Survey of Family Growth
ROS-	Reactive Oxygen Species
HCV-	Hepatitis C Virus
HBV-	Hepatitis B Virus
HIV-	Human Immunodeficiency Virus
ASA-	Anti-Sperm Antibodies
PMN-	Polymorphonuclear
FSH-	Follicle stimulating hormone
ICSI -	Intracytoplasmic sperm injection
LH-	Luteinizing hormone
UI-	University of Ghana
UCH-	University college hospital
PFAAs-	Perfluoroalkyl acids
DNA-	Deoxyribonucleic acid
PR-	Progressive motility
NP-	Non-progressive motility
PMN-	Polymorphonuclear

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

It was in 17th century that Antoni van Leeuwenhoek first described semen (spermatozoa) after it was found in the vas deferens and testes and he then said that the primary purpose of testis is sperm production (Ruestow, 1983). Since 1928 sperm has been found to be associated with fertility potential, different semen analysis and parameters have been developed with the intention to categorize men into those that can impregnate their partner or those that cannot (Crha et al., 2009). The male reproductive functions can be divided into three namely: spermatogenesis which means the formation of sperms, execution of the male sexual act and management of male reproductive functions by individual hormones. Linked with these reproductive functions are the influences of the male sex hormones on the accessory sexual organs, cellular metabolism, growth and other functions of the body (Hall, 2006). Infertility is a disease of the reproductive system manifested by inability of couples to achieve pregnancy within one year following regular sexual intercourse (WHO, 2016). Semen analysis provides the basic information on the spermatogenesis, the functionality of the gonads and potentiality of the male genital tract (Vasan, 2011). Semen analysis makes up of series of descriptive semen parameters that help to determine semen quality such as concentration, motility, volume, morphology, vitality and pH and male infertility are not unconnected with the parameters (Kordum-Skelin & Turek, 2010; Rrumbullaku, 2011). Several factors such as environmental, chemical, genetics, infections etc have been implicated in semen abnormalities in males. Examples of environmental factors include heat, toxins, heavy metal etc. The mode of operation of these factors is by heating up the testes and thereby impairing semen production and damaged some. Certain type of lifestyle has been

implicated in male infertility by direct effect on semen quality e.g., alcoholism, tobacco smoking, drug use etc. Tobacco smoking generates high level of reactive oxygen species (ROS) which cumulatively increase oxidative stress within the cells and later lead to the death of such cell. Alcoholism lowers testosterone levels in males and thereby causing decrease sperm production. Genetic abnormality, chromosome defects in which a male inherited two X chromosomes and one Y chromosome. This leads to abnormal growth of male reproductive organ. Male infertility can also result from hormone imbalances e.g., low testosterone which leads to low production of semen. Certain infections can interfere the production of healthy sperm or can block the passage of sperm. Examples include epididymitis (inflammation of the epididymis), some sexually transmitted infections. Oxidative stress has also contributed to defective spermatogenesis and eventually leads to poor semen quality associated with idiopathic male factor infertility. Semen analysis and testicular biopsy form significant screening tools in the appraisal of an infertile male. In a study conducted on 50 infertile males with sterility of 2-6 years duration, it was found that hypospermatogenesis and maturation arrest are the most common lesion (Webster RA, 2007). Recent studies suggested that ancestral connection is highly correlated with rare genetic sperm-defect syndromes involving the sperm head or sperm tail. As a result of this, assisted reproductive technologies especially intracytoplasmic sperm injection (ICSI) should be carried with caution (Inhorn et al., 2009). It has been suggested that if multiple semen analysis show azoospermia, oligospermia or any other semen abnormality, then hormone test should be performed to really know the cause of the specific dysfunction. Low level of testosterone together with increased level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) suggests primary testicular failure. If normal testosterone, LH and FSH levels accompany oligospermia or azoospermia, then seminal fluid fructose should be checked (Dohle et al.,2005). Shivendra, 2014 conducted a

study on 50 cases of which 23 smoked more than 5 cigarettes/day and 14 smoked less than 5 cigarettes/day. It was discovered that majority of the smokers had oligospermia.

The cases of infertility in the world stood between 40-50% and it affects approximately 7% of all men (Brugh et al., 2004). Globally, 15% to 20% of couples are said to be suffering from infertility (Sabanegh & Agarwa, 2011). Infertility among couples in Africa ranks highest in the world between 15% and 30% as against 5% to 10% in Europe and 4% to 7% in US (Jean et al., 2013). It affects 10–30% of couples in Nigeria (Chimbatata & Malimba, 2016); men account for 20 to 30% of cases and contribute 50% of mixed infertility cases (Martinez, Daniels & Chandra, 2010). The factors responsible for male infertility can be divided into three major categories pre-testicular, testicular and post-testicular (Bayasgalan et al., 2004; Sandro, Miyaoka & Agarwal, 2011). In Nigeria, study conducted in southwest reported 24.7% of sperm abnormality among infertile couples (Ikechebelu et al., 2003). The task force on the diagnosis and treatment of infertility set up by WHO reported that up to 15% of the population suffers either primary or secondary infertility (WHO, 2010).

In this study, the minimum sample size (N) was calculated using Cochran's (1977) formula and the prevalence of sperm abnormality among infertile couples in southwest, Nigeria was 24.7% (Ikechebelu et al., 2003).

Regionally, Africa has in some instances been known for high incidence and prevalence of communicable and non-communicable diseases. According to the World Health Organization Task Force on the Diagnosis and Treatment of Infertility, average infertility in Africa is 10.1% of couples with as high as 32% in some countries in Africa and with some certain tribes more cases of infertility than others. Ibadan being a cosmopolitan city with different ethnic groups coupled with other factors causing male infertility mentioned above, residents of Ibadan are predisposed to one or more factors causing infertility in males. Urology clinic was purposively

selected because it serves as both inpatient and outpatient and accept referrals. The clinic is being manned by consultants in subspecialties of urology. The surgical research laboratory was considered for this study because it is directly attached to the clinic and carries out the semen analysis for the patients and being manned by qualified and certified medical laboratory scientists. This study aimed to describe the semen profile of infertile male, the prevalence of infertility among the attendees and semen morphological abnormality among the users of this clinic during the period under review.

1.2 RATIONALE FOR THE STUDY

The impacts of infertility both psychologically and the social wellbeing are not only on the index patient but also on his or her partner cannot be overemphasised. Semen analysis is very important examination for the diagnosis of male infertility and is a predictor of fertility capability of males. It is carried out by examining semen on several parameters such as concentration, motility, morphology, vitality etc. In the recent times, there has been an increased demand in the use of infertility treatment and a decline in fertility in some countries which have generated concerns whether human fertility is declining or has declined. This scene of decline in semen quality over the past of a century has been proposed (Kumar, 2011). Infertility affects 10–30% of couples in Nigeria (Chimbatata & Malimba, 2016), men account for 20 to 30% of cases and contribute 50% of mixed infertility cases (Martinez, Daniels & Chandra, 2010). The problem of male infertility can be improved by using preventive, management and curative interventions. However, the efficiency of these interventions will depend on accurate and correct identification of the risk factors. More importantly, the understanding of the damaging effects of these risk factors on the semen quality and quantity in males in Nigeria, so that workable interventions can be designed to address the onset of male infertility. Although studies have been done on infertility among couples, but this study was out to profile changes in the semen parameters of already infertile

males seen in this clinic, to describe the relative contribution of the various clinical conditions to types of infertility and their modal age of occurrence.

1.3 CONCEPTUAL FRAMEWORK FOR SEMEN PROFILE OF INFERTILE MALE

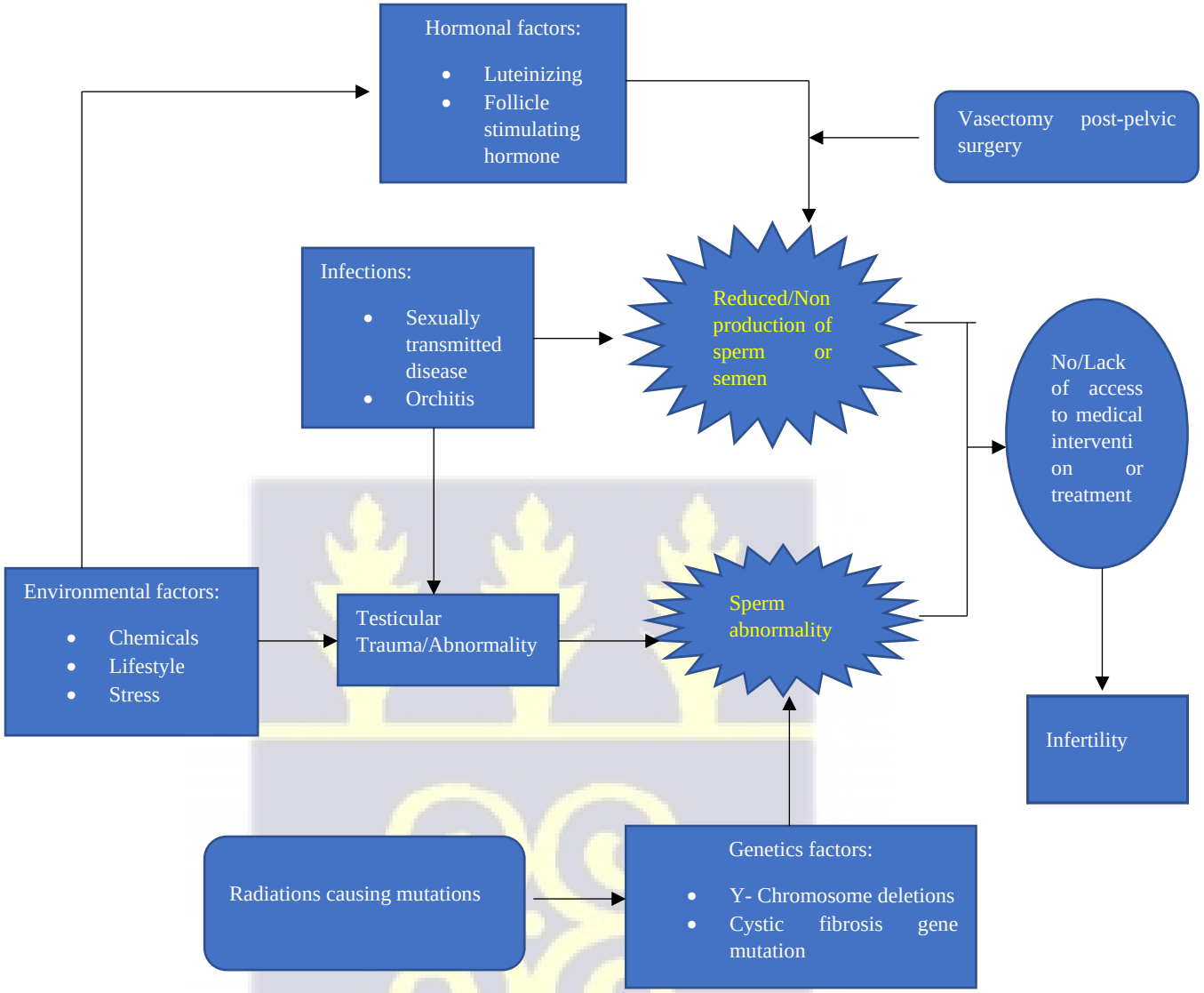


Figure 1.1 Conceptual framework

The possible factors responsible for either reduced/non-production of semen, semen abnormalities, or both in infertile male are environmental, hormonal, chemicals, infections, radiations and genetics, and these factors either interact with each other or singly to affect semen parameters either the same way or differently.

Environmental factors such as chemicals (herbicides, insecticides), lifestyle (alcoholism, drug abuse) and stress can cause either hormonal imbalance or testicular trauma/abnormality. If the damage caused is hormonal imbalance, then there may be reduced/non-production of sperm/semen but if testicular abnormality, the resultant effect will be sperm abnormality. These two scenarios are depicted above with stars (24 points stars). Infections such as sexually transmitted diseases, orchitis can cause testicular abnormality which in turn affect sperm morphology or the infections directly have effect on the production of sperm/semen. Radiations cause DNA damage in male reproductive system and can be inherited though this quite different from genetic disorder such as Y chromosome deletions. The combined effects of these two scenarios are either on sperm abnormality or semen/sperm production or both. Vasectomy is a post-pelvic surgery where the testes still produce sperm but they are soaked up by the body thereby causing reduced (low volume) of ejaculate. No or lack of intervention or medical treatment eventually leads to infertility. Therefore, this study focuses on the two highlighted yellow (24 points stars) in the conceptual framework.

1.4 RESEARCH QUESTIONS

1. What is the prevalence of male infertility among the attendees to the urological clinic (infertility case/all attendees)?

2. What type of infertility commonly reported to the clinic between September 2016 – August 2018?

3. What are the profiles of semen from these patients and do they differ by the cause of infertility?

1.5 OBJECTIVES OF THE STUDY

GENERAL OBJECTIVE:

The study aimed at describing the semen profile of male patients attending the urology clinic in University College Hospital, Ibadan.

SPECIFIC OBJECTIVES:

1. To describe the types of infertility among the attendees at the Urological Clinic.

2. To identify the causes (types) of infertility commonly reported to the clinic between September 2016 – August 2018.

3. To describe the profiles of semen abnormalities of infertile male patients presented at this clinic during the period under review.

CHAPTER TWO

LITERATURE REVIEW

2.1 SPERMATOGENESIS

It is a process by which a single cell of spermatozoon (diploid, 2n) developed from germ cells in the seminiferous tubules of the testis and begins with the mitotic division of the stem cells at the basement membrane of the tubules. This process starts at early life and continuing throughout life but decreasing in old age *(*Kretser et al., 1998*)*. These cells are called spermatogonial stem cells. The division of the nucleus (mitosis) produces two types of cells namely type A cells which replenish the stem cells, and type B cells which differentiate into primary spermatocytes. The primary spermatocyte undergoes cell division (meiosis) that leads to emergence of four daughter cells, each has half the number of chromosomes of the parent cell into two secondary spermatocytes and each secondary spermatocyte divides into two equal haploid spermatids during Meiosis 1 and Meiosis II. The transformation of spermatids into spermatozoa is called spermiogenesis and these develop into mature spermatozoa, also known as sperm cells. Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes and the two secondary spermatocytes by their subdivision produce four spermatozoa and four haploid cells (Sharma & Hanukoglu, 2018). The DNA methylation and histone modification have been implicated in the regulation of this process of spermatogenesis (Song et al., 2011).

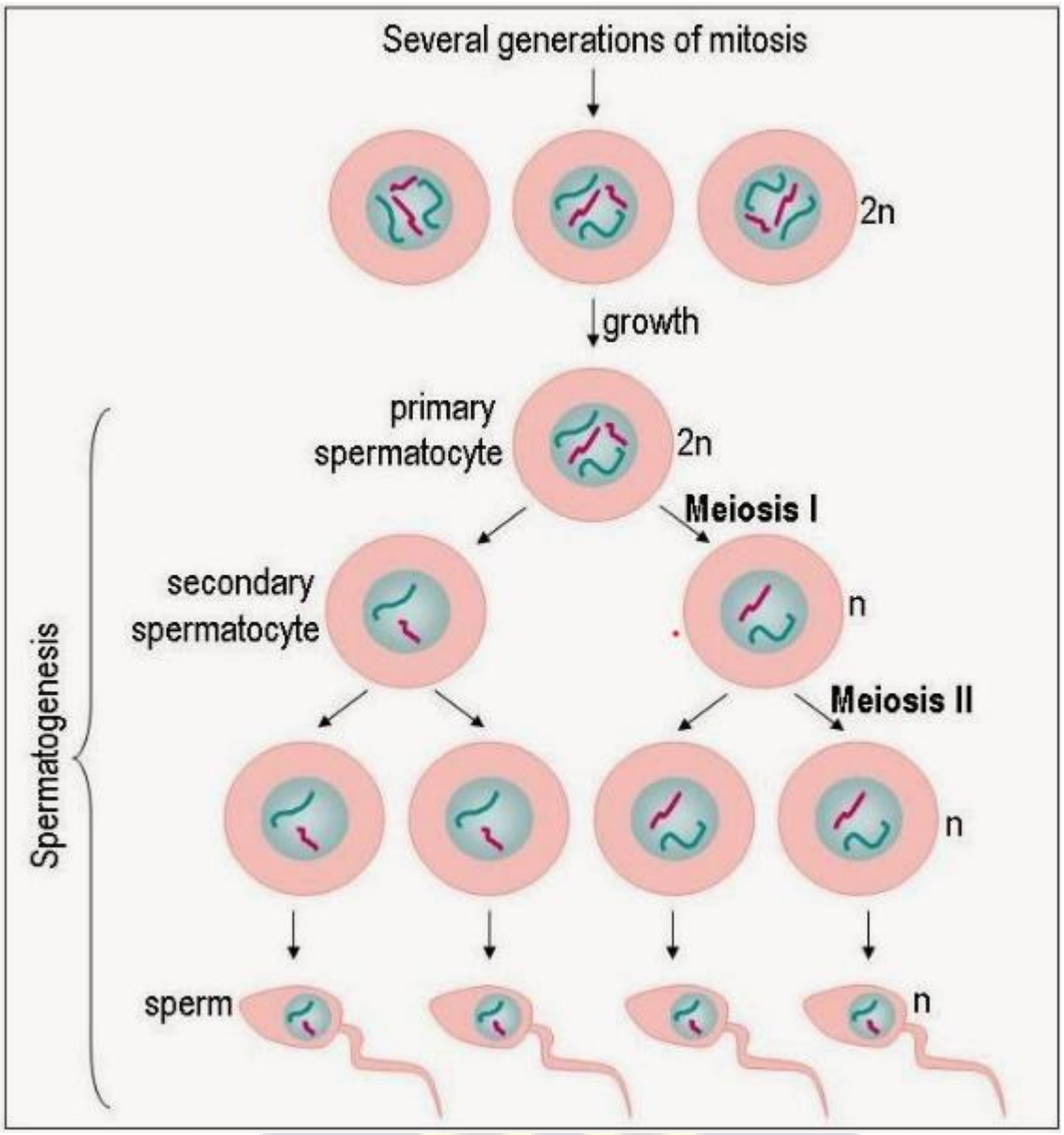


Figure 2.1 Phases of spermatogenesis

2.2 INFERTILITY

Skakkebaek et al., (2006) defined infertility as the inability of couple to achieve pregnancy after 12 months period of regular unprotected sexual intercourse. The Disabilities Act of 1998 in America recognised infertility as a disease and this recognition has led to the increased awareness of infertility and that it should be identified, registered and treated (Sabanegh & Agarwal, 2011).

The first epidemiologic study on infertility was conducted by Matthews Duncan (1866) in his book titled: Fecundity, Fertility and Sterility and not until recently when a population-based study on infertility was conducted (Templeton, 1992). To estimate the prevalence of male infertility is not easy, because population-based studies are few (Greenhall & Vessey, 1990; Thonneau & Spira, 1990) and until recently when United States (US) set up National Survey of Family Growth (NSFG) mainly to obtain periodic information on pregnancy, infertility, contraception, marriage and divorce among women, but in its sixth cycle, two thousand and two (2,002) men were included (Mosher & Pratt, 1991). Studies on the prevalence of male infertility are mostly clinic-based and these lead to low estimation of the prevalence of infertility (male) since only couples who sought medical intervention are recruited (Thonneau, Marchand & Tallec, 1991).

Suggestions have come from different authors to categorize infertility into ‘resolved’ infertility (those who get pregnant after a subfertile episode) and 'unresolved' infertility (those who do net pregnant) (Greenhall & Vessey, 1990; Mosher & Pratt, 1991).

2.3 EPIDEMIOLOGY OF MALE INFERTILITY

It has been reported that about 40-50% cases of infertility worldwide are because of male factors (CDC, 2013). The burden of male factors in infertility has been met with difficulty due to few large-scale population studies on male infertility (Winters & Walsh, 2014) and nevertheless with the available diagnostic techniques, male factors in infertility is common

(Collins et al., 1983). Philippov et al., (1988) conducted study on 2,000 married women using the WHO questionnaire reported 16.7% prevalence for couple infertility and 6.4% for male infertility while another study conducted on 1,686 couples in a French region revealed 14.1% infertility in the population, 39% due to male and female factors and 20% due to male infertility (Thonneau, Marchand & Tallec, 1991).

Bayasgalan et al., (2004) conducted a retrospective study in Mongolia and found out that male factors accounted for 25.6 % of all infertility. In North America, the estimated prevalence of male infertility was 4.5% to 6%, 9% in Australia and was 8% -12% in Eastern Europe (Agarwal et al., 2015).

Southeast Asia and Sub-Sahara Africa countries have high number of cases of infertility and various factors and different types of infertility were reported in the different African countries (Mascarenhas et al., 2012).

In developing countries, epidemiological study on male infertility has been less documented (Cates, Farley, & Rowe, 1985). In southwest Nigeria, a study conducted on 314 couples between 1997 to 1998 revealed 27.4% prevalence of sperm abnormality among infertile couples and female factors accounted for 28.5% (Ikechebelu et al., 2003).

In Ghana, Fiander (1990) recorded 45% male factor while Christian & Patrick (2017) reported 85.5% for male factor while male and female factors accounted for the remaining 14.5%.

Reports have shown differences in the prevalence of both primary and secondary infertility, North Africa and the Middle East, especially Morocco have recorded high rate of both primary and secondary infertility while Yemen recorded a low prevalence of secondary infertility. However, the Central and Eastern Europe and Central Asia have high prevalence of secondary infertility and low rate of primary infertility (Mascarenhas et al., 2012).

Differences in male infertility due to geographical variations have been documented. Eisenberg (2013) reviewed the NSFG and found that Caucasian men mostly undergo infertility evaluation.

On the other hand, reports from the United States Veterans administration revealed that Hispanics followed by African Americans and Caucasians have the highest number of occurrences for male infertility treatment (Meacham et al., 2007).

Templeton, Morris, & Parslow (1996) recognized that various epidemiological factors have contributed to couple's infertility and these include: age, a major determinant of infertility in female (the influence of age is less understood in male infertility) but the effects of smoking by both partners is highly relevant (smoking reduces sperm concentrations) (Joffe & Li, 1994).

2.4 CAUSES (TYPES) OF MALE INFERTILITY

Male infertility can be grouped into pre-testicular, testicular and post-testicular.

(i) Pre-testicular: This includes hypogonadism, erectile dysfunction, retrograde ejaculation, anejaculation, genetic factors, chromosomal abnormalities.

(ii) Testicular disorder: Examples of testicular disorder are testicular tumour, orchiectomy, undescended testes and atrophic testes. Another example is varicocele which weakens testicular thermoregulation due to interruption of the pampiniform venous plexus, the heat regulator; chemical toxins may cause epididymal dysfunction, epididymal cysts, epididymitis, or may be idiopathic (Stillman, 1982)

(iii) Post-testicular: It comprises injury of the seminal tract, inflammatory diseases, absence of the vas deferens congenitally, premature ejaculation and erectile dysfunction (Hikim et al., 2000).

2.4.1 MALE INFERTILITY AND VARICOCELE

A population prevalence of greater than 10% was observed in a survey of more than 10, 000 military recruits (Damonte et al., 1984) while in men with primary infertility is about 21%–41% and secondary infertility is 75%–81% (Will et al., 2011). It has been found to be a common cause of low sperm counts affecting 11% with normal semen and 25% with abnormal

semen (WHO, 1992a) and causes abnormal testicular temperature regulation (Goldstein & Eid, 1989).

2.4.2 MALE INFERTILITY AND INFECTIONS

Neisseria gonorrhoea has been implicated as an aetiological agent in obstructive azoospermia (Jequier & Holmes, 1984) and one of the effects of this infection is increased seminal white blood cell (leukocytosis) (Eggert-Kruse, Probst, & Rohr, 1995) and high level of white blood cell count (leukocytosis) leads to high production of reactive oxygen species (ROS) (Krausz et al., 1992). A prospective study conducted by Aitken, Krausz, & Buckingham (1994) shown that high levels of ROS generation in couples are likely to affect conception either spontaneously, or in in-vitro fertilization. Poor semen quality had been reported in men with prostatitis due to *Chlamydia trachomatis* (Mazzoli, Cai, & Addonisio, 2010) and C. *trachomatis* infection in males was associated with a low sperm count in Kuwait (Al-Sweih, Al-Fadli, & Omu, 2012) and Nigeria (Okoror & Agbonlahor, 2012). The effects of infection due to Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) on semen quality have been assessed and found that sperm concentration, sperm motility and sperm viability was decreased in HCV seropositive and HBV seropositive and HCV-HIV seropositive (people who live with either HCV, HBV, or HIV) males compared to controls (Lorusso, Palmisano, & Chironna, 2010).

2.4.3 MALE INFERTILITY AND SYSTEMIC AND IATROGENIC FACTORS

Certain medical disorders e.g., diabetes have direct or indirect effect on male infertility; cryptorchidism (undescended testes) generally impairs germ cell development leading to infertility in adulthood (Chung & Brock, 2011). In a study involving men with history of herniorrhaphy, 49 (56.3%) had testicular atrophy and low serum testosterone (<5 nmol/l), 52 (59.8%) had azoospermia and another 31 (35.6%) had severe oligozoospermia (Omu, Al-Azemi & Al-Jassar, 2013).

2.4.4 MALE INFERTILITY AND OCCUPATIONAL FACTORS

Reduced sperm count has been associated with working in hot environments, exposure to ionizing radiation or high temperature (Fleurian et al., 2009). A reduction in sperm densities has been observed in men who sit for long time (more than 8 hours) without standing, because of increase in scrotal temperature by 0.7ºC, and this impairs spermatogenesis thus resulting in abnormal semen parameters (Stoy, Hjollund, & Mortensen, 2004).

2.4.5 MALE INFERTILITY AND ENVIRONMENTAL FACTORS

Eaton, Schenker, & Whorton (1986) reported that certain heavy metals e.g., cadmium, lead, arsenic, zinc impair spermatogenesis, and certain pesticides and herbicides are known to hinder spermatogenesis. Consumption of fruits and vegetables with high remnant of pesticide have caused low sperm counts in men compared to men who consumed produce with lower remnant of pesticide (Yu-Han, Audrey, & Paige (2016). Endocrine-disrupting chemicals e.g., Bisphenol A found in plastics and food packages has effect on sperm counts because it causes errors in spermatogenesis (Liza, 2016); phthalates**,** found in vinyl plastic and personal care products do disrupt enzymatic activities involved in spermatogenesis thereby affecting sperm quality (Jung, Sultan, & Curtis, 2002) and perfluoroalkyl acids (PFAAs) found in non-stick cookware and water-repellent textiles have the ability to reduce numbers of normal sperm in men with high blood level of it compared to men with lower blood level of it, indicating that PFAAs disrupt spermatogenesis and alter sperm morphology (Ulla et al., 2009).

2.4.6 MALE INFERTILITY AND IMMUNOLOGICAL FACTORS

In about 8–10% of couples, men are more prone to causing ‘male immunological infertility’ because infertile men show autoimmunity to sperm, and that Anti-sperm antibody (ASA) interferes with the productive capability of spermatozoa. Therefore, ASA affects the motility of the spermatozoa, or the penetration of the oocyte (Vickram et al., 2019). Moreover, ASA

impairs the process of fertilization at the various levels e.g., acrosome reaction, zona pellucida recognition and penetration and sperm–vitellus interaction (Andrea et al., 2016).

2.4.7 MALE INFERTILITY AND GENETIC FACTORS

Genetic abnormalities account for 15-30% and may cause partial or complete irreversible spermatogenic arrest (Yoshida, Miura, & Shirai, 1997). Genetic causes of azoospermia include chromosomal abnormalities e.g., Y chromosome microdeletions and specific mutations or deletions of several Y chromosomal genes (Miyamoto et al., 2015). Human *SYCP3* (Synaptonemal Complex Protein 3) gene at 12q23 causes azoospermia by meiotic delay (Miyamoto, Hasuike, & Yogev, 2003).

2.5 SEMINAL ABNORMALITIES

Normal seminal fluid contains secretions from the testis, epididymis, Cowper's glands, periurethral glands and seminal vesicles. This fluid is made available from the glands in a stepwise manner during ejaculation. Major part of the ejaculate contains the largest amount of sperm, other part come from the epididymis, vas deferens in addition to some prostatic and seminal vesicle fluids. Semen liquefaction is brought about by the activity of proteases in the prostatic fluid and the plasminogen activator (Sabanegh & Agarwal, 2012).

Seminal vesicles produce 60% of the total volume which includes Fructose with range of 1.5-6.5mg/dl, Phosphorylcholine, Ergothioneine, Ascorbic Acid, Flavins and Prostaglandins. The prostate gland contributes 20% of total volume: Spermine, Citric acid, Cholesterol (phospholipids), Fibrinolysin (fibrinogenase) and Buffers: Phosphatase, Bicarbonate and Hyaluronidase (Barrett et al., 2012).

The diagnosis of male infertility is based on the examination of semen/sperm profiles, following standardised guidelines and these profiles include ejaculatory volume, sperm concentration, their motility, the vitality and the morphological appearances (WHO, 1992b). Estimation and detection of cells other than sperm and detection of anti-sperm antibodies are

also parts of semen analysis (Rrumbullaku, 2011).

2.5.1 VOLUME

After 2-7days of sexual abstinence, the normal volume of ejaculate is about 2-6ml. Hypospermia means less than 2ml of ejaculated semen while hyperspermia means greater than 6ml of ejaculated semen. In any semen analysis, the exact volume is essential, because it is used to calculate the total number of spermatozoa and cells other than semen in the ejaculate (WHO, 2010).

2.5.2 PH

It balances the pH values of the different secretions, secretion from seminal vesicles which is alkaline and the acidic secretion from prostate. The pH of normal fresh semen lies between 7.9 and 8.1 and measured by using pH paper (Vasan, 2011).

2.5.3 COLOUR

Semen has a homogeneous, grey-opalescent appearance and if the sperm concentration is very low, it becomes less opaque. The colour may also be different, i.e., red-brown when stained with red blood cells and referred to as haemospermia, or yellow in a man who is jaundiced or has taken certain vitamins or drugs (WHO, 2010).

Changes in semen colour may be due to the following reactions:

(a) Presence of urine: Urine may contaminate the semen, and this usually happens in men with the problem in bladder neck function and ejaculation. This can be detected by a high urea content in the semen sample. Semen contaminated by urine will contain either poorly motile or even completely immotile sperms, because of the lethal effect of urine on it.

(b) Presence of blood: Freshly blood-stained semen turned pinkish while presence of large amount of blood will impact it brighter. Infection of the seminal vesicles and prostate, trauma and malignancy of the testis may make blood present in semen sample. Semen may be coloured brown where bleeding had occurred in the genital tract few hours or even days

previously.

(c) Presence of bilirubin: Yellow colouration of semen may be due to the presence of bilirubin and have little effects on semen quality, but the associated liver disease can severely disturb spermatogenesis and may affect both the sperm count and motility (Vasan, 2011).

2.5.4 LIQUEFACTION

Typically, semen is semi-solid and within few minutes of ejaculation at room temperature the semen usually become thinner (liquified). At initial point, the fluid contains variety of lumps and as liquefaction continues, the semen becomes more thinner and quite watery. Complete liquefaction at room temperature is within 15-20 minutes and does not go up to 60 minutes or more (WHO, 2010). Liquefaction is usually assessed visually. Unliquefied semen contains a semi-solid coagulum and partially liquefied semen contains many small gels-like clots. In fully liquefied semen no such clots are seen and the semen appears completely watery. Alpha-amylase (α-amylase) is used to liquefy unliquefied semen (Vasan, 2011).

2.5.5 VISCOSITY

Completely liquefied semen sample leaves the pipette drop by drop. In viscosity abnormality, a drop of semen with the aid of pipette will form a thread more than 2 cm long, semen exhibits uniform stickiness, and the semen remains in this state with time. High viscosity can be recognized when semen strongly adheres to itself while attempts made to pipette it and this is due to the elastic properties of the sample (WHO, 2010).

2.5.6 SPECIFIC GRAVITY

The average specific gravity of normal semen sample is 1.028 (Godkar & Godkar, 2010).

2.5.7 SPERM MOTILITY

It is a forward movement of spermatozoa and good to be assessed within 25-30 minutes after liquefaction of the sample and at most between 1 hour after discharge. This is to restrict the

influence of drying (dehydration), pH, or changes in temperature on motility. The motility of each spermatozoon is assessed as follows:

(i) Progressive motility (PR): Active movement of spermatozoan either straight forward or in a circular movement irrespective of speed.

(ii) Non-progressive motility (NP): All other patterns of movement with an absence of progressive motility.

(iii) Immotile (IM): No movement (WHO, 2010).

Methods used to assess sperm movement:

1. Slide technique (by using grease-free coverslip preparation): minimum of 200 sperms should be counted.
2. By using Markler or Horwell chambers (Vasan, 2011).

2.5.8 SPERM VITALITY

It is used for the assessment of the viability and the integrity of the sperm cell's membrane. This test provides a check on the motility evaluation. The percentage of living (viable) cells normally exceeds that of motile cells. The dye exclusion principle is used since spoilt plasma membranes, such as those found in dead cells which then allow stains to enter (WHO, 2010).

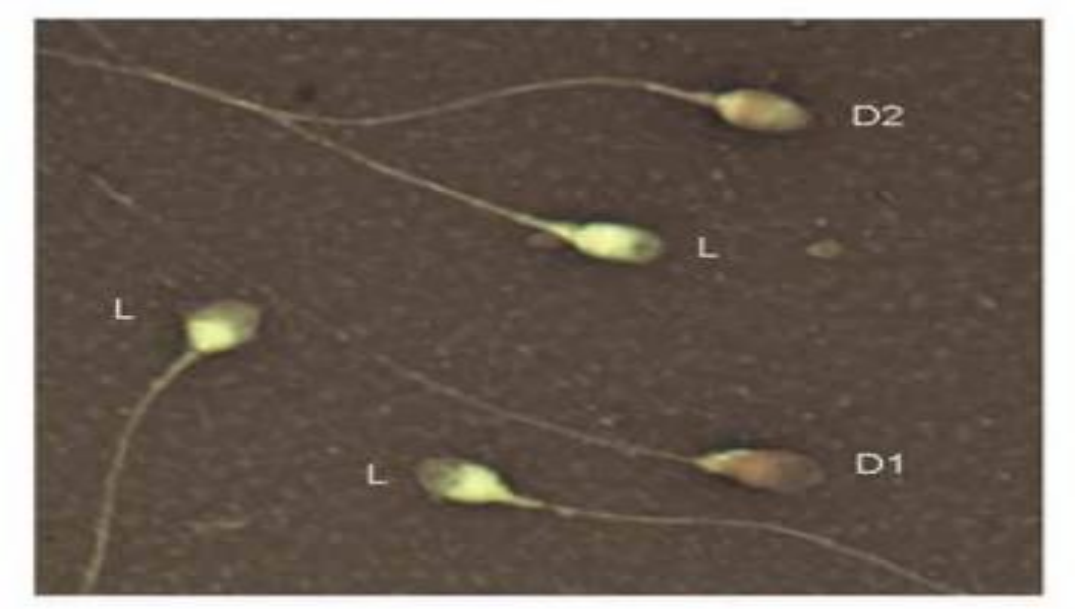


Figure 2.3 Sperm vitality result: L: Live, D1: Dead

2.5.9 SPERM MORPHOLOGY

Due to the predictive value as a fertility determinant, sperm morphology in the recent years has attracted attention. Morphologically abnormal sperms show multiple defects and refer to as teratozoospermic index which is a predictor of sperm function both in vivo and in vitro. WHO classification, or by Kruger's strict criteria classification can be used to score semen morphology Leushuis et al., (2010).

(i) Head defects: These include large, small, tapered, pyriform, round, amorphous, vacuolated, double heads and a combination of any of the above.

(ii) Neck and midpiece defects: These include bent neck, fusion of the midpiece into head, thick, uneven midpiece, irregular thin midpiece and a combination of these.

(iii) Tail defects: These include short, multiple, hairpin, broken, bent, kinked, coiled tails and combinations of any of these.

(iv) Cytoplasmic droplets: Cytoplasmic droplets occupy greater than one-third of the area of a normal sperm head (Barrett et al., 2012).

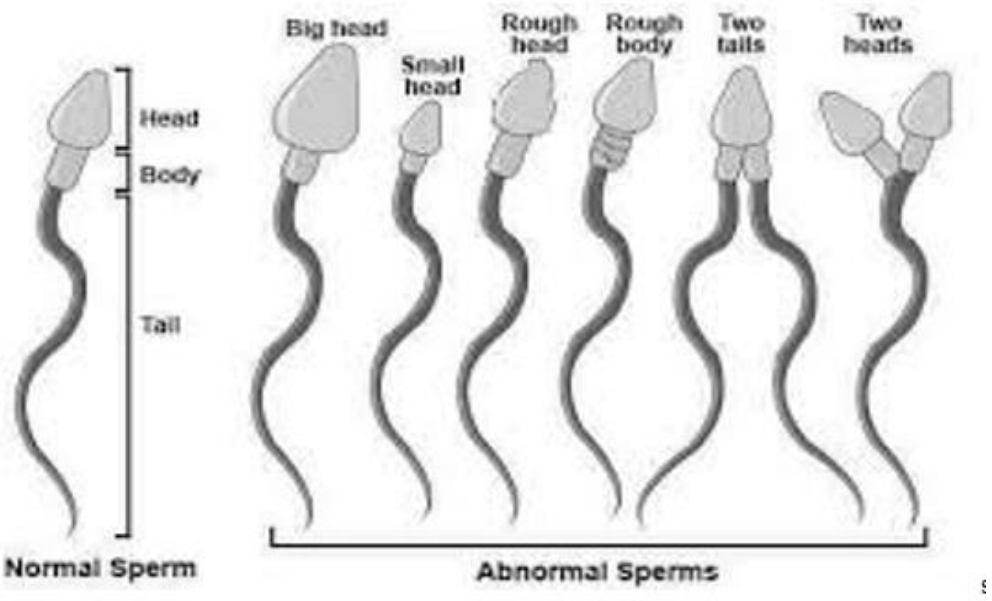


Figure 2.2 Normal and abnormal sperm cells

2.5.10 SPERM AGGLUTINATION

Agglutination refers to motile spermatozoa sticking to each other e.g., head-to-head, tail-to-tail or in a mixed way. Agglutination affects motility, and at times when the spermatozoa are so agglutinated that their motion is curtailed.

Agglutinations are graded thus:

(i) Grade 1: isolated <10 spermatozoa per agglutinate.

(ii) Grade 2: moderate 10–50 spermatozoa per agglutinate.

(iii) Grade 3: large agglutinates of >50 spermatozoa.

(iv) Grade 4: gross, all spermatozoa agglutinated, and agglutinates intertwined ((Rose, 1976)

2.5.11 SPERM COUNT

The total sperm count (per ejaculate) and the sperm concentration (per unit mL) are related to pregnancy (Slama, 2002; Zinaman, 2000). When there is no obstruction in the male tract and the abstinence time short, the total number of spermatozoa in the ejaculate is mutually related with testicular volume which is a measure of the ability of the testes to produce spermatozoa (Behre, 2000; MacLeod & Wang, 1979). Total sperm count refers to the total number of

spermatozoa in the entire ejaculate which is obtained by multiplying the sperm concentration by the total volume of semen ejaculated while sperm concentration refers to the number of spermatozoa per unit volume of fluid diluting them (WHO, 2010).

2.5.12 LEUKOCYTES IN SEMEN

Polymorphonuclear (PMN) leukocytes (neutrophile, eosinophils and basophils) are present in most human ejaculates and the abnormal count of leukocytes in semen is referred to as pyospermia and suggestive of genital tract inflammation (Johanisson, 2000). Round cells (lymphocytes & immature germ cells) are different from PMN leukocytes, round cells are peroxidase negative whereas PMN are peroxidase-positive granulocytes (Barrett et al., 2012).

2.5.13 SPERM FUNCTION TESTS

These include:

(I) SPERM-CERVICAL MUCUS PENETRATION TEST: It is a after intercourse (post–coital) test designed to describe the effect of a woman's cervical mucus on sperm, and usually carried out within 2-24 hours after intercourse, when ovulation should have started. Cervical mucus is collected with the aid of a spatula and examined under a microscope, and if no surviving sperm or no sperm at all is observed, then the cervical mucus should be cultured for the presence of bacteria. This test is not used to assess sperm movement from the cervix into the fallopian tubes or the sperm's ability to fertilize an egg (Kumar, 2012).

(II) MICRO-PENETRATION ASSAY TEST: It is used to demonstrate if sperm can enter hamster eggs that have had the covering removed. If less than 5-20% of the eggs are penetrated by the sperm, infertility is diagnosed. It may be used for determining the best assisted reproductive treatment options for men with infertility (Kumar, 2012).

2.6 WHO DIAGNOSTIC REFERENCE

Table 2.1 **Diagnostic reference values/terminologies (WHO, 2010)**

Semen Parameter	**Interpretation**
Semen volume	1.5- 6.0 mL)
Total sperm number	33-46 million/ejaculate
Sperm concentration	15 million/mL
Total motility (PR + NP)	>40%
Vitality (live spermatozoa)	>58%
Sperm morphology (normal forms)	4-48%
pH	7.2-8.0
Necrozoospermia	No viable (living) sperm
Aspermia	No ejaculate
Globozoospermia	Acrosome round head defect
Leucocytospermia	$>1x10^6$/ml (white cell count)
Azoospermia	No sperm cells in the ejaculate
Teratozoospermia	Abnormal sperm morphology <15%
Oligospermia	Sperm count $<15x10^6$/ml
Seminal fructose	≥13 µmol/ejaculate

CHAPTER THREE

METHODS

3.1 RESEARCH METHODOLOGY

This chapter describes the methods used for this study, including the study design, study area and sampling and sample size considerations, study procedure, data collection, data analysis in addition to ethical considerations. Both dependent and independent variables were categorised as shown below.

3.2 STUDY DESIGN

A retrospective record review was conducted to study the semen profile of male patients aged between 18- and 65-years attending urology clinic for infertility. A multi-stage sampling method was employed. The first stage involved was the purposive selection of both the urology clinic and the surgical research laboratory. In the second stage, laboratory registers were screened for eligibility and registers covering September 2016 – August 2018 were selected. Patients' results in the selected registers were screened for inclusion criteria, sampling interval was calculated and simple random sampling was performed. The data source reviewed was laboratory registers at the study site. A pre-tested data abstraction guide that covered the age, sperm concentration, motility, morphology, vitality, sperm cytology, volume, clinical and pH conditions was used to collect information of the patients. Data were subjected to appropriate statistical analysis and inferences were drawn. The outcomes of interest include no sperm cells in ejaculate, low sperm concentration, no ejaculate, low quantity of semen, increased quantity of semen, spermatozoa abnormalities and the clinical conditions of the patients.

3.3 STUDY SITE

The study was carried out in Surgical Research Laboratory situated within the College of Medicine, University of Ibadan/University College Hospital, Ibadan. It is a Federal Teaching

Hospital established in 1952 by Act of Parliament. Currently, the hospital has 1,000 bed spaces and 200 examination couches with occupancy rates ranging from 65 to 70%. The capital of Oyo State in south-western Nigeria is Ibadan, it is 128 kilometres northeast of Lagos and 530 kilometres southwest of Abuja, the federal capital of Nigeria. Ibadan is the third largest metropolitan city in Nigeria, with population of 1,338,659 according to the 2006 census (National Population Commission, 2006). The Urology clinic is being conducted by consultants in various subspecialties of urology and involve in the training of medical doctors and other health professionals. In terms of volume of patients, the Urology clinic receives patients both within its environment and accept referrals from both public and private health facilities. Factors considered in choosing the surgical research laboratory was because it serves the clinic and therefore expected to have more results on semen analysis and reasonably maintained high records keeping. Also, considered were the facts that qualified and certified laboratory personnel processed their samples.

3.4 STUDY POPULATION

The target population comprised male patients seen at Urology Clinic, University College Hospital while sampled population was male patients aged between 18 and 65 years who had semen sample processed at the study site between the period under review.

3.5 STUDY VARIABLES

3.5.1 OUTCOME/DEPENDENT VARIABLES

The outcome of interest is the semen profile of the target respondents operationalised as the proportion with non-/inadequate production of semen/sperms which include azoospermia (no sperm cells in ejaculate), oligospermia (low sperm concentration), aspermia (no ejaculate), hypospermia (low quantity of semen), hyperspermia (increased quantity of semen) and spermatozoa abnormalities which include morphology, pH, vitality, and motility. The clinical

conditions of the patients e.g., varicocele, trauma, erectile dysfunction etc were also used to analyse the sperm profile and categorised.

- Ejaculatory volume: Discrete variable but was categorised as decreased (<1.5mL), normal (1.5-6.0), increased (>6.0)
- pH: It is continuous variable but categorised as acidic, neutral, and basic
- Sperm concentration: Discrete variable but categorised as low (<1million/mL), normal (1-15million/mL), high (≥15million/mL).
- Morphology: Discrete variable but dichotomised as normal (≥4%) and abnormal (<4%)
- Motility: Discrete variable but categorised as dead, progressive, and non-progressive.
- Vitality: Discrete variable but dichotomised as normal (≥58%) and abnormal (<58%).
- Viscosity: Categorised as decreased, normal and increased
- Duration of abstinence: Categorical but grouped as 2-3days, 4-5days and >5days.
- Varicocele: Categorised as palpable and impalpable.
- Trauma: Categorised as blunt and penetrating
- Inflammation: Categorised as acute and chronic
- Erectile dysfunction: Categorised as mild and severe.

3.5.2 INDEPENDENT OR EXPOSURE VARIABLES

The independent variables among the respondents are environmental factors, radiations, genetics factors, hormonal factors, vasectomy, and infections.

Social Demographic

- Age: A continuous variable, but was categorised into age groups thus: <30, 30-50, and >50

Environmental factors:

- Chemicals: herbicides, insecticides
- Lifestyle: Alcoholism, drug abuse
- Stress

Hormonal factors:

- Follicle stimulating hormone
- Luteinizing hormone

Genetic factors:

- Y chromosome deletions
- Chromosomal translocations
- Cystic fibrosis gene mutation

Infections:

- Sexually transmitted diseases
- Orchitis

Testicular trauma/abnormality

Vasectomy post- pelvic surgery: It is post-pelvic surgery where the testes still produce sperm, but they are soaked up by the body thereby causing reduced (low volume) of ejaculate

3.6 SAMPLE SIZE DETERMINATION

The minimum sample size (N) was calculated using Cochran's (1977) formula and the prevalence of sperm abnormality among infertile couples in southwest, Nigeria was 24.7% (Ikechebelu et al., 2003).

The minimum sample size (N) was calculated using Cochran's (1977) formula.

$N = \underline{Z^2pq}$

d^2

where,

N= the minimum sample size required.

Z= the standard normal deviation set at 1.96 which corresponds to 95% confidence level.

p= the prevalence of sperm abnormality among infertile couples in southwest, Nigeria 24.7% (Ikechebelu et al., 2003).

q= 1-p

q= 1-0.247 (24.7/100)

q=0.753

d= the level of significant set at 0.05.

Therefore,

$$N = \frac{1.96^2 \times 0.247 \times 0.753}{0.05^2}$$

$$= \frac{3.8416 \times 0.247 \times 0.753}{0.0025}$$

$$= \frac{0.7145}{0.0025}$$

= 285.8

Therefore 286 patients' results were abstracted into this study.

3.7 DATA COLLECTION TOOL

Data abstraction guide was employed to obtain information on patient's result from the source documents on the following age, year of the test, clinical diagnosis, volume, morphology, count, motility, vitality, pH and abstinence.

3.8 INCLUSION CRITERIA

Eligible patients must meet the following inclusion criteria: (i) the patient aged between 18 and 65 years; (ii) no previous history of vasectomy; (iii) semen produced through masturbation; (iv) abstinence within 2 and 7 days; and (v) sample processed between September 2016- August 2018.

3.9 EXCLUSION CRITERIA

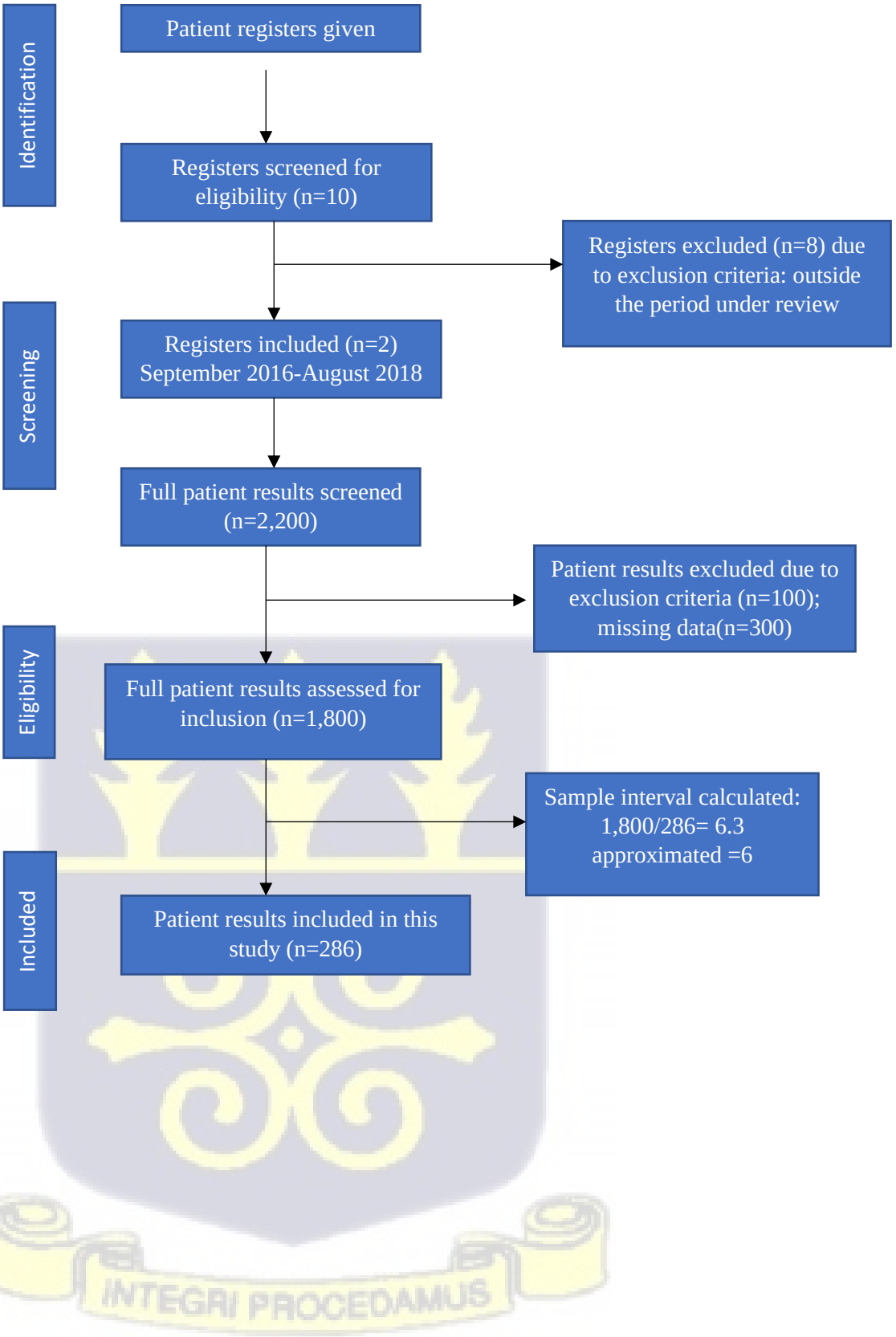
Patients who have no record of time of abstinence, sample produced through coitus interruptus (withdrawal method) and registers outside the period under review were excluded.

3.10 DATA COLLECTION PROCEDURE (TECHNIQUE)

Registers for patients' results were requested from the laboratory personnel and ten registers were given. Registers were screened for eligibility and eight registers were excluded because they were outside the period under review. The registers included in this study are those that covered between September 2016 - August 2018. The total results screened between September 2016 - August 2018 were two thousand, two hundred (2,200); one hundred (100) results were excluded due to exclusion criteria and three hundred (300) results due to missing data. The remaining one thousand eight hundred (1,800) results met the inclusion criteria. Sampling interval was calculated by dividing the total results that met the inclusion criteria by the desired sample size (286), then 6.3 approximated to the nearest whole number, 6. Numbering to sampling interval was done i.e., 1, 2, 3, 4, 5, & 6 and then simple random sampling was performed. For example, if No 2 was selected by simple random sampling, then the name of

the patient on the register (among 1,800 results that met the inclusion criteria) stating from September 2016 corresponding to No 2 became the first result abstracted on this study. To abstract the second result on the study, 2 (the first-person number) was added to the sampling interval, 6 which became 8 (2+6), then the person on No 8 became the second result abstracted on this study. This was repeated until the last result for the sample size was abstracted. Data abstracted included age, sperm concentration, motility, morphology, vitality, sperm cytology, volume, and pH. Each data point was assigned a unique serial number when they are abstracted. Below is the flow chart showing identification, screening, eligibility, and selection of patient result for this study.

Figure 3.5 Flow chart showing selection of patients' result for this study.



3.11 DATA PROCESSING

Data were first entered into the Microsoft excel version 2019 and exported to Stata 2016 for analysis mean, mode, frequencies and percentages. Data were checked for completeness, duplicates, range, and consistency e.g., participant age less than 18 years old was invalid. Cleaned data exported to STATA for analysis.

3.12 DATA ANALYSIS

Descriptive statistics was performed for all continuous variables e.g., age, semen parameters and presented as mean, median, standard deviation, percentages and range. Pie chart, frequency table etc was used to present and summarize patient's result. The Chi square was used to the test the association of categorical variable (clinical diagnosis) and continuous variables (age, semen parameters). Logistic models were fitted to the data to determine the strength of relationship between variables. Statistically significance was assessed at the 5% level of 95% Confidence Interval were constructed around point estimate.

3.13 ETHICAL CONSIDERATIONS

Ethical approval was obtained from UI/UCH Ethics Committee with approval number: UI/EC/21/0193. Also, permission was sought and granted from the Head of Surgery Department, College of Medicine, University of Ibadan for data access.

3.13.1 CONFIDENTIALITY OF DATA

Strict confidentiality was maintained throughout the study. Information extracted from the source documents was kept secret and without traceable to any of the patient.

3.13.2 BENEFICENCES TO PARTICIPANTS

The research will provide information on semen abnormalities seen to the prescribers of seminal fluid analysis which will be used to address challenges arising from morphological abnormalities seen in male infertility.

3.14 QUALITY CONTROL

Data abstraction guide was prepared to reflect the parameters usually reported for semen analysis and clinical conditions. Before data collection, the data collection form was presented to the laboratory personnel at the study site to cross check with their laboratory report and it was adjudged good. In ensuring the quality of the data, data validation was set on Excel before data entry for patient's hospital number to avoid duplications of patient's result. Each data point was assigned a unique serial number when they are abstracted.

3.15 CATEGORIZATION OF SEMEN PROFILES AND CLINICAL CONDITIONS

The figure below shows the categorization of semen profiles and the clinical conditions and these were used to explain the results in this study.

Figure 3.6 Categorization of semen profiles and clinical conditions

Azoospermia	No/absence of sperm cells in semen produced/ejaculated
Oligospermia	Low sperm count in one ejaculate
Vitality	Sperm cells being alive/active
Ejaculatory volume	Quantity of semen per ejaculate
Sperm concentration	No of sperm cells in one ejaculate
Sperm motility	No of sperm cells capable of moving either progressively or non-progressively
Viscosity	Thickness/stickiness of semen
Sperm morphology	Shapes/structures of sperm cells in the ejaculate
pH	State of being Acidic, Neutral or Basic
Varicocele	Enlargement of the veins that hold the testicles (scrotum)
Erectile dysfunction	Inability to keep/sustain erection
Primary infertility	Pregnancy has never been achieved
Secondary infertility	At least one prior pregnancy has been achieved
Trauma	Physical injury to the testicles or scrotum
Infection	Growth of germs
Inflammation	Inflamed scrotum

3.16 LIMITATION OF THE STUDY

- A spermogram is the biological test of the semen which is a key biological test in the first-line assessment of male infertility. But this test was not part of laboratory request carried out at the study site therefore, not found in the laboratory registers.

CHAPTER FOUR

RESULTS

INTRODUCTION

This chapter presents the analysis and interpretations of finding from this study and are presented objective by objective in tables and graphs showing percentages, frequencies, and associations.

4.1 DISTRIBUTION OF PATIENTS ACCORDING TO RESULTS OF SEMEN ANALYSIS AND CLINICAL CONDITIONS

The age of patients ranged from 18 to 64 years with mean age of 38.0 ± 7.6 years. The modal age group was 30-50 years 243 (85.0%). The clinical condition most reported among the patients was the enlargement of the veins that hold the testicles 143 (49.3%), followed by inability to keep/sustain erection 42 (14.7%) and physical injury (trauma) to the scrotum had 39 (13.6%). Patients with decreased thickness/stickiness of semen was 153 (53.5%), normal stickiness 115 (40.2%) and increased thickness was 18 (6.3%). In this study, semen with acidic pH was 24 (8.4%), neutral pH was 205 (71.7%), and basic pH was 57 (19.9%). The study of the duration of sexual abstinence revealed that 92 (32.2%) patients had 2-3 days, 185 (64.7%) had 4-5 days and 9 (3.2%) had >5 days The study of the number of sperm cells capable of moving either progressively or non-progressively revealed that 61 (26.6%) of patients recorded all sperm cells dead, 49 (21.4%) had progressive movement and 119 (52.0%) had non-progressive movement. No of sperm cells in one ejaculate shown that 57 (19.9%) of patients were diagnosed of no/absence of sperm cells in the semen ejaculated, 143 (50.0%) had low sperm count in one ejaculate while 86 (30.1%) had normal sperm count. In this study, patients with decreased quantity of semen produced per ejaculate was 64 (22.4%), normal quantity was 213 (74.5%) and increased quantity was 9 (3.2%).

Table 4.1 **Distribution of patients according to the results of semen analysis and clinical diagnoses.**

Parameters	Frequency	Percent
Age group (yrs)		
<30	35	12.2
30-50	243	85.0
>50	8	2.8
Mean (±SD)	38.0±7.6	
Range	18-64yrs	
Varicocele	141	49.3
Trauma	39	13.6
Inflammation	26	9.1
Infection	18	6.3
Erectile dysfunction	42	14.7
Testicular torsion	20	7.0
Viscosity		
Decreased	153	53.5
Normal	115	40.2
Increased	18	6.3
pH		
Acidic	24	8.4
Neural	205	71.7
Basic	57	19.9
Motility (%)		
Dead	61	26.6
Progressive	49	21.4
Non-progressive	119	52.0
Vitality (%)		
Normal	176	61.5
Abnormal	110	38.5
Duration of Abstinence		
2-3 days	92	32.2
4-5 day	185	64.7
>5 days	9	3.1
Sperm concentration (M/mL)		
Low	57	19.9
Normal	143	50.0
High	86	30.1
Ejaculatory volume (mL)		
Decreased	64	22.4
Normal	213	74.5
Increased	9	3.1
Total	**286**	**100**

4.2 ASSOCIATION BETWEEN CLINICAL CONDITIONS AND SPERM MORPHOLOGY

Out of the 286 patients, 107 (37.4%) had the enlargement of the veins that hold the testicles (scrotum). From these 84 patients, 14 (16.7%) had abnormal palpable enlargement of the veins that hold the testicles (scrotum). Association between shapes/structures of sperm cells in the ejaculate and the enlargement of the veins that hold the testicles (scrotum) was statistically significant (x^2 = 15.04, p-value <0.001). Out of the 286 patients, 31 (10.8%) had history of physical injury to the testicles. Of these 31 patients, 5 (38.5%) was blunt physical injury with abnormal structures of sperm cells in the ejaculate. This was statistically significant (x^2 = 12.71, p-value =0.001). The other two variables, inflamed scrotum, and inability to sustain erection were not statistically significant at p=0.05

Table 4.2 **Association between clinical conditions and sperm morphology**

Clinical condition	Total	Normal	Abnormal	Test Statistic	P-value
Varicocele				**15.04**	**<0.001***
Impalpable	23	14(60.9)	9(39.1)		
Palpable	84	70(83.3)	14(16.7)		
Trauma				**12.71**	**0.001†***
Blunt	13	8(61.5)	5(38.5)		
Penetrating	18	16(88.9)	2(11.1)		
Inflammation				0.64	0.999†
Acute	15	13(86.7)	2(13.3)		
Chronic	5	4(80.0)	1(20.0)		
Erectile dysfunction				1.58	0.399†
Mild	31	24(77.4)	7(22.6)		
Severe	8	5(62.5)	3(37.5)		

*Significant (p<0.05) using chi-square test; †Fisher's exact test

4.3 ASSOCIATION BETWEEN MOTILITY, VITALITY, VOLUME, VISCOSITY, PH, SPERM MORPHOLOGY, AND DURATION OF ABSTINENCE AND SPERM CONCENTRATION.

It was revealed that total number of motile sperm cells (progressive and non-progressive) (x^2 = 8.11, p-value 0.02) and sperm cells that are alive (x^2 = 4.52, p-value 0.04) were statistically significant, therefore, have association with number of sperm cells in one ejaculate sperm. However, quantity of semen per ejaculate (x^2= 1.50, p-value 0.23), thickness/stickiness of semen (x^2 = 0.14, p-value 0.93), state of semen being acidic, neutral, or basic (x^2= 0.69, p-value 0.71), shapes of sperm cells per ejaculate (x^2 = 3.24, p-value 0.08) and days of abstinence (x^2 = 1.10, p-value 0.58) were statistically not significant and therefore have no association with number of sperm cells (sperm count) in the ejaculate.

Table 4.3 Association between motility, vitality, volume, viscosity, pH, sperm morphology, and days of abstinence and sperm concentration

Variables	Sperm Concentration		Total	Test Statistic	P-value
	Normal	Abnormal			
Semen ejaculatory volume				**1.50**	**0.23**
Normal	66(28.8)	119(52.0)	185(80.8)		
Abnormal	20(8.7)	24(10.5)	44(19.2)		
Viscosity				**0.14**	**0.93**
Decreased	48(21.0)	80(34.9)	128(55.9)		
Normal	33(14.4)	53(23.1)	86(37.6)		
Increased	5(2.2)	10(4.4)	15(6.6)		
pH				**0.69**	**0.71**
Acidic	7(3.1)	11(4.8)	18(7.9)		
Neutral	59(25.8)	105(45.9)	164(71.6)		
Basic	20(8.7)	27(11.8)	47(20.5)		
Motility (%)				**8.11**	**0.02****
Dead	0(0.0)	4(1.7)	4(1.7)		
Progressive	3(1.3)	18(7.9)	11(9.2)		
Non-progressive	83(36.2)	102(44.5)	204(89.1)		
Vitality (%)				**4.52**	**0.04***
Normal	72(31.4)	38(16.6)	110(48.0)		
Abnormal	14(6.1)	105(45.9)	119(52.0)		
Sperm morphology (%)				**3.24**	**0.08**
Normal	72(31.4)	105(45.9)	177(77.3)		
Abnormal	14(6.1)	38(16.6)	52(22.7)		
Days of abstinence				**1.10**	**0.58**
2-3 days	29(12.7)	49(21.4)	78(34.1)		
4-5days	54(23.6)	92(40.2)	146(63.8)		
>5days	3(1.3)	2(0.9)	5(2.2)		

*Significant ($p<0.05$) using chi-square test; †Fisher's exact test

4.4 TYPES OF INFERTILITY AMONG THE ATTENDEES

It was shown that out of 286 patients that came to the urology clinic the period under review, 159 (55.6%) had never achieved pregnancy while 127 (44.4%) had at least achieved one prior pregnancy.

Table 4.4 Types of infertility among the attendees

Types of infertility	Frequency	Percent
Primary	159	55.6
Secondary	127	44.4
Total	**286**	**100**

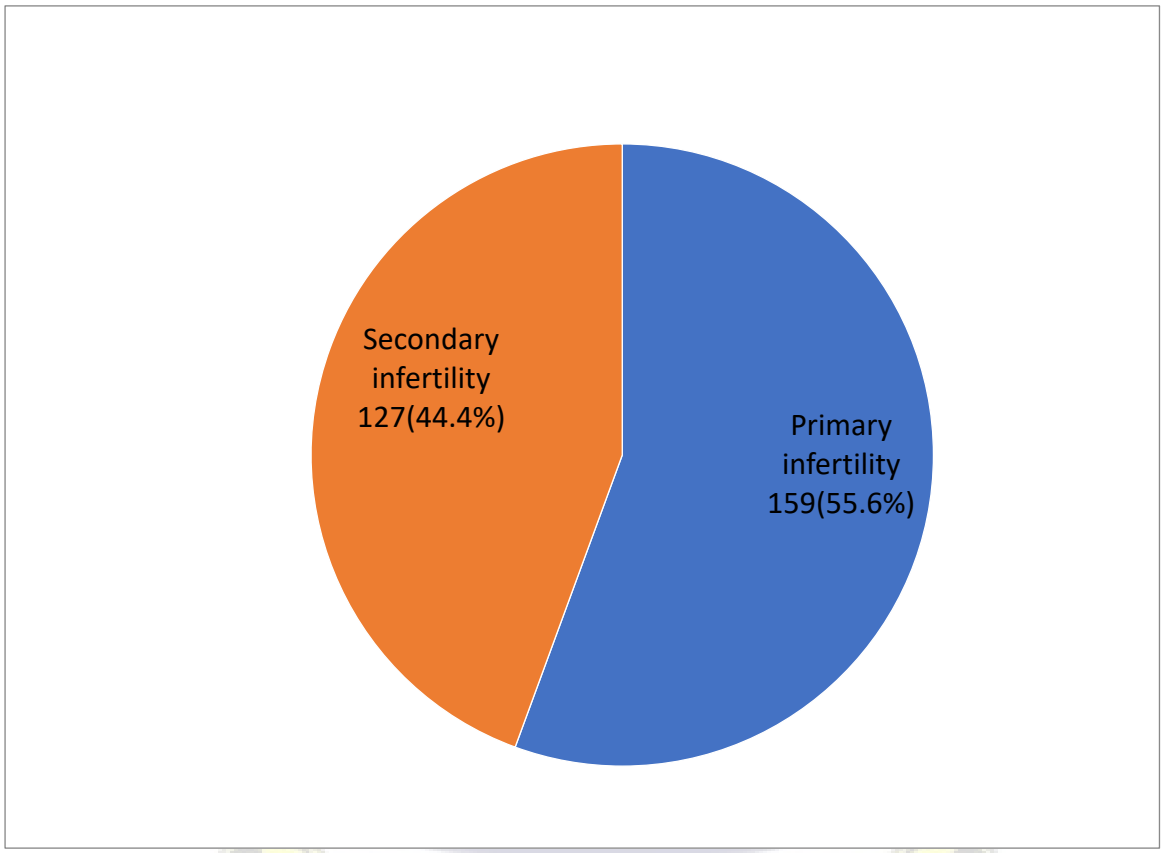


Figure 4.7 Pie chart showing types of infertility

4.5 THE CAUSES (TYPES) OF INFERTILITY COMMONLY REPORTED TO THE CLINIC

It was revealed from this study that the enlargement of the veins that hold the testicles (scrotum) 141 (49.3%) accounted for the overall cause of inability to impregnate. It was responsible for primary infertility 79 (27.6%) and secondary infertility 62 (21.7%). This was followed by inability to keep/sustain erection with overall cause of infertility 42 (14.7%) and 23 (8.0%) for primary infertility and 19 (6.6%) for secondary infertility while physical injury to the scrotum had overall cause of 39 (13.6%) and 20 (7.0%) for primary infertility and 19 (6.6%) for secondary infertility. Other factors considered were abnormal sperm structures in the ejaculate which contributed 52 (22.7%) cause of infertility among the patients while the percentage of low sperm count in patients was 62.2% and occurred more in primary infertility 79 (34.5%) than in secondary infertility 64 (27.9%).

Table 4.5 Causes (types) of infertility commonly reported to the clinic

Variables	Types of infertility		Total
	Primary	Secondary	
Clinical condition			
Varicocele	79(27.6)	62(21.7)	141(49.3)
Trauma	20(7.0)	19(6.6)	39(13.6)
Inflammation	19(6.6)	7(2.5)	26(9.1)
Infection	7(2.4)	11(3.9)	18(6.3)
Erectile dysfunction	23(8.0)	19(6.6)	42(14.6)
Testicular torsion	11(3.9)	9(3.2)	20(7.1)
Sperm morphology (%)			
Normal	97(42.4)	80(34.9)	177(77.3)
Abnormal	31(13.5)	21(9.2)	52(22.7)
pH			
Acidic	12(4.2)	12(4.2)	24(8.4)
Neutral	117(40.9)	88(30.8)	205(71.7)
Basic	30(10.5)	27(9.4)	57(19.9)
Sperm concentration (m/mL)			
Normal	49((21.4)	37(16.1)	86(37.5)
Low	79(34.5)	64(28.0)	143(62.5)
Motility			
Dead	34(11.9)	27(9.4)	61(21.3)
Progressive	7(2.4)	14(4.9)	21(7.3)
Non-progressive	118(41.3)	86(30.1)	204(71.4)
Total	**159(55.6)**	**127(44.4)**	**286(100)**

4.6 MULTIPLE LOGISTIC REGRESSION ANALYSIS FOR FACTORS ASSOCIATED WITH SEMEN ABNORMALITY

Multiple logistic regression analysis was performed on all variables that were statistically significant at 95% CI and p-value < 0.05 as well as those with strong theoretical or empirical basis for their addition were used as explanatory variables in the multivariate logistic regression model.

These variables were quantity of semen ejaculated, motility, no of sperm cells alive/active, sperm structures, enlargement of the veins that hold the testicles (scrotum), physical injury to scrotum, inflamed scrotum, and inability to keep erection. Out of these variables, only 3 were

statistically significant and had an association with infertility in the multiple logistic regression model (p-value< 0.05).

Table 4.6 below indicates how the related factors are associated with infertility using simple and multiple logistic regression analysis to determine the crude and adjusted odds ratios, their corresponding 95% CI and p-values. After adjusting for other variables in the multiple logistic regression model, there was a 64% decrease in odds of infertility among those with sperm motility >40% (a OR = 0.36, 95% CI = 0.17 - 0.78).

All the other variables were not significantly associated with at the multiple logistic regression analysis level at 95% CI and a p-value <0.05. Details are as shown in the table below

Table 4. 6 Multiple logistic regression analysis for factors associated with sperm abnormality

Variables	Type of infertility		Unadjusted	p-value	Adjusted	p-value
	Primary	Secondary	OR (95% CI)		OR (95% CI)	
Semen ejaculatory volume						
Normal	66(28.8)	119(52.0)	1		1	
Abnormal	20(8.7)	24(10.5)	0.81(0.46 - 1.42)	0.46	0.89(0.45 - 1.79)	0.75
Motility						
Dead	34(11.9)	27(9.4)	1		1	
Progressive	7(2.4)	14(4.9)	1.94 (0.88 - 4.28)	0.10	-	
Non-progressive	118(41.3)	86(30.1)	0.82 (0. .45 - 1.47)	0.51	0.36(0.17 - 0.78)	**0.009***
Vitality (%)						
Normal	72(31.4)	38(16.6)	0.82(0.44 - 1.51)	0.52	0.96(0.46 - 2.02)	
Abnormal	14(6.1)	105(45.9)	1			0.92
Sperm morphology (%)						
Normal	97(42.4)	80(34.9)	1			
Abnormal	31(13.5)	21(9.2)	0.84 (0.45 - 1.55)	0.57	0.58(0.28 - 1.20)	0.14
Varicocele						
Impalpable	14(60.9)	9(39.1)	1		1	
Palpable	70(83.3)	14(16.7)	2.06(1.45 - 7.82)	**<0.001***	7.37(3.23 - 10.76)	**<0.001***
Trauma						
Blunt	8(61.5)	5(38.5)	1		1	
Penetrating	16(88.9)	2(11.1)	1.71(1.04 - 8.92)	**0.001***	2.71(1.26 - 5.43)	**0.021***
Inflammation						
Acute	13(86.7)	2(13.3)	1		1	
Chronic	4(80.0)	1(20.0)	0.31(0.10 - 1.02)	1.00	1.32(0.34 - 3.98)	0.09
Erectile dysfunction						
Mild	24(77.4)	7(22.6)	1		1	
Severe	5(62.5)	3(37.5)	0.20(0.14 -1.81)	0.40	1.42(0.42 - 4.23)	0.23

CHAPTER FIVE

DISCUSSIONS

This chapter provides discussions on the findings from this study and relating the findings to other works done on the subject matter. Cases of male infertility are on the increase and semen quality decreasing; therefore, this study was set out to describe the semen profile of already infertile males attending urology clinic for infertility in Nigeria.

In this study, the number of patients who had never achieved pregnancy was higher than those who had at least achieved one prior pregnancy. This was inconsonance with another study conducted (Christian, Patrick & Adofo, 2017). The most common clinical condition was the enlargement of the veins that hold the testicles (scrotum) and occurred higher in primary infertility than in secondary infertility. We found a little difference in the number of patients with the enlargement of the veins that hold the testicles (scrotum) reported here when compared with other studies (Christian, Patrick & Adofo, 2017; Sandro, Ricardo & Ashok, 2011). This difference could be as a rest of male patients that sought for medical intervention for their ill health. The public health impact of this is that there is an undulating prevalence and incidence cases of infertility among the population, and this calls for national action for the detection, prevention and management of infertility.

Furthermore, we observed that half of the patients under review had low sperm cells count per ejaculate (oligospermia). Among the patients with low sperm cells count per ejaculate, severe oligospermia recorded highest frequency followed by moderate, and the least was mild oligospermia. No/absence of sperm cells in the semen was least observed amongst the patients under review. When compared with other studies, our study revealed higher occurrence of mild and severe low sperm counts than theirs (Christian, Patrick & Adofo, 2017). According to

WHO, increased quantity of semen is considered when semen volume exceeds 6.0mL (>6.0mL) and decreased quantity of semen when less than 1.5mL (<1.5mL). Therefore, the percentage of decreased quantity of semen in this study was higher than increased quantity of semen. This was like another study conducted in India (Pradeep, 2018), but slightly differ from the one conducted in Ghana (Christian, Patrick & Adofo, 2017). This low quantity of semen could be because of incomplete ejaculation due to a dysfunction of the ejaculatory reflex, or insufficient secretions of one or more of the hormones (Robin et al., 2008). Other possible factors are environmental, hormonal, chemicals, infections, radiations and genetics, and these factors either interact with each other or singly to affect semen parameters either the same way or differently as explained in the conceptual framework.

The public health implication of this is that there likely to be an increase demand for assisted reproductive techniques e.g., in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) which are not readily available in our environment and where available are not within the reach of the commoners. Increased quantity of semen could be explained because of prolong abstinence. Mamor (1993) has proved that increase in sperm volume can contribute to infertility.

The ideal duration of abstinence as recommended by WHO duration is between 2-3 days. From our study, larger percentage of patients under review had 4-5 days duration of abstinence followed by 2-3 days duration of abstinence and the least number of patients were in the >5 days. The number of patients who complied with the recommended duration of sexual abstinence was lower here than in another study conducted (Pradeep, 2018). The pattern of seminal pH observed in this study ranging from the most to the least was neutral pH, basic pH, and acidic pH. We observed a slight difference in the number of semen samples with acidic pH range when compared with another study conducted in Algeria, (Anissa, 2020). The pH

alkalinity can be due to prostatic insufficiency or the presence of infection whereas the pH acidity may be due to seminal vesicular dysfunction (Okonofua, 2003).

Furthermore, it was found that more than half of the patients under review had decreased semen thickness/stickiness followed by normal sperm viscosity while the incidence of increased sperm thickness was least. An increased stickiness could suggest a prostatic dysfunction. Our result was not congruent with other studies (Anissa, Malika & Tewfik, 2020; James, 2019).

In this study, the modal age group was 30-50 years with the highest frequency. The impact of this is that ageing male expresses changes in sexual function which has been collaborated with in different studies. Another possible effect of this ageing is that the seminiferous tubules will begin to produce low spermatids as reported that men in their 20s and 30s produced more spermatids compared with men in their 40s and 50s and pronounced in semen volume drops from a median of 2.80mL in men aged 45-47.8 years to 1.95mL in men aged >56.6 years (Hellstrom et al., 2006; Sasano & Ichijo, 1969).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

This chapter provides highlights of the major findings from the study and recommendations are also provided.

6.1 CONCLUSION

This study concludes that:

i. More than half of the cases of infertility among males attending the Urology Clinic at the University College Hospital had never achieved pregnancy.

ii. Enlargement of the veins that hold the testicles (scrotum) was the commonest cause of inability to impregnate followed by inability to keep/sustain erection.

iii. More than six out of ten attendees had low sperm cells count per ejaculate and this was commoner among those who had never achieved pregnancy than those who had at least achieved one prior pregnancy and in more than four of the six, the low sperm cells count was severe. Indeed, one out of five had low sperm cells count.

iv. The no of sperm cells in one ejaculate observed showed equal distribution of counts 1-14million/mL and those above that.

We conclude therefore that varicocele, erectile dysfunction, low volume of ejaculate and sperm count are the commonest presentations of men with infertility at the Urology Clinic and were also strongly associated with infertility in males.

6.2 RECOMMENDATIONS

The following recommendations can be made from the findings from this study:

i. Low sperm count, low volume of ejaculate and erectile dysfunction was observed among the patients in this study therefore, patients should take their medications as prescribed and

report any improvement or otherwise to their physicians.

ii. The commonest clinical condition reported was enlargement of the vein that hold the testicles and recorded more in primary infertility than in secondary infertility. Therefore, it is imperative that early monitoring of this vein for early detection of possible enlargement should be encouraged in our hospitals. For this reason, the Ministry of Health as a matter of necessity should engage/sensitise the general populace on the possible danger of this clinical condition.

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APPENDICES

1.1 Data abstraction guide

Instruction: Copy or check the box.

Patient Index No…….

Serial No: ……. Date: …….

1. Age: ……years 2. Duration of abstinence: ……days

3. Date (sample collected) ……

4. Clinical condition: …….

5. Type of infertility: Primary ☐ Secondary ☐

6. Method of production: Masturbation ☐

7. Ejaculatory volume: ……mL

8. Viscosity: Increase ☐ Normal ☐ Decreased ☐

9. pH: ……

10. Sperm concentration: ……million/mL

11. No sperms in the ejaculate: Azoospermia ☐

12. Sperm morphology: Normal ……. % Abnormal ……. %

13. Motility: Motile……. % Immotile……%

14. Vitality: …… (%)

15. Varicocele: Palpable ☐ Impalpable ☐

16. Trauma: Blunt ☐ Penetrating ☐

17. Inflammation: Acute ☐ Chronic ☐

18. Erectile dysfunction: Mild ☐ Severe ☐

1.2 Standard Operating Procedure (SOP) for data abstraction

- Each patient's result was checked for inclusion criteria and completeness of information, and those that met these conditions were recruited.
- The next patient on the register (laboratory) was either included or excluded based on the above conditions.
- Each patient was assigned unique serial number when recruited.
- This was repeated until the last patient for the sample size was reached.

1.3 Ethical Approval

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)
College of Medicine, University of Ibadan, Ibadan, Nigeria.

Director: **Prof. Catherine O. Falade,** *MBBS (Ib), M.Sc., FMCP, FWACP*
Tel: 0803 326 4593, 0802 360 9151
e-mail: cfalade@comui.edu.ng lillyfunke@yahoo.com

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Semen Profile of Male Patients Attending Urology Clinic for Infertility: Analysis of Laboratory Records at Surgical Research Laboratory, Ibadan, Nigeria.

UI/UCH Ethics Committee assigned number: UI/EC/21/0193
Name of Principal Investigator: **Julius A. Adediji**
Address of Principal Investigator: Department of Surgery
College of Medicine
University of Ibadan, Ibadan

Date of receipt of valid application: 07/05/2021

Date of meeting when final determination on ethical approval was made: **N /A**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

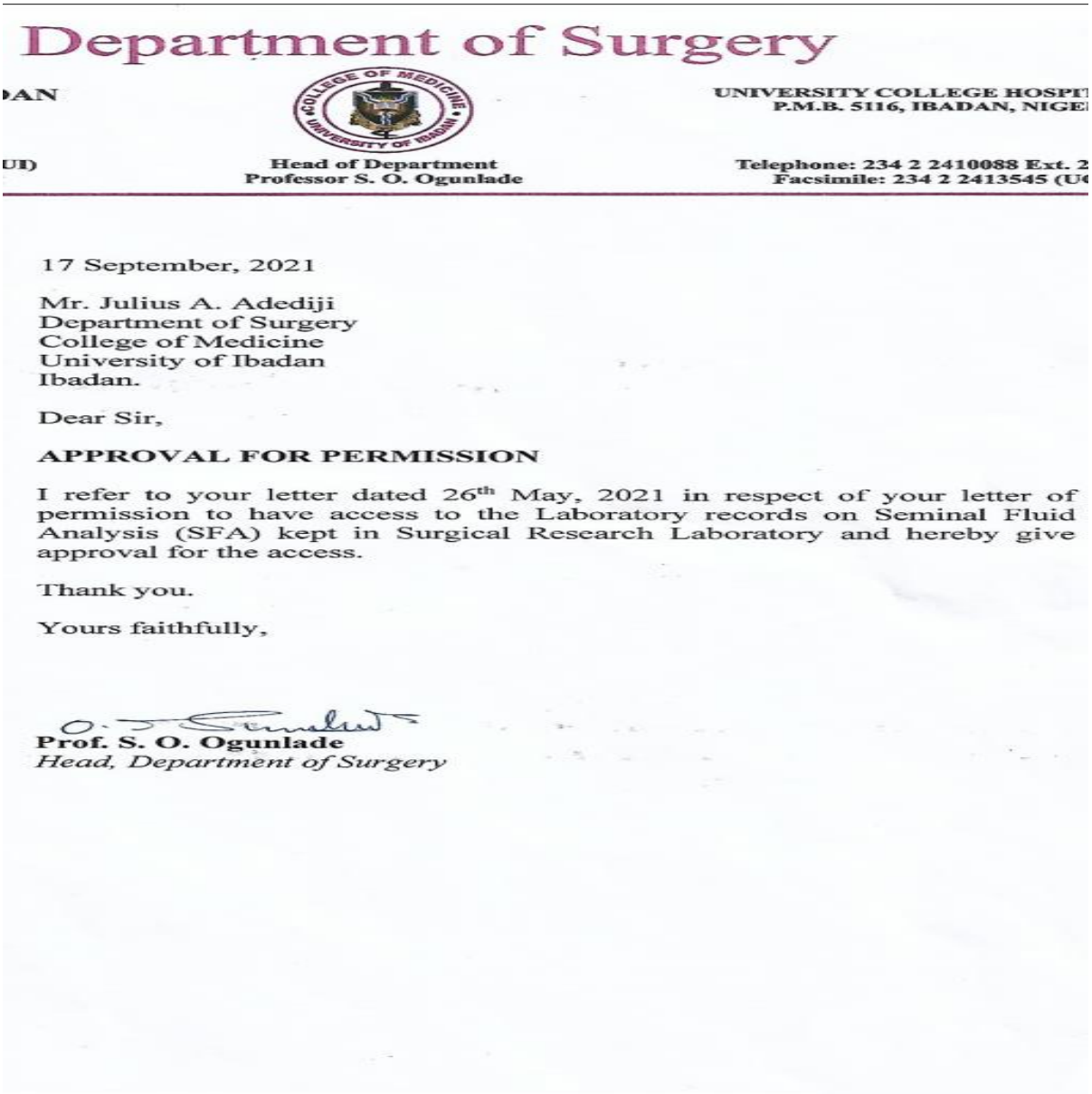
This approval dates from **21/05/2021 to 20/05/2022.** If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

UI/UCH EC
21 MAY 2021
APPROVED
COLLEGE OF MEDICINE - U.I.

Professor Catherine O. Falade
Director, IAMRAT
Chairperson, UI/UCH Research Ethics Committee
E-mail: uiuchec@gmail.com

1.4 Approval for permission



Department of Surgery

DAN

UNIVERSITY COLLEGE HOSPI
P.M.B. 5116, IBADAN, NIGE

UI)

Head of Department
Professor S. O. Ogunlade

Telephone: 234 2 2410088 Ext. 2
Facsimile: 234 2 2413545 (U

17 September, 2021

Mr. Julius A. Adediji
Department of Surgery
College of Medicine
University of Ibadan
Ibadan.

Dear Sir,

APPROVAL FOR PERMISSION

I refer to your letter dated 26th May, 2021 in respect of your letter of permission to have access to the Laboratory records on Seminal Fluid Analysis (SFA) kept in Surgical Research Laboratory and hereby give approval for the access.

Thank you.

Yours faithfully,

Prof. S. O. Ogunlade
Head, Department of Surgery